Contemporary Nephrology

Volume 4

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Contemporary Nephrology

Volume 4

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Preface

Volume 4 of *Contemporary Nephrology* summarizes major advances in 16 different areas of nephrology during the years 1985 and 1986. Major changes in the composition of the Editorial Board and authorship of the different chapters have occurred in this volume. Six distinguished contributors have retired from the Editorial Board. They include Dr. Zalman A. Agus, Philadelphia; Dr. Robert Anderson, Denver; Dr. Eli Friedman, Brooklyn; Dr. Richard Glassock, Torrance, California; Dr. James Schafer, Birmingham, Alabama; and Dr. Gordon Williams, Boston. We are grateful to them for their outstanding contributions to the first three volumes of this series and for their advice and suggestions as members of the Editorial Board. They certainly deserve substantial credit for the success of this series.

Seven outstanding academicians have joined the Board. They include Dr. Vito M. Campese, Professor of Medicine at the University of Southern California, who contributed the chapter on "Recent Advances in the Role of the Renal Nervous System and Renin in Hypertension"; Dr. William G. Couser, Professor of Medicine and Head of the Division of Nephrology at the University of Washington in Seattle ("Immunologic Aspects of Renal Disease"); Dr. Garabed Eknoyan, Professor of Medicine and Vice Chairman of the Department of Medicine at Baylor College of Medicine ("The Uremic Syndrome"); Dr. H. David Humes, Associate Professor of Medicine and Chief of the Nephrology Section at the University of Michigan Medical School, Veterans Administration Medical Center ("Acute Renal Failure and Toxic Nephropathy"); Dr. Luis Reuss,

Professor and Chairman of the Department of Physiology and Biophysics at the University of Texas Galveston Branch ("Isosmotic Fluid Transport across Epithelia"); Dr. Wadi N. Suki, Professor of Medicine and Physiology at Baylor College of Medicine ("The Kidney in Systemic Disease"); and Dr. Roger A. L. Sutton, Professor of Medicine and Head of the Division of Nephrology at the University of British Columbia ("Mineral Metabolism"). We would like to welcome these distinguished clinician–investigators as members of the Editorial Board and look forward to working with them in the next three volumes of this series.

A new chapter on "Congenital Renal Disorders and Kidney Tumors" is included in this volume. Dr. Manuel Martínez-Maldonado, who was previously responsible for the chapter on "The Kidney in Systemic Disease," contributed this new chapter. In addition, the volume includes contributions by previous members of the Editorial Board: Dr. William M. Bennett ("Drugs and the Kidney"); Dr. Michael J. Dunn ("Renal Prostaglandins"); Dr. Lee W. Henderson ("Dialysis"); Dr. Franklyn G. Knox ("Renal Hemodynamics and Sodium Chloride Excretion"); Dr. Neil A. Kurtzman ("Acid–Base Physiology and Pathophysiology"); Dr. William E. Mitch ("Nutrition in Renal Disease"); Dr. Anton C. Schoolwerth ("Renal Metabolism"); and Dr. Terry B. Strom ("Renal Transplantation").

It is our belief that this series continues to serve well the original design of the editors, to provide the reader with an update of important developments in the major areas that comprise modern nephrology. It has become evident in recent years that the explosion of knowledge in different areas of nephrology has accelerated, and it is becoming more and more difficult to keep abreast of developments in the different areas of the subspecialty. This volume provides a summary, written by recognized experts, of major advances that have occurred in their particular areas of interest in the last 2 years. The editors would again like to express their deep appreciation to the members of the Editorial Board, those who have participated since the beginning of the series and those who have just joined, for their contributions to the success of this series. At the same time, we would like to thank the different authors for their contributions, which made this volume possible. We again expect our readers to provide us with comments and criticisms so that we may better serve their needs in future volumes of this series.

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Isosmotic Fluid Transport across Epithelia

Luis Reuss and Calvin U. Cotton

1. Introduction

The purpose of this chapter is to review water transport mechanisms in proximal renal tubule and in other epithelia that either participate in water homeostasis or are good model systems for experimental studies of isosmotic water transport. First, we shall review basic principles and definitions, in order to provide a theoretical framework for analysis of the experimental data, and then we shall discuss the particular case of isosmotic transepithelial water transport, using the mammalian renal proximal tubule as the main, but not exclusive model.

2. Basic Principles

This section follows closely the introductory chapters in the recent book by Finkelstein,[1] which should be consulted for derivations of equations and further details. The classic book by House[2] is also an excellent comprehensive reference in this field.

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2.1. Osmotic Equilibrium

The simplest system that can be used to illustrate the principle of *osmotic equilibrium* consists of a semipermeable membrane separating water from a solution that contains a nondissociating solute (Fig. 1). A semipermeable membrane has a finite permeability to water and is impermeable to the solute. In such a system, at thermodynamic equilibrium there is no driving force for *net* water flow across the membrane. It has been shown that for dilute solutions this condition of equilibrium is approximately described by van't Hoff's law:

$$\Delta P = \pi = RTC_s' \tag{1}$$

where ΔP is the hydrostatic pressure difference between the two compartments ($P' - P''$), R and T are the gas constant and the absolute temperature, respectively, C_s' is the molar concentration of the solute, and π is the *osmotic pressure* of the solution, i.e., the pressure that must exist in that solution, relative to that of the water-filled compartment, for the net water flow across the semipermeable membrane to be zero.

When the semipermeable membrane separates two solutions, the relationships between P, π, and C_s on each side of the membrane can be described by Eq. (1). Combining the two equations yields

$$P' - P'' = \Delta P = RT(C_s' - C_s'') = \pi' - \pi'' = \Delta\pi \tag{2}$$

The magnitude of the pressure generated across the membrane at equilibrium depends on the molar concentrations of the impermeant solute and the number of particles (n) that each molecule yields in solution. Osmolality of a solution, measured in osmoles/kg H_2O is given by

$$\text{Osm} = nC_s \tag{3}$$

At room temperature, a 1-Osm solution has an osmotic pressure of about 24.6 atm. Although osmolality is a measure of concentration of particles, not of pressure, because of the above equivalence it is frequently used to denote osmotic pressure.

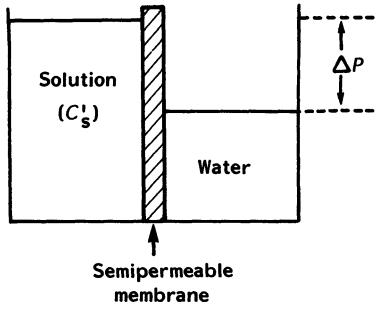

Fig. 1. Osmotic equilibrium. The semipermeable membrane separates a solution (left) from water (right). At equilibrium, there is no net water flow across the membrane, and $\Delta P = \pi' = RTC_s'$.

The mechanism of generation of ΔP upon addition of impermeant solute to one side of the membrane can be understood by considering its effect on the water chemical potential (μ_w), which is given by

$$\mu_w = \mu_w^\circ + RT \ln X_w + P\bar{V}_w \tag{4}$$

where μ_w° is the standard chemical potential, X_w is the water mole fraction [moles of water/(moles of water + moles of solute)], and $\bar{V}_w$ is the partial molar volume of water; the other symbols have been defined. Addition of solute to one side reduces the chemical potential of water in that side (μ_w') because the water is "diluted" by the solute. Hence, a difference in water chemical potential is created ($\Delta\mu_w = \mu_w'' - \mu_w'$), which is the "driving force" for water flow toward the side of higher osmolality (and lower μ_w). Depending on the physical properties of the system, this flow can result in a change in ΔP across the membrane (which was initially 0). For instance, if both compartments are open to the atmosphere, ΔP will result from changes in the height of both compartments. If one of the compartments is closed, its pressure will change in proportion to the water flux, with a proportionality constant dependent on the compliance of the compartment.

No particular mechanisms of water transport need be invoked for the preceding analysis. The derivation of equations (1) and (2) is based on thermodynamics and is thus independent of mechanistic considerations.

2.2. Osmotic Water Flow

For small deviations from equilibrium, the volume flow is linearly related to the driving force, according to

$$J_v = L_p(\Delta P - \Delta\pi) \tag{5}$$

where J_v is the volume flow (volume·area^{-1}·time^{-1}), L_p is the *hydraulic permeability coefficient* of the membrane, and ΔP and $\Delta\pi$ are the differences in hydrostatic and osmotic pressure, respectively. The L_p is usually expressed in cm·sec^{-1}·(osmoles/kg)$^{-1}$. In most cases, a *filtration* or *osmotic permeability coefficient* is used instead of L_p. The coefficient is related to L_p by

$$P_f = P_{os} = \frac{L_p RT}{\bar{V}_w} \tag{6}$$

The units of P_f and P_{os} are cm·sec^{-1}. It is clear from the above relationships that ΔP and $\Delta\pi$ are equivalent as "driving forces" in determining osmotic water flow. The reason for such equivalence is not at all obvious; understanding it requires consideration of the nature of the membrane under study and the mechanism of osmotic water transport.

2.2.1. Osmotic Water Flow across Lipid Membranes

The mechanism of osmotic water flow across homogeneous lipid membranes is referred to as *solubility–diffusion.* Water moves from the solution into the membrane, across the lipid phase and into the other solution, by independent, random motion. In the absence of a net driving force between the two aqueous phases, that is, when $\Delta P = \Delta\pi$, the two diffusive fluxes are equal, and there is no net flux (or flow) of water across the membrane. When a net driving force exists, i.e., when $\Delta P - \Delta\pi \neq 0$, a net flux arises. The mechanisms of water flow can be better explained if we consider the effect of ΔC_s and ΔP on the chemical potential of water in the two compartments.

A net diffusive water flux can only result from a difference in water chemical potential within the membrane. If the membrane is homogeneous in composition, a steady flux results when the chemical potential gradient throughout the membrane thickness is constant. When there is a difference in osmotic pressure between the two solutions, the water mole fractions and hence the water concentrations *just inside* the membrane are different at the two sides. If water transport across the membrane–solution interfaces is faster than water diffusion in the membrane, then the water chemical potential just inside the membrane is equal to that in the adjacent layer of solution, and therefore water is at equilibrium across the interfaces. Since μ_w is inversely related to C_s, a gradient of water concentration will exist across the membrane if the concentrations of impermeant solute in the aqueous phases differ.

Similarly, when initially $\Delta\pi$ is 0, but ΔP is not 0, the water chemical potentials in the two solutions are different (because $P' > P''$) and hence the flux of water into the membrane from side $'$ is greater than that from side $''$, creating a difference in water concentration (and water chemical potential) within the membrane.

The osmotic water permeability coefficient of a lipid membrane is given by[1]

$$P_{os} = \frac{D_w^m \beta_w \bar{V}_w}{d\bar{V}_{oil}} \tag{7}$$

where D_w^m is the water diffusion coefficient in the membrane, β_w is the oil/water water partition coefficient, d is the membrane thickness, and $\bar{V}_{oil}$ is the partial molar volume of the membrane lipid.

2.2.2. Osmotic Water Flow across a Porous Membrane

For simplicity, let us consider a rigid membrane of a water-impermeable material that contains n pores per unit area. The pores are water-filled cylinders of length L and radius r. The solute does not penetrate

the pores. Water flow through membrane pores can be assigned to one of three modes, based on the radius of the pores: (1) the pores are large enough that the water flow can be described by Newtonian mechanics; (2) the pores are of molecular dimensions, in which case there is no clear-cut theoretical treatment of the water flow; (3) the pores are so narrow that there is single-file water transport; i.e., water molecules in the pore cannot slip past each other. We will consider these three cases separately.

2.2.2.1. Large Pores. In the case of large pores, water flow driven by a hydrostatic pressure is described by Poiseuille's law, originally derived to describe water flow in thin capillaries:

$$J_v = n\frac{(\Pi)r^4}{8L\eta}\Delta P \tag{8}$$

where η is the viscosity of water and the symbol (Π) has been used to denote the constant 3.1415 . . . , to avoid confusion with π = osmotic pressure.

Poiseuille's law applies to steady flow, when pore access effects can be neglected. The pressure gradient (dP/dL) remains constant along the length of the pore, as depicted in Fig. 2A.

The mechanism of water flow is not obvious when the only driving force is a difference in osmotic pressure. In this case, the water concentration in the pore is the same as in the water-filled compartment, since both contain pure water, but there is a sharp transition at the interface between the pore interior and the solution, with the latter having a lower water concentration. If water transport across the membrane interfaces is faster than water transport across the membrane itself, equilibrium

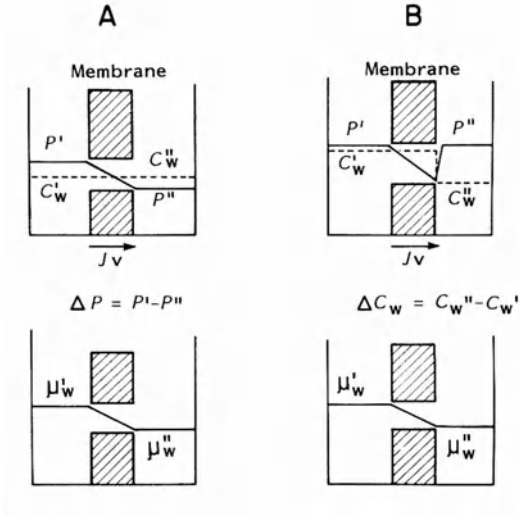

Fig. 2. Mechanisms of water flow across a porous membrane. Top: A: Flow driven by a difference in hydrostatic pressure (ΔP). The water concentration (C_w) is the same on both sides of the membrane. B: Flow driven by a difference in osmotic pressure, i.e., in concentration of impermeable solute. The water concentrations differ, but the pressures are the same on both sides. Bottom: The gradients of water chemical potential (μ_w) in the steady state are equal for both conditions.

exists at the two interfaces, i.e., at the pore openings. In terms of chemical potential of water

$$\mu_w(O) = \mu_w' \text{ and } \mu_w(L) = \mu_w'' \tag{9}$$

where O and L denote values just inside the pore, at the water and solution interfaces, respectively. The hydrostatic pressure difference between the water-filled compartment and the adjacent pore opening, given by $P' - P(O)$, is zero, since there is no difference in water concentration. However, $P(L)$ will be less than P'' because there is a difference in water concentration between the pore interior and the solution. At equilibrium, that is, if $\Delta\mu_w$ is zero across the pore opening, then

$$P'' - P(L) = RTC_s \tag{10}$$

In other words, the water concentration difference between pore interior and solution is "balanced" by a fall in the pressure inside the pore, created by net water flux out of the pore. This flux causes a state of "tension" in the fluid inside the pore.[3] In the steady state, the pressure profile inside the pore is linear, as depicted in Fig. 2B. Since there is equivalence between ΔP and $\Delta\pi$ as driving forces for water flow, P_f (or P_{os}) for both cases can be expressed in terms contained in Poiseuille's law:

$$P_f = P_{os} = n\frac{(\Pi)r^4RT}{8L\eta\bar{V}_w} \tag{11}$$

2.2.2.2. Pores of Molecular Dimensions. As shown by Bean,[4] the above formulation is valid for pores of $r \geq 115$ nm. For pores of $r < 15$ nm, several ad hoc corrections have been formulated, including the addition of a diffusive water flow term, a correction for steric hindrance of water penetration into the pore (because for small r, the size of the water molecule cannot be neglected, as in the Poiseuille formulation), and a term corresponding to the frictional interaction of water with the pore wall. As pointed out by Finkelstein,[1] such corrections are based on assumptions valid for continuum mechanics, but do not necessarily apply in this case. No satisfactory theory exists to treat the case of pores of $r < 15$ nm, but an interesting simulation study by Levitt[5] suggests that Poiseuille's law provides a satisfactory description of water transport in small pores as well.

2.2.2.3. Single-File Pore. For a single-file pore, P_{os} is given by[1,6]

$$P_{os} = n\frac{\bar{v}_w kTN}{\gamma L^2} \tag{12}$$

where $\bar{v}_w$ is the volume per water molecule, k is the Boltzmann constant ($=R/N_A$, where N_A is Avogadro's number), N is the number of water

molecules in the pore, and γ is the frictional coefficient per water molecule. If the water densities inside the pore and in bulk solution are equal,

$$N = \frac{(\Pi)r^2L}{\bar{v}_w} \tag{13}$$

and, since $kT/\gamma = D_w$, equation (12) reduces to

$$P_{os} = n\frac{(\Pi)r^2D_w}{L} \tag{14}$$

which is exactly the result expected if osmotic water flow through a single-file pore can be described as a diffusive flux.

2.3. Diffusion

In contrast with the discussion of osmotic water flow, we consider now a lipid or porous membrane exposed to solutions identical in composition, except for partial replacement of water with tracer water at a concentration C_w^* (Fig. 3). There are no differences in water or solute concentration or in pressure between the two aqueous compartments. The solutions are of infinite volume and perfectly mixed (C_w^* at the membrane surface = C_w^* in the bulk). The tracer water flux is given by

$$J_w^* = P_{dw}\Delta C_w^* \tag{15}$$

where P_{dw} is the diffusive water permeability coefficient of the membrane and ΔC_w^* is the difference in tracer water concentration ($C_w^{*\prime} - C_w^{*\prime\prime}$). Again, we consider separately the cases of lipid and porous membranes.

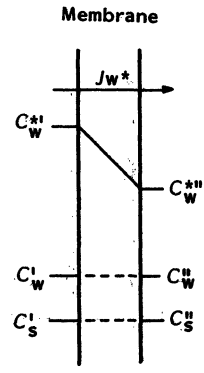

Fig. 3. Diffusion of tracer water across a lipid membrane. Tracer water concentration denoted as C_w^*. At the steady state, the gradient of tracer concentration in the membrane is constant. In the case depicted, the oil/water partition coefficient of the tracer is 1.0. If it were less, the tracer concentrations in the membrane would be lower than in the respective adjacent solutions.

2.3.1. Lipid Membrane

Since the tracer water flux is by solubility–diffusion, P_{dw} is given by

$$P_{dw} = \frac{D_w^m \beta_w \bar{V}_w}{d\bar{V}_{oil}} \tag{16}$$

where all parameters have been defined above. The above expression for P_{dw} is identical to that for P_f (or P_{os}) for a lipid membrane [equation (7)]. Hence,

$$P_{os}/P_{dw} = 1 \text{ (lipid membrane)} \tag{17}$$

2.3.2. Porous Membrane

In the case of pores that obey Poiseuille's law, the diffusive flux of water through the pores is described by

$$J_w^* = \frac{n(\Pi)r^2 D_w}{L} \Delta C_w^* \tag{18}$$

where the area for tracer water diffusion is equal to the cross-sectional area of pores $[n(\Pi)r^2]$ and D_w is the water self-diffusion coefficient (in the membrane, tracer water diffuses in the water-filled pores). Inspection of equation (18) gives an expression for P_{dw}:

$$P_{dw} = \frac{n(\Pi)r^2 D_w}{L} \tag{19}$$

and the relationship between P_{os} and P_{dw} [compare equations (11) and (19)] becomes

$$P_{os}/P_{dw} = \frac{RT}{8\eta D_w \bar{V}_w} r^2 + 1 \tag{20}$$

where the 1 comes from the contribution of the diffusive water flow through the pores. From equation (20), the equivalent pore radius of a porous membrane can be calculated from the experimentally determined values of P_{dw} and P_{os}. At 25°C, $[RT/(8\eta D_w \bar{V}_w)]$ has a value of $8.04 \cdot 10^{-14}$ cm^{-2}.

In the case of pores of $r < 15$ nm, approximate solutions to calculate pore radius have been proposed.[7]

In the case of single-file pores, the diffusive water flux is given by

$$J_w^* = nP_{dw}\Delta n^* \tag{21}$$

where Δn^* is the difference in tracer water concentration between the two compartments, in molecules per unit volume. P_{dw} is given by

$$P_{dw} = \frac{n\bar{v}_w kT}{dL^2} \tag{22}$$

and by comparing equations (12) and (22) it can be seen that the ratio between P_{os} and P_{dw} is equal to the number of water molecules in the pore:

$$P_{os}/P_{dw} = N \text{ (for single-file pores)} \tag{23}$$

The explanation for this result is that P_{dw} in a single-file pore is proportional to $1/L^2$, whereas P_{os} is proportional to $1/L$. This is in contrast to the situation in a larger pore, where diffusion is proportional to $1/L$. The dependency on $1/L^2$ can be understood as a violation of the independence principle in the single-file pore. Water movement in these pores is no longer independent of the movement of other water molecules, since for an individual tracer molecule to move through the pore, all other molecules must move as well. Each diffusive step of a tracer water molecule must be accompanied by a diffusive step of another water molecule; since both depend on $1/L$, the whole process depends on $1/L^2$.

2.4. Unstirred Layers

Unstirred layers at membrane–solution interfaces are layers of fluid that are not mixed (by convection) with the bulk solution. The solute concentrations in the unstirred layers are determined by diffusion, can be different from that of the adjacent bulk solutions, and depend on depth in the unstirred layer. The presence of unstirred layers in series with biological membranes introduces errors in the determination of both P_{dw} and P_{os}. If unstirred layers are neglected, such errors can lead to mistaken conclusions about the existence of aqueous pores.

2.4.1. Effects of Unstirred Layers on the Estimation of P_{dw}

In an experiment such as that depicted in Fig. 4, there are in fact three barriers for diffusion of tracer water between the two bulk solutions—the membrane and two unstirred layers in series (of thicknesses δ_1 and δ_2, respectively). Hence, the observed diffusive water permeability of the system differs from the membrane diffusive permeability according to

$$\frac{1}{P^o_{dw}} = \frac{1}{P_{dw}} + \frac{1}{D_w/\delta_1} + \frac{1}{D_w/\delta_2} \tag{24}$$

where P^o_{dw} is the measured value. Solving equation (24) for P^o_{dw}, taking $\delta_1 = \delta_2 = \delta$, one obtains

$$P_{dw}^{o} = \frac{1}{1 + P_{dw}(2\delta/D_w)} P_{dw} \tag{25}$$

which indicates that $P_{dw} = P_{dw}^{o}$ only when $\delta = 0$. For a typical set of values of $D_w = 2.4 \cdot 10^{-5}$ cm^2·sec^{-1}, $2\delta = 0.024$ cm (120 μm on each side), and $P_{dw} = 10^{-3}$ cm·sec^{-1}, the true P_{dw} of the membrane is underestimated by 50%.

2.4.2. Effects of Unstirred Layers on the Estimation of P_{os}

In a system consisting of a semipermeable membrane separating equimolar NaCl solutions, addition of another solute to one side only causes osmotic water flow across the membrane (Fig. 5). This flow causes concentration changes in the unstirred layers: salt is concentrated in the right-hand side and both salt and the other solute are diluted in the left-hand side, relative to their bulk solution concentrations. At the steady state, the concentration profiles in both unstirred layers remain constant, because the effects of the water flux and of solute diffusion balance each other. At the membrane surface, for the "diluted" side

$$C_m = C_b \exp(-v\delta/D_s) \tag{26}$$

and for the "concentrated" side

$$C_m = C_b \exp(v\delta/D_s) \tag{26a}$$

where C_m and C_b are the solute concentrations at the membrane surface and in the bulk solution, respectively, v is the flow velocity of water

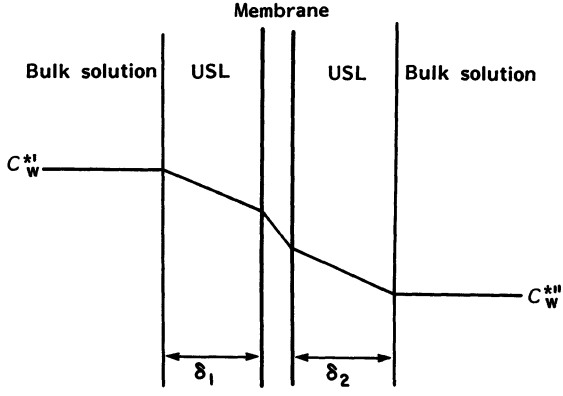

Fig. 4. Effects of unstirred layers on the estimation of P_{dw}. C_w^* denotes the tracer concentration. The presence of unstirred layers causes the steady-state concentration difference across the membrane to be less than the concentration difference between the two bulk solutions.

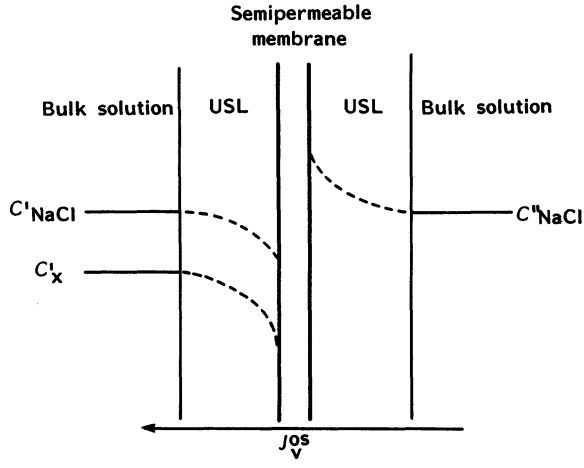

Fig. 5. Effects of unstirred layers on the estimation of P_{os}. C_{NaCl} and C_x denote the concentrations of NaCl and the osmotic solute, respectively. Both are assumed impermeant for simplicity. The water flow ($J_w = J_v$) in response to the osmotic gradient causes "dilution" of both solutes in the unstirred layer on the hyperosmotic side and "concentration" of the NaCl in the unstirred layer on the other side.

(perpendicular to the membrane surface), and D_s is the diffusion coefficient of either solute in water.

According to equation (26), the relationship between the measured value, P^o_{os}, and the true value, P_{os}, is given by

$$P^o_{os} = P_{os} \exp(-v\delta/D_s) \tag{27}$$

For small volume flows and planar membranes, the value of v is small, and the exponential term approaches 1. However, in epithelia, where water flow could be funneled via preferential pathways (such as the lateral intercellular spaces), v can easily be one or two orders of magnitude larger than the value predicted for the same J_v in a planar membrane; hence P_{os} can be significantly underestimated.[8,9]

Equations (26–27) consider only the effect of J_v on the concentration of impermeant solutes. If permeant solutes are also present, their permeability must be taken into account.[8,9]

2.5. Solute Reflection Coefficient

The preceding analysis of osmotic water flow becomes more complicated if the solute is permeant. In this case (Fig. 6), the volume flow, J_v, will be less than if the solute were impermeant, and will be described by

$$J_v = L_p(\Delta P - \sigma_s \Delta\pi) \tag{28}$$

where σ_s is the reflection coefficient of the solute. As explained below, σ_s is related to the ratio of the membrane permeabilities for water and the solute. Its value can be 1 or less.

2.5.1. Lipid Membrane

In case of a lipid membrane, in which both water and solute move by solubility–diffusion, the reduction in J_v is easily explained by the coexistence of two volume flows in opposite directions, one of water (J_w) and one of solute (J_s):

$$J_v = J_w + J_s = \overline{V}_w\phi_w + \overline{V}_s\phi_s \tag{29}$$

where ϕ denotes flux (moles · cm^{-2} · sec^{-1}), $\overline{V}$ denotes partial molar volume, and the subscripts w and s indicate water and solute, respectively. Since the fluxes are diffusive, i.e., $J = P_d \cdot C$, equation (29) becomes

$$J_v = -(P_{dw}\overline{V}_w - P_{ds}\overline{V}_s)\Delta C_s \tag{30}$$

where P_{ds} is the solute diffusive permeability coefficient. By comparison with equation (28), σ_s is given by

$$\sigma_s = 1 - \frac{P_{ds}\overline{V}_s}{P_{dw}\overline{V}_w} \tag{31}$$

Clearly, $\sigma_s = 1$ when P_{ds} is 0, $\sigma_s = 0$ when $P_{ds}\overline{V}_s = P_{dw}\overline{V}_w$, and σ_s is negative when $P_{ds}\overline{V}_s > P_{dw}\overline{V}_w$. In other words, the solute reflection coefficient can be 1 or less, depending on the solute permeability and partial molar volume compared with those of water. If a solute has the same diffusive permeability and partial molar volume as water, its re-

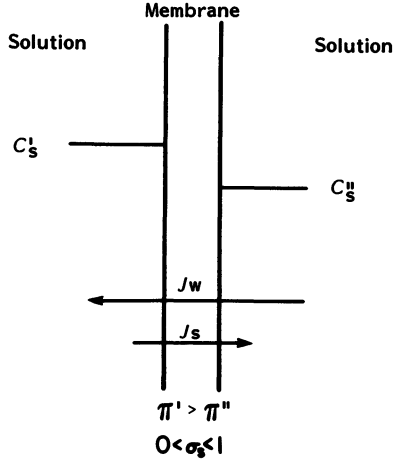

Fig. 6. Osmotic flow caused by a permeant solute ($0 < \sigma_s < 1$). C_s denotes solute concentration. The volume flow across the membrane is the difference between the net water flow (J_w) toward the left-hand side and the net solute flow (J_s) toward the right-hand side.

flection coefficient is zero; that is, its addition to one side of the membrane causes no transmembrane volume flow. In such a case, there is a water flux toward the solute-containing side (of the same magnitude as that observed with an impermeant solute), but it is exactly balanced by a solute flux in the opposite direction. Addition of a solute with a negative reflection coefficient will cause a net volume flow in a direction opposite to that of the water flow, a phenomenon referred to as negative osmosis.[1]

2.5.2. Porous Membrane

A detailed analysis of the interpretation of reflection coefficients in porous membranes is beyond the scope of this chapter, and consequently this discussion will be qualitative. For a complete analysis of the problem, the reader is referred to Anderson and Malone[10] and Finkelstein.[1]

In the case of large pores, the problem can be understood by considering the distribution of solute particles near the pore opening. If the solute particles are larger than the water particles, the solute is excluded from a region in the periphery of the pore. Near the axis of the pore, at its opening, C_s is equal to that in the external solution, and therefore C_w at this site is less than at the periphery of the pore. Hence, there is a water concentration gradient from the periphery to the axis of the pore. At equilibrium, the water concentration gradient is balanced by a drop in hydrostatic pressure in the periphery [see equation (4)]. Since there is a longitudinal solute concentration gradient, a longitudinal hydrostatic pressure gradient is generated along the periphery of the pore, which will result in water flow toward the side with the higher C_s. The water flow through the outer annulus of the pore is non-Poiseuillian. The larger the solute, the thicker the annulus, until, when the solute cannot penetrate the pore, the value of J_v reaches a maximum and $\sigma_s = 1$. If the solute is equal in size to water, it is distributed within the cross-section of the pore exactly as water. Hence, no pressure gradients exist, and the two flows (J_w and J_s) are strictly diffusive, equal in magnitude, and opposite in direction. If the solute particles are smaller than the water particles, the situation reverses; J_v is in the direction of J_s, since water is partially excluded from the region of the pore near the wall, and solute is driven in the opposite direction by the pressure gradient within the pore.

In a single-file pore, for the case of the solution dilute enough so that the pores cannot contain more than one molecule of solute, at any time two populations of pores will exist: (1) pores that contain only water, in which case net water flow occurs toward the concentrated solution, and (2) pores that contain solute, in which case the solute and the water contained in the pore are transported in the opposite direction by single-

file diffusion. If both the solute and the water in the pore are subject to the single-file restriction, σ_s is given by[1,11]

$$\sigma_s = 1 - \frac{P_{ds}\bar{V}_p^s}{P_{dw}\bar{V}_p} \tag{32}$$

where $\bar{V}_p^s$ and $\bar{V}_p$ are the molar volumes of solute-containing and solute-free pores, respectively. Note the similarities between this expression and equation (31).

2.6. Two Solutes with Different Reflection Coefficients

Net water (and volume) flow can take place between two solutions with equal total solute concentrations (and osmolalities), if there are concentration gradients of the individual solutes across the membrane, and if the reflection coefficients of the solutes differ. For instance (see Fig. 7), if $\Delta C_s = -\Delta C_x$, $\sigma_s = 0$, and $\sigma_x = 1$, a net volume flow will result although the total osmolalities of the solutions are the same. If at time 0 the pressures are the same in both compartments,

$$J_v = L_p RT(\sigma_x \Delta C_x - \sigma_s \Delta C_s) \tag{33}$$

Since $\sigma_s = 0$, the volume flow will be toward the solution with higher concentration of impermeant solute (C_x). The product $\sigma RT\Delta C$ has been called the "effective osmolality" of the solution. In the case of epithelia, which can perform active solute transport, asymmetries in solution composition can be established which in principle can result in net water

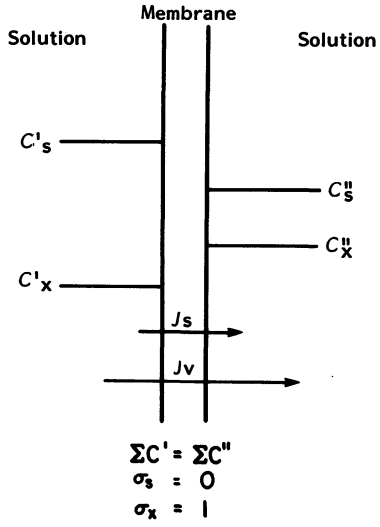

Fig. 7. Volume flow between solutions of equal total osmolalities ($\Sigma C' = \Sigma C''$). The concentration differences across the membrane are the same for solutes s and x. However, since $\sigma_s = 0$, whereas $\sigma_x = 1$, a net volume flow (J_v) toward the compartment containing the impermeant solute occurs. The steady-state J_v is the algebraic sum of two identical water fluxes and a net flux of permeant solute (J_s) from the more concentrated side.

transport in the absence of differences in bulk solution osmolality (see Section 3.3.1.).

2.7. Solvent Drag

When a net water flow is present across a porous membrane and a solute is present that also permeates the pores, the water flow causes a solute flux in the same direction. This flux has been attributed to frictional interaction between the water and the solute in the pores. For large pores with Poiseuillian flow, if $C_s' = C_s'' = C_s$, and J_v is elicited by either a hydrostatic pressure gradient or by asymmetrical addition of an impermeant solute, the solute flux due to solvent drag is given by

$$J_s = J_v C_s (1 - \sigma_s) \tag{34}$$

Accordingly, demonstration of solvent drag has been used as an argument for the presence of pores in membranes. When the permeable solute is a nonelectrolyte, present at the same concentration on both sides of the membrane, "uphill" transport can be demonstrated, which is always in the same direction as the water flow. However, such demonstration does not prove unequivocally that the mechanism of the net solute flux is frictional interaction with the solvent in the pores. If unstirred layers are present in series with the membrane, J_v will cause changes in the solute concentrations at the membrane surfaces [see equations (26) and (26a)]. If the membrane is permeable to the solute, for instance by a solubility–diffusion mechanism, a diffusive solute flux will result, according to

$$J_s = P_{ds} C_s[\exp(v\delta/D_s) - \exp(-v\delta/D_s)] \tag{35}$$

where the two exponential terms denote the fractional concentration and dilution of the solute on the two sides of the membrane, respectively. This phenomenon has been referred to as "pseudo solvent drag."[9] Demonstrations of solvent drag and the accompanying conclusion of a pore-mediated transport mechanism must take this possibility into account.

3. Isosmotic Water Transport in Epithelia

3.1. Theories of Water Transport

Water transport in proximal renal tubule and in other fluid-absorbing epithelia is usually referred to as isosmotic, since it can occur in the absence of osmotic pressure differences between the bulk luminal and basolateral solutions. Water can also be absorbed against its chemical

gradient, that is, from a concentrated to a dilute solution.[12] It is generally accepted that the net transport of water is coupled to solute transport in the same direction, as shown first in small intestine,[12] although there is at least one instance in which the dependence of proximal tubule water absorption on salt transport is doubtful.[13]

The mechanisms and pathways of water transport in epithelia are largely unknown. Assuming that the water flow is coupled to salt transport, the driving force could be either osmotic or electroosmotic. The possibility of a small transport compartment that bypasses the cytosol has also been proposed. In this scheme, water transport would be by fluid-phase pinocytosis, i.e., endocytosis at the luminal membrane and exocytosis at the basolateral one. In principle, the water transport pathway could be transcellular, paracellular, or some combination.

Before addressing the problems relevant to water transport mechanisms and pathways in proximal tubules, we shall discuss the principal theories pertaining to the mechanisms of transepithelial isosmotic water transport in general. Because of its simplicity, the gallbladder has frequently been used as a model system for this kind of study. Several interesting, and sometimes colorful, reviews on this topic have recently been published.[8,14–16]

3.1.1. Pinocytosis

Frederiksen and Leyssac[17] claimed that energy consumption by the epithelium of rabbit gallbladder was dependent on the fluid transport rate, but not on solute transport *per se*. These workers proposed the existence of a small, membrane-enclosed intracellular compartment, which would serve as a pathway for transepithelial fluid transport. Further, they proposed the tubulocisternal endoplasmic reticulum as this compartment.[18] In recent years, several objections to this hypothesis have been raised.[14,15] First, one of their arguments is based on a dissociation between changes in the rates of Na^+ and water absorption in response to alterations in external salt concentration.[17] However, this argument is flawed, because Na^+ transport was not measured directly, but was assumed to follow the changes in NaCl concentrations. Under such conditions, delays in the effects on Na^+ transport can be expected.[14] Second, to account for fluid transport by pinocytosis, extensive membrane recycling would be required. Such recycling has not been observed in electron microscopic studies. Third, the theory does not explain the strict ionic requirements of fluid transport, particularly as described for flat epithelia, such as small intestine and gallbladder. It should be noted, however, that in the snake proximal renal tubule strict ionic requirements for fluid absorption have not been demonstrated.[13] Fourth, recent

electrophysiological studies in *Necturus* gallbladder have demonstrated that either altering the ionic composition of apical or basolateral solutions or exposing the tissue to transport inhibitors causes rapid changes in cell volume and also in intracellular ionic activities. The rates of change of the intracellular contents are close to those predicted from measurements of transepithelial transport, which strongly suggests that salt and water mix in a cellular transport pool that includes the entire cytosol, not only a restricted compartment.[19,20]

3.1.2. Electroosmosis

In electroosmosis, water transport is linked to the presence of a net electrical potential on the surface of the wall of the transport pathway (e.g., junctional or cell membrane pores). This results in an excess of counterions in the immediate vicinity of the wall. If there is a voltage difference along the length of the pathway, ions migrate, dragging water in the same direction.

Electroosmosis was proposed as a possible mechanism of transepithelial osmotic water transport by Hill,[21] who felt that the osmotic theory, as proposed by Diamond and Bossert[22] (see below), was based on unreasonable assumptions about the P_{os} of the cell membrane, the dimensions of the lateral intercellular spaces, or both. Electroosmosis, however, cannot fully account for transepithelial water transport, as illustrated by the following calculation. In mammalian epithelia, an electroosmotic mechanism would require the transfer of 1 liter of solution per 310 mosmoles of solute, i.e., a coupling ratio (J_v/J_s) of 3.3 liters/osmole. However, experimental determinations of J_v and J_s elicited by an applied transepithelial electrical potential difference in mammalian gallbladder[23] yield a J_v/J_s of only 0.5 to 1 liter/osmole. Moreover, this coupling ratio is likely to be an overestimate, since the J_v caused by imposing a transepithelial voltage could be in part due to changes in ion concentrations in unstirred layers.[24]

Although electroosmosis might not fully account for isosmotic water transport, it is possible that in epithelia with narrow lateral intercellular spaces the fluid movement along the spaces is in part electroosmotic, provided that the lateral cell membranes have fixed net charges.[25]

3.1.3. Osmosis

If water transport is coupled osmotically to salt transport, a difference in effective osmolality must exist across each of the barriers to transepithelial water flow, such that $\Delta\pi$, in the case of absorbing epithelia, favors water flow from the lumen to the interstitial compartment. De-

pending on the water transport pathway, such osmotic pressure differences might be effective across the junctional complexes or the cell membranes. In principle, it is not necessary that the total osmolalities of the solutions be different. As discussed in Section 2.6, if the solutions differ in composition, and the reflection coefficients of the constituent solutes are also different, an effective osmolality difference will be present.

If the driving force for isosmotic fluid absorption is a difference in total osmolality, solute transport must result in a decrease in luminal solution osmolality, an increase in the osmolality of a basolateral compartment, or a combination of both. In fact, depending on the epithelium, transport of a hyperosmotic fluid from lumen to interstitial compartment can result in predominant luminal hypotonicity or basolateral hypertonicity, but strictly speaking both must exist (see Section 3.4).

Early analyses of water transport in planar epithelia concentrated on the possible existence of an intraepithelial compartment in the basolateral region, namely, the lateral intercellular spaces, which would be rendered hyperosmotic, relative to the bathing solution, by active salt transport. Water would then flow into this compartment driven by $\Delta\pi$. Salt and water transport into the basolateral bathing solution would be by bulk flow, driven by the increase in hydrostatic pressure caused by fluid transport into the lateral spaces, and by diffusion of solute down its chemical gradient. Such a model, proposed by Curran and MacIntosh[26] to explain *uphill* transepithelial water transport, is shown in Fig. 8. The membranes bounding the middle compartment have different properties: one is semipermeable; i.e., it has a high water permeability and a low solute permeability; whereas the other one is porous, i.e., highly permeable to both solute and water. When the middle compartment is hyperosmotic relative to compartment 1, *water* flows from 1 to 2. Since the pressure increases in 2, there is flow of *solution* from 2 to 3. Hence, when $\pi_2 > \pi_1$, J_v will be from 1 to 3, regardless of the value of π_3. When $\pi_3 < \pi_1$, water transport from 1 to 3 will be an uphill process. No thermodynamic laws are violated in this process, inasmuch as work is necessary to maintain π_2 higher than π_1.

The model of Curran and MacIntosh explains osmotic coupling and

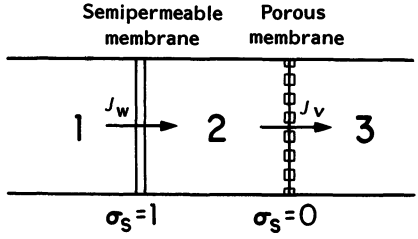

Fig. 8. Three-compartment model of Curran and MacIntosh.[26] $C_s(2) > C_s(1)$, which causes osmotic water flow (J_w) from compartment 1 to compartment 2. The elevation of the hydrostatic pressure in compartment 2 causes viscous flow of solution (J_v) into compartment 3, regardless of the solute concentration (and hence osmotic pressure) in the latter.

uphill water transport, but the fluid transported from 2 to 3 is always hyperosmotic relative to that in compartment 1, since such $\Delta\pi$ is in fact the driving force for J_v. Thus, isosmotic fluid transport cannot be explained by this simple model.

An elegant refinement of the model of Curran and MacIntosh was the notion of osmotic equilibration within an unstirred compartment, which is the basis of the standing-gradient hypothesis of Diamond and Bossert,[22] depicted in Fig. 9. Solute diffusion along the lateral intercellular space is restricted by its geometry, allowing for osmotic water flow into the space to reduce progressively the osmolality, eventually to near-isotonic levels. Diamond and Bossert analyzed mathematically the case of a long, narrow, blind channel, with the following simplifying assumptions: impermeability of the junctions (to both salt and water), active transport of salt restricted to the apical region, and no convective mixing of the fluid in the channel. The osmolality of the emerging fluid becomes closer to that of the bulk solution as the channel's length increases, the channel's width decreases, D_s decreases, and the cell membrane P_{os} increases.

Since the formulation of the standing-gradient hypothesis, two of these assumptions have been proven incorrect. The junctions were demonstrated to have high ionic permeability,[27,28] and the active transport sites, i.e., the Na^+ pumps, were shown to be distributed homogeneously in the basolateral membrane.[29] The contention was advanced[15,30,31] that only unrealistic dimensions of the lateral intercellular spaces (very long and narrow), or a much higher P_{os} than previously estimated, would allow for osmotic equilibration of the absorbate. Thus, the absolute value of the P_{os} of the structure across which water transport takes place became a crucial test of the hypothesis.

In another theoretical study it was demonstrated that the osmolality of the absorbate is highly dependent on the osmolality of the luminal solution in the immediate vicinity of the epithelial surface.[32] Salt absorption makes this solution hyposmotic, and the absorbate, which remains hyperosmotic relative to this "dilute" apical solution, can become nearly isosmotic compared to the bulk solution.

3.2. Transepithelial and Cell Membrane Osmotic Water Permeabilities

To establish the mechanisms and pathways for isosmotic water transport in epithelia such as the renal tubule, several biophysical parameters need to be determined experimentally, including the water permeabilities of the presumed barriers to water flow, the driving forces across these barriers, and the precise, microscopic mechanism of water trans-

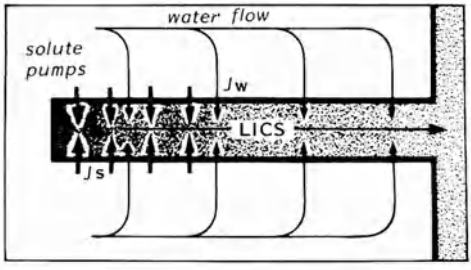

Fig. 9. Standing-gradient hypothesis of Diamond and Bossert.[22] Solute transport (thick arrows) into the channel (LICS, lateral intercellular space) causes a local increase in osmolality; water flows osmotically (J_w) across the bounding membranes (thin arrows), "diluting" the solution in the channel. Transport toward the open end is by viscous flow and diffusion.

port across each of these barriers. A complete analysis is certainly not available in proximal renal tubule or in any other epithelium, but significant progress has been made in recent years with the development of new experimental techniques. In the next sections, we shall concentrate on experimental data obtained in proximal renal tubule and gallbladder epithelium, the two preparations used for such studies. We shall discuss the measurements of transepithelial and cell membrane osmotic water permeabilities and possible routes and driving forces for water transport. Much of the information presented in these sections has been previously reviewed, in the case of proximal tubule by Berry[33] and in the case of the gallbladder by Spring.[16] The reader is referred to these reviews for additional details. Here, we shall attempt to discuss the bases of the measurements, the principal results, and the difficulties involved in their interpretation. We do not intend to offer an exhaustive review of the literature.

3.2.1. Transepithelial P_{os}

The measurement of the transepithelial P_{os} (P^t_{os}) does not constitute by itself a test of the osmotic theory. However, it is experimentally useful for two reasons: First, given the simple relationship $J_v = L_p \cdot \Delta\pi$, experimental determination of P_{os} and J_v (the spontaneous transport rate) allows estimation of the driving force for water transport ($\Delta\pi$). Second, the value of P^t_{os} can be used in conjunction with that of one of the two possible transepithelial pathways—transcellular or paracellular—to obtain a rough estimate of the P_{os} of the remaining pathway. If P^c_{os} and P^p_{os}, i.e., the transcellular and paracellular osmotic water permeabilities, were independent of each other, P^t_{os} would be simply the result of their arrangement in parallel; therefore, if P^t_{os} and P^c_{os} were known, P^p_{os} could be calculated from

$$P^t_{os} = P^c_{os} + P^p_{os} \tag{36}$$

In fact, however, the situation is more complicated because of the complicated geometry, particularly of the basolateral membrane. Since this membrane is in series with the lateral intercellular space, the resistance to water flow is distributed.

The determination of P^t_{os} is technically difficult both in tubular and in flat epithelia. In tubular epithelia, the major problems stem from the cylindrical geometry of the preparation. The changes in concentration and driving force imposed experimentally depend on position along the length of the perfused segment, making the analysis of the results mathematically complicated. A major advantage in working with renal tubules is that the thickness of the unstirred layers on both sides of the epithelial cells can be kept small. In flat epithelia, such as gallbladder, reduction of unstirred layer thickness is more difficult. However, the solute concentrations in the external solutions and the driving forces depend only on distance from the membrane surface, making formal analysis of the results easier.

3.2.1.1. P^t_{os} in Proximal Renal Tubules. P^t_{os} has been measured in mammalian and amphibian proximal tubules by a variety of techniques. We shall briefly discuss these techniques before summarizing the results of the measurements.

3.2.1.1a. Luminal perfusion in vivo with anisosmotic solutions. In these experiments,[34,35] L_p (and P_{os}) are calculated from the rate of fluid transport across the wall (J_v) and/or the osmolalities of perfusate, collected fluid, and plasma. If at the site of collection the luminal fluid has not achieved osmotic equilibrium, the L_p of the wall can be calculated from

$$L_p = \frac{C_o V_o r}{2RTC_p{}^2} b \tag{37}$$

where

$$b = \frac{1}{x}\{ln[1-(C_p/C_o)/(1-(C_p/C_x))] + C_p(1/C_o - 1/C_x)\}$$

C_o, C_p, and C_x are the osmolalities of the lumen perfusate, the capillary perfusate, and the collected fluid, respectively; r = tubule radius; x = distance between tubule perfusion and collection sites; V_o = linear velocity of the perfusion fluid. This equation was originally derived by Ullrich *et al.*,[34] assuming that only water flow across the tubule wall contributes to osmotic equilibration, and that the approach to isotonicity is by a single exponential decay from the perfusion site.* The second

* In the paper by Green *et al.*[35] there is a typographical error in the numerator of the equation taken from Ullrich *et al.*[34] The correct numerator is C_oV_or instead of C_oV_{or}.

of these assumptions is probably correct if the tubule is axially homogeneous with respect to its transport properties. However, the first assumption is more difficult to evaluate. Generally, in these experiments a low [Na^+] is used in the lumen to assure that under control conditions Na^+ transport across the wall does not contribute to volume flow. However, solvent drag in response to the osmotic gradient has not been ruled out. In addition, there are several nontrivial technical problems. (1) If the rate of perfusion is low, osmotic equilibration occurs within a short distance, and therefore the sampling site has to be close to the perfusion site (proximal to the site of osmotic equilibration), since otherwise P_{os} cannot be calculated from equation (37). (2) If the perfusion rate is high, sampling is possible at longer distances, but the changes in the concentration of the volume marker in the collected fluid become smaller and hence difficult to measure accurately. (3) The osmolality can be measured with reasonable accuracy in both perfusate and collectate, but it is, strictly speaking, unknown in the peritubular compartment. It is assumed that the interstitial osmolality equals that in the peritubular capillary, which in turn equals that in arterial blood. The magnitude of the potential error involved in this assumption is unknown. These and other problems with this technique have been discussed by Berry.[33]

3.2.1.1b. Dependence of J_v on luminal perfusion rate. In these experiments, tubules are perfused at varying rates (V_o), an osmotic gradient is imposed, and the reabsorption rate (J_v) is calculated using inulin as a luminal volume marker.[36,37] J_v is plotted as a function of V_o and extrapolated to infinite V_o. In this condition, $\Delta\pi$ across the tubule wall should be independent of position, and hence $J_v = L_p \cdot \sigma\Delta\pi$, from which L_p can be easily calculated. The approach is ingenious, but at high perfusion rates the fractional error in estimating J_v is very high, because the changes in collected fluid (inulin concentration) are small. In addition, the data of Schafer *et al.*[36] clearly show that when the technique is applied to long segments (ca. 3.29 mm), the calculated P_{os} is less than when short tubule segments (ca. 0.86 mm) are used.

3.2.1.1c. Relationship of spontaneous J_v to measured transepithelial $\Delta\pi$. Two approaches have been utilized that take advantage of either the small volume of the tubule lumen,[38,39] by perfusing isolated segments *in vitro* using salt solutions on both sides, or the small volume of the peritubular compartment,[40] by studying tubules perfused *in vitro* with a physiologic salt solution, but immersed in oil. In the first experiment, a slow perfusion rate is used, allowing for the development of luminal hypotonicity which is measured cryoscopically in the collected fluid. In the second experiment, the tubule is perfused at a fast rate, the fluid absorbed is

gathered from droplets that form on the basolateral surface, and its hypertonicity is determined. In the first case, $\Delta\pi = \pi(\text{perf}) - \pi(\text{coll})$; in the second case, $\Delta\pi = \pi(\text{drop}) - \pi(\text{perf})$. In both cases, J_v is measured and L_p calculated. Both approaches are elegant and difficult. In the measurements of luminal hypotonicity, two problems are apparent: (1) the reliability of the determinations of such small differences in osmolality of the collected fluid, and (2) the lack of a quantitative consideration of the possible hyperosmolality of the basolateral side of the epithelium: although it is clear that the resistance of the lateral intercellular spaces to NaCl diffusion is small,[41,42] mass balance considerations dictate that if fluid transport results in luminal hypotonicity, it must also cause antiluminal hypertonicity. If such hypertonicity is sizable, the L_p has been overestimated in these calculations. The experiments under oil are intriguing and raise a number of technical questions which have been, at least in part, addressed in the original publication,[40] namely, the possibilities of abnormal hydrostatic pressure gradients, loss of absorbed water into the oil phase, or alterations of the transport properties of the tubule. Although J_v values with different perfusates were similar to those measured in tubules bathed in physiologic salt solutions, controls with nontransported salts or transport inhibitors were not carried out.

3.2.1.1d. Results. As pointed out in the preceding discussion, the measurement of L_p^t in proximal renal tubules are subject to a number of uncertainties and potential errors. Since different techniques have been employed, the sources of possible errors differ. It is therefore remarkable that, with very few exceptions, the estimated values are extremely close, ranging between 0.1 and 0.2 $cm \cdot sec^{-1}$ for P_{os} (see Berry[33]), values that are equivalent to a range of $1.8 \cdot 10^{-3}$ to $3.6 \cdot 10^{-3}$ $cm \cdot sec^{-1} \cdot (osmoles/kg)^{-1}$ for L_p. If a range of 0.1 to 0.2 $cm \cdot sec^{-1}$ is accepted as correct for P_{os}, and if J_v is of the order of 1.0 $nl \cdot mm^{-1} \cdot min^{-1}$, equivalent to $0.24 \cdot 10^{-4}$ $cm^3 \cdot cm^{-2} \cdot sec^{-1}$, then, if the driving force is osmotic, $\Delta\pi$ must be between 6.6 and 13.2 mosmoles/liter. Luminal fluid hypotonicity (9.2 mosmoles/kg water) was reported in free-flow micropuncture studies in rat proximal convoluted tubule.[38] Furthermore, reabsorbate from rabbit proximal convoluted tubules perfused under oil was found to be hypertonic (15.8 mosmoles/kg water).[40] These observations are at least consistent with values predicted for osmotic coupling of salt and water transport.

3.2.1.2. P_{os}^t in Gallbladder Epithelium. Inasmuch as measurements of transepithelial J_v are difficult to make with good time resolution, determinations of P_{os}^t in epithelia bounded by unstirred layers of significant thickness are quite uncertain. This was first demonstrated in studies in

rabbit gallbladder,[43] in which, upon imposition of a transepithelial osmotic gradient, J_v fell slowly, reaching a steady state only after several minutes. These very long transients are probably an artifact related to the experimental technique employed in these studies[44] (gallbladders were mounted as bags and J_v was measured gravimetrically). Using a rapid-mixing chamber, a flat preparation, and measuring volume flow with capacitance probes, steady-state J_v values were obtained within seconds,[45] and P_{os}, corrected for unstirred layer effects, was 0.05 cm·sec^{-1}. For J_v values[24] of 50–100 μl·cm^{-2}·hr^{-1}, an osmotic water transport mechanism would require a $\Delta\pi$ of 20–40 mosmoles/kg. The unstirred layer correction for P_{os} was based on the assumption that the change in transepithelial voltage caused by $\Delta\pi$ was entirely due to changes in salt concentration in the unstirred layers, which cause a transepithelial (paracellular) diffusion potential. The assumption has been criticized, because the voltage change could be due in part to true electroosmosis.[15] Neglecting the above correction, P_{os} was $9.3 \cdot 10^{-3}$ cm·sec^{-1}. In conclusion, determinations of P_{os}^t in flat epithelia are difficult to make because of the presence of unstirred layers and the uncertainties involved in correcting for them. The calculated value of the $\Delta\pi$ necessary to drive spontaneous fluid absorption is difficult to reconcile with the observation of near-isotonicity of the transported fluid. It is likely that the measured value of P_{os}^t is an underestimate.

3.2.2. Cell Membrane P_{os}

3.2.2.1. Cell Membrane P_{os} in Proximal Tubule. Two different approaches have been used to obtain estimates of cell membrane P_{os} in proximal renal tubule preparations: measurements of changes in cell volume in response to osmotic gradients and estimates of volume changes of preparations of apical or basolateral membrane vesicles, also elicited by osmotic gradients. We will discuss these two sets of studies separately.

3.2.2.1a. P_{os} measurements from changes in cell volume. In the last few years, two groups of investigators have made direct measurements of cell membrane P_{os} using isolated proximal renal tubules. Both groups employed collapsed rabbit proximal convoluted or straight tubules for measurements of basolateral membrane P_{os}.[46–49] For measurements of luminal membrane P_{os}, straight portions of the proximal tubule were perfused with solutions of varying osmolalities while immersed in oil.[50] Cell volume changes in response to alterations in basolateral solution osmolality were assessed by optical measurements of the outer tubule diameter, whereas those elicited by changes in the osmolality of the luminal solution were determined from measurements of the inner tub-

ule diameter. The osmotic water permeability coefficient was estimated either from the initial rate of change in cell volume (assuming no change in intracellular osmolality)[48] or from the complete time course of the cell volume change (assuming no change in intracellular solute content).[46,47,49,50]

Basolateral P_{os} determinations have been carried out with improving resolution. Initially, manual adjustment of an image-splitting eyepiece was employed.[46,47] Later, the measurements of diameter were made on unstained images stored in a video recorder.[48] Recently, an elegant technique has been developed, which permits rapid automated measurements of the external diameter of a tubule segment stained with a supravital dye to increase contrast.[49] This technique averages many simultaneous measurements of tubule diameter, with a fractional error of only 10^{-3} and a detection limit of 0.05 μm.

The results of these measurements, summarized in Table I, indicate that P_{os} is quite high and hence support the notion that osmotic transepithelial water transport may be predominantly or exclusively transcellular. In spite of the recent technical advances, it is possible that the P_{os} values are still underestimated. For instance, the measurements of basolateral membrane P_{os} may be in error because the width of the lateral intercellular spaces may be reduced in collapsed tubules. Such a reduction in width could create a series resistance to diffusion of the osmotic probe into or out of the lateral spaces and solute polarization. Carpi-Medina *et al.*[49] estimated the error induced by unstirred layer effects using equation (26). This is formally incorrect on three grounds: first,

Table I. Cell Membrane Osmotic Water Permeability in Proximal Renal Tubules

Segment	Method	P_{os} (cm · sec^{-1})		Reference
		Apical	Basolateral	
PST	A	—	0.14	González *et al.*[46]
PCT	A	—	0.23	Carpi-Medina *et al.*[47]
PCT (S_1,S_2,S_3)	A	—	0.30–0.55	Welling *et al.*[48a]
PST	A	—	0.28	Carpi-Medina *et al.*[49]
PST	B	0.13	—	González *et al.*[50]
Cortex	C	0.40	—	Verkman *et al.*[51]
Cortex	C	—	0.50–0.60	Verkman and Ives[52]

[a] The values of Welling *et al.*[48] were computed from the inital rates of cell volume change. The remaining ones were obtained by analysis of the volume change over several seconds. Values of P_{os} are expressed with respect to an ideal cylindrical tubule area. Methods: (A) Optical measurements of diameter of tubules with occluded lumen. (B) Optical measurements of diameter of tubules perfused with aqueous solutions, immersed in oil. (C) Light scattering of brush border or basolateral membrane vesicles.

it can be applied only to the steady state, i.e., it does not take into account the diffusional delay for buildup of the osmotic gradient; second, it neglects the contribution of the sweeping-away effect, e.g., the dilution of the external solute (not only the osmotic probe) in a cell shrinkage experiment; and third, it does not consider the possibility of funneling of the J_v in the lateral intercellular spaces, which could increase v by two orders of magnitude in equation (26). In the experiments of Welling *et al.*,[48] who also measured basolateral membrane P_{os}, the resolution of the measurements was less. Their observation of P_{os} values independent of the imposed $\Delta\pi$ provided an excellent validation for their use of initial rates of change in cell volume, but it is possible that the $\Delta\pi$ at the beginning of the transient was less than estimated, because of unstirred layer effects. Finally, in the measurements of apical membrane P_{os} of González *et al.*,[50] the data obtained near the onset of the cell volume change are in error, because while the volume is measured every 17 msec, it takes 125–200 msec for the experimental solution to travel the 100 μm of tubule length over which the measurements are made.

Regardless of these limitations, these studies have provided important data concerning the pathways and mechanisms of osmotic water transport in proximal tubule. On the whole, the results tend to support the notion of a dominant transcellular pathway. However, as pointed out by González *et al.*,[50] the apical membrane P_{os} value is highly uncertain because water flow in response to changes in luminal osmolality could be at least in part via the junctions, changing the osmolality of the fluid in the lateral intercellular spaces, and hence causing shrinking or swelling across the basolateral membrane. As in the case of gallbladder epithelium, discussed in Section 3.2, the issue cannot be resolved without direct measurements of junctional osmotic water permeability.

3.2.2.1b. P_{os} measurements in isolated membrane vesicles. Using light-scattering techniques in a stop-flow apparatus, Verkman *et al.* have measured directly the changes in average volume, elicited by changes in external osmolality, in vesicle preparations enriched in either apical[51] or basolateral membranes.[52] Apical membrane vesicles were obtained from rabbit kidney cortex and basolateral membrane vesicles were from either rat or rabbit kidney. To calculate P_{os}, the vesicles were modeled as spheres of 0.3 and 0.5 μm internal diameter, for apical and basolateral membrane, respectively. Taking into account folding factors for each membrane, the respective P_{os} values at 37°C, expressed with respect to an ideal cylindrical tubule area, were 0.4 and 0.5 cm·sec^{-1} (Table I). These elegant studies support the conclusion of high cell membrane P_{os} values, and hence of predominant transcellular water transport. However, some questions remain, such as the degree of heterogeneity of the preparations, in terms of both origin and geometry of the vesicles and the

possibility of changes in properties of the membranes attributable to the isolation procedure.

Studies of the temperature dependence of P_{os} and measurements of membrane fluidity by the same group[52] suggest the possibility that osmotic water transport is via pores in the basolateral membrane and by solubility–diffusion in the apical membrane.

3.2.2.2. Cell Membrane P_{os} in Gallbladder Epithelium. Three groups of investigators have recently obtained estimates of cell membrane P_{os} in *Necturus* gallbladder epithelium. In all three cases, P_{os} was calculated from the initial changes in cell volume (V_c) elicited by rapid alterations in the osmolality of the bathing solution. What differs is the technique used to measure changes in V_c. Persson and Spring[19] used quantitative light microscopy. Zeuthen[53] used ion-selective microelectrodes to follow the changes in intracellular Cl^- or Na^+ activities, assuming that these ions can be used as cell volume markers, i.e., that there was no net flux of either ion during the osmotic transient. Reuss[54] loaded the epithelial cells with tetramethylammonium (TMA^+), by transiently exposing the apical surface of the tissue to the pore-forming antibiotic nystatin, and measured, also with microelectrodes, the intracellular activity of TMA^+. The results obtained with these techniques are summarized in Table II.* It is likely that these results represent minimum estimates of P_{os}, primarily because of the presence of unstirred layers (see Section 2.4). Cotton and Reuss[55] have corrected for this effect using an extracellular marker (tetrabutylammonium) with a diffusion coefficient similar to that of the osmotic probe (sucrose), so that the concentration of the latter at the membrane surface could be estimated simultaneously with the cell volume measurement. With this correction (see Table II), the P_{os} of the apical membrane was calculated to be 0.50 $cm \cdot sec^{-1}$. For a spontaneous fluid transport rate of 12 $\mu l \cdot cm^{-2} \cdot hr^{-1}$, the difference in osmolality required to drive the water across the apical membrane would be only ca. 0.37 mosmole/kg. If the P_{os} of the basolateral membrane is higher than that of the apical membrane, as suggested by the results of Persson and Spring[19] and Zeuthen,[53] the steady-state difference in osmolality across the basolateral membrane would presumably be smaller than that across the apical membrane.

However, because the junctional complexes in this epithelium have a much higher ionic permeability than that of the cell membranes, the possibility of junctional water flux, both under spontaneous transporting conditions and during osmotic experiments, cannot be ruled out *a priori*. It is conceivable that part of the cell volume change elicited by alterations

* In the papers by Reuss[54] and Cotton and Reuss,[55] the conversions from L_p to P_{os} are in error. The correct P_{os} values are 10-fold greater than those given.

Table II. Cell Membrane Osmotic Water Permeability in Gallbladder Epithelium[a]

Method	P_{os} (cm · sec^{-1})		Reference
	Apical	Basolateral	
Optical	0.06	0.12	Persson and Spring[19][b]
Intracellular ionic activities (aCl_i, aNa_i)	0.04	0.09	Zeuthen[53]
Intracellular TMA^+ activity	0.06–0.17	—	Reuss[54]
Intracellular TMA^+ activity	0.50	—	Cotton and Reuss[55][c]

[a] All values were obtained in *Necturus* gallbladder.
[b] Persson and Spring[19] extrapolated to $\Delta\pi = 0$. In the remaining studies, initial rates of cell volume change were used.
[c] Cotton and Reuss[55] also corrected for the time course of $\Delta\pi$.

in apical solution osmolality reflects water flow across the junctions and the basolateral membrane and not through the apical membrane.[54] Direct measurements of the P_{os} of the junctions are essential, but have not yet been carried out.

3.3. Water Transport Pathway

The pathway for isosmotic transepithelial water transport has not been established. In principle, water flow could occur via the cell membranes, the paracellular route (i.e., the junctions in series with the lateral intercellular spaces), or both pathways. The role of the lateral intercellular spaces in transepithelial fluid transport was first recognized in studies in rabbit gallbladder in which a direct correlation was demonstrated to exist between lateral space width (measured in electron microscopic specimens) and the fluid transport rate measured before fixation.[56,57] However, in frog gallbladders examined with differential interference contrast microscopy, under transporting conditions, the spaces were found to be closed, but appeared opened during fixation, suggesting that the electron microscopic studies are artifactual.[58] Spring and Hope[59] were able to visualize open lateral intercellular spaces in transporting *Necturus* gallbladder and observed a rapid reduction in width upon removal of NaCl from the mucosal solution. Collapse of spaces has also been observed in preparations mounted in chambers when oxygen was deleted from the bathing media.[60] Thus, it is likely that the lateral intercellular

spaces constitute a pathway for transepithelial isosmotic fluid transport, but this does not prove that the water transport pathway includes the junctions. Inasmuch as the lateral spaces are in series with both the junctions and the lateral cell membrane, fluid transported via either pathway would finally reach the spaces.

3.3.1. The Paracellular Pathway

The possibility that the paracellular pathway is a major route for water transport is suggested by the high electrodiffusive ion permeability of this pathway. If the junctions are highly permeable to ions, and if the junctions are aqueous pathways,[61] then the junctional water permeability may also be high. This argument has been questioned by Berry,[33] who points out that when data from different renal tubule segments are considered, no correlation is found between transepithelial electrical conductance, assumed to reflect junctional ion permeability, and transepithelial P_{os}. A second argument frequently raised by proponents of a paracellular water transport pathway is the large value of P_{os}/P_{dw}, which, as discussed in Section 2.3, can be construed as evidence for the existence of pores. By itself, this argument does not address the question, because the cell membranes can also contain pores. It can become valid, however, if the calculated pore radius is large, i.e., incompatible with the known permeability properties of the cell membranes. Although this argument has been raised, it is not valid, since the available measurements of transepithelial P_{dw} (see Berry[33] for references) are probably erroneous because of lack of corrections for unstirred layers.

Stronger arguments in favor of a junctional water transport route have been proposed: (1) P^t_{os} is much greater than P^c_{os}, (2) there is solvent drag, and this could only occur via the junctional pathway, and (3) the driving force for osmotic water flow is the *effective* transtubular osmotic gradient, which implies solvent drag of a salt with a low reflection coefficient.

The first argument, that the P_{os} of the cell membranes in series is too low to account for the measured transepithelial P_{os}, has been made by Whittembury and associates for proximal tubule[50] and by Hill for gallbladder.[15] The argument depends on the quantitative accuracy of both transepithelial and cell membrane P_{os} determinations. As discussed in Section 3.2, there are unresolved experimental uncertainties which make such measurements tenuous at this time.

The second argument in support of paracellular water transport is based on the claim of solvent drag, which has been made in both proximal tubule[62] and gallbladder.[63,64] These experiments consist of measuring transepithelial fluxes of hydrophilic solutes of diverse molecular size as

a function of fluid transport rate. A positive correlation between the solute and volume fluxes is taken as evidence for solvent drag, which implies a frictional interaction between water and solute fluxes in a hydrophilic pathway, assumed to be paracellular. However, such results can be explained equally well by pseudo solvent drag.[8,9] In other words, the net solute transport "coupled" to J_v could be diffusive, i.e., due to concentration gradients generated in the unstirred layers by the water flux. It is in principle possible to distinguish between solvent drag and pseudo solvent drag by measuring fluxes of both hydrophilic and lipophilic solutes. Pseudo solvent drag will enhance both solute fluxes, but only the flux of hydrophilic solutes would be elevated by true solvent drag,[9] since permeation of the lipophilic molecules is largely transcellular. In the absence of control experiments designed to rule out pseudo solvent drag, suggestions of true solvent drag in proximal tubule and gallbladder epithelium are inconclusive.

The third argument in favor of paracellular fluid absorption has been made for the specific case of the late proximal tubule. The hypothesis is that a fraction of the luminal fluid is reabsorbed by an osmotic mechanism dependent on the asymmetry of salt composition and salt reflection coefficients between luminal and peritubular fluids. Because of the preferential reabsorption of $NaHCO_3$, the luminal $NaHCO_3$ concentration is lower, and the luminal NaCl concentration is higher than the respective concentrations in the peritubular fluid. If the $NaHCO_3$ reflection coefficient is higher than that of NaCl, an effective osmotic gradient will exist that will favor water absorption. Inasmuch as the driving force implies a sizable NaCl permeability of the water transport pathway, NaCl will be transported with the water, by solvent drag. This proposal, supported initially by several groups,[65–67] is strictly based on the reflection coefficient argument. However, recent studies suggest that the NaCl and $NaHCO_3$ reflection coefficients in late proximal tubules are not significantly different.[68,69]

3.3.2. The Transcellular Pathway

Those who claim that the predominant pathway for water transport is transcellular base their contention on three major arguments. First, in rabbit gallbladder, measurements of the transepithelial osmotic water permeability coefficient at different temperatures indicate a high activation energy,[70] which would be consistent with water permeation across cell membranes, either by solubility–diffusion in the lipid bilayer or by permeation across small pores. However, in intact tubules the existence of a junctional route of water transport that is highly temperature-sensitive (e.g., permeation via small pores in a lipid matrix) cannot be ruled

out *a priori*. The possibility of water permeation through pores is supported by the observation of a reversible decrease in proximal tubule basolateral membrane P_{os} by the sulfhydryl reactive agent *p*-chloromercuribenzenesulfonic acid.[71] However, this effect was not confirmed in P_{os} measurements in isolated membrane vesicles.[52]

Second, direct measurements of cell membrane P_{os} in proximal tubule and gallbladder epithelium (see Tables I and II) yield very high values, thus requiring only small osmotic gradients to account for transepithelial water flow. The problem is that the estimates of apical membrane P_{os} are based on the implicit or explicit assumption that changes in cell volume elicited by changing luminal solution osmolality are entirely caused by water flow across the apical cell membrane. As discussed before,[50,54] if the junctions have a sizable water permeability, junctional water flow could rapidly change the osmolality of the fluid in the lateral intercellular spaces, causing osmotic water flow across the basolateral membrane, which appears to be more permeable to water than the apical membrane (Tables I and II). Hence, in the absence of proof that the junctional water flow is insufficient to account for the cell volume changes during alterations of luminal osmolality, the argument for transcellular water transport is circular.

Third, electron microscopic studies and measurements of nonelectrolyte permeability suggest that the junctional area available for water permeation is insufficient to account for a P_{os} value of the order of magnitude measured for either the cell membranes[16] or the whole epithelium.[33] Such calculations are frequently based on an "equivalent pore radius" estimated from transepithelial nonelectrolyte fluxes. The result is that there are relatively few pores of large radius. P_{os}, calculated from pore density and size, is found to be small. This argument may be flawed because the pore size could be distributed, including the existence of narrow pores that would allow only water to permeate. Nevertheless, calculations for proximal tubule, allowing the entire junctional area to be water-conductive, yield a P_{os} of 0.13–0.60 $cm \cdot sec^{-1}$, but, as discussed by Berry,[33] the assumed width of the junctions (3–5 nm) is incompatible with the observed sucrose permeability.[72]*

In conclusion, the arguments in favor of transcellular water transport appear more convincing because of the recent estimates of high values of cell membrane P_{os}. However, uncertainties persist, and the issue of the transepithelial pathway for osmotic water flow and isosmotic

* In footnotes 3 and 4 of the review by Berry,[33] the equations used to calculate the P_f of lateral intercellular spaces and junctions are in error. In both, the denominator should be $\underline{\Delta X} \cdot 3\eta$ instead of $\Delta X^3 \cdot \eta$. In addition, the factor for conversion from L_p to P_f (i.e., $RT/\overline{V}_w$) was omitted.

transepithelial water transport will not be resolved until direct measurements of junctional P_{os} are made.

3.4. Driving Forces for Isosmotic Water Transport

If water transport in proximal tubule and gallbladder is by osmosis, the driving forces required to account for the spontaneous fluid transport rates are of at most a few milliosmoles in the case of the gallbladder, and between 1 and 20 milliosmoles in the case of the proximal tubule, depending on the segment and the P_{os} values chosen for the calculation. Neglecting differences in the salt reflection coefficients in the late proximal tubule (see Section 3.3), osmotic water absorption would require development of luminal hypotonicity, basolateral hypertonicity, or a combination of both.

Luminal hypotonicity has been proposed as a driving force for water reabsorption in rabbit proximal tubule[73] and has been demonstrated in rat proximal tubule *in situ*.[38,39,72] Whether its magnitude accounts for the water transport rate is doubtful, as discussed in Section 3.2. In transporting *Necturus* gallbladder, luminal solution hypotonicity has been also suggested, on the basis of measurements of fluid electrical conductivity *in situ*.[75]

Basolateral hypertonicity was the essential idea in both the Curran and MacIntosh[26] and the Diamond and Bossert[22] models. Theoretical objections of these models[15,30,31] were based on calculations which indicate that isotonicity of the emergent fluid cannot be achieved when the luminal solution is isosmotic. However, as stated in Section 3.2, if the luminal fluid becomes hypotonic, then the transported fluid can be near-isotonic to the bulk solution, but hypertonic to the "dilute" solution bathing the apical cell surface. The best available evidence for development of basolateral hypertonicity is provided by the elegant experiments of Barfuss and Schafer[40] in isolated, perfused proximal tubules. The magnitude of the hyperosmolality in the lateral spaces, when the tubule is exposed to an aqueous solution on the basolateral side, *in situ* or *in vitro,* is uncertain, because the resistance of the spaces to salt diffusion is low.[41,42]

In *Necturus* gallbladder epithelium, on the basis of the cell membrane P_{os} estimates of Cotton and Reuss[55] (about 0.50 cm·sec^{-1}), a space hypertonicity of less than 1 mosmole/kg would suffice to account for the average transepithelial rate of fluid absorption. These conclusions are in excellent agreement with indirect estimates based on the observation of lack of significant changes in ionic activities in the lateral spaces during application of transepithelial current.[76] The spaces were observed to change in width under these conditions, suggesting that transport of

ions followed by osmotic water flow did take place. If such water flow was across the basolateral membrane, its P_{os} would be of about 0.56 $cm \cdot sec^{-1}$.

In conclusion, the increasingly higher estimates of P_{os} of cell membranes of epithelia that transport fluid at low rates by an isosmotic process suggest that very small differences in osmolality across the cell membranes might account for the measured rates of transepithelial water transport. The osmotic gradients between the apical unstirred layer, the cell interior, and the fluid in the lateral intercellular spaces are perhaps undetectable by current techniques. In the proximal convoluted tubule, which has a higher J_v, particularly in the rat, it is possible that a measurable gradient develops under normal transporting conditions. The contribution of luminal hypotonicity to such gradient seems to be quantitatively more important than that of basolateral solution hypertonicity.

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2

Renal Hemodynamics and Sodium Chloride Excretion

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1. Renal Hemodynamics

The regulation of renal hemodynamics is influenced by a number of intrinsic and extrinsic control mechanisms. While the extrinsic control mechanisms can be important in many cases, much attention has been focused on intrinsic control systems involved in the autoregulation of renal blood flow and glomerular filtration rate (GFR).

Autoregulation of renal blood flow and GFR may be defined as the intrinsic capability of the kidney to maintain a constant level of blood flow and glomerular filtration in the face of considerable variations in renal perfusion pressure. The actual pressure range of autoregulation may vary from one species to another, but is usually between 70 and 180 mm Hg in mammals. It is uncertain whether distinct mechanisms regulate renal blood flow and glomerular filtration; however, the two can be dissociated under certain conditions.[1–4]

Two primary mechanisms explain the phenomenon of renal autoregulation. One mechanism, called the myogenic mechanism, is mainly

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a phenomenon manifested by the renal vasculature. A second mechanism is tubuloglomerular feedback.

1.1. Myogenic Mechanism

This mechanism is thought to respond to instantaneous changes in vascular wall tension. The explanation of this phenomenon is based on Laplace's law, which states that the wall tension in a vessel is equal to the product of transmural hydrostatic pressure difference and radius of the vessel. According to this theory, the wall tension of a vessel is held constant. An increase in the transmural pressure (for example, due to an increase in renal perfusion pressure) results in an increase in wall tension. Under these circumstances, the radius of this vessel decreases instantaneously to maintain a constant wall tension. The reduction in vessel radius results from vasoconstriction which leads to an increase in vascular resistance. Therefore, the controlled variable in the myogenic mechanism is wall tension. Blood flow is regulated as a secondary event by changes in vascular resistance.

Most studies that support the myogenic mechanism have been through exclusion of various other mechanisms rather than through direct support. Studies by Gilmore *et al.*[5] and Källskog *et al.*,[6] however, demonstrated direct evidence for the myogenic phenomenon in regulating renal hemodynamics in the renal vasculature of hamsters and rats. The observation by Gilmore *et al.*[5] that the radius of transplanted hamster renal afferent arterioles is very responsive to extravascular pressure supports the proposal that some purely physical parameter that depends on transmural pressure is responsible for the alteration of smooth muscle contractile activity. Edwards[7] investigated the interaction between lumen diameter and intraluminal pressures of interlobular arteries and superficial afferent and efferent arterioles isolated from rabbit kidneys. In these isolated microvessels it was found that increasing intraluminal pressure from 70 to 180 mm Hg resulted in decreases in lumen diameters of interlobular arteries and afferent arterioles. In contrast, the efferent arterioles responded in a passive manner to increases in intraluminal pressure by dilation. Thus, the results of this study provide some evidence for the involvement of the myogenic mechanism in regulating renal blood flow by the preglomerular vessels.

Mathematical analyses of the myogenic hypothesis with particular reference to autoregulation of renal blood flow have been developed by Oien and Aukland[8] and Lush and Fray.[9] In the latter myogenic model it is argued that the vascular smooth muscle contraction is initiated by stretch-induced changes in calcium permeability. The model predicts an upward and to-the-right shift of the autoregulatory pressure flow curve

in response to increased tissue hydrostatic pressure. In this model, the autoregulatory mechanism senses stretch, but simply responds to it rather than attempting to regulate it. Most of the constituent parts of the model have experimental support except the hypothesis that stretch controls intracellular calcium.

Further evidence for the importance of the myogenic mechanism in regulating renal hemodynamics has been demonstrated by Young and Marsh[10] and Sakai and Marsh.[11] The authors analyzed the transient and frequency responses of renal blood flow autoregulation and hydrostatic pressure wave propagation along the nephron in rats. The results of these studies provide strong support for the existence of a fast-acting component in renal autoregulation. This component was attributed by these investigators to an intrinsic myogenic response of the renal vessels. In a recent study, Moore[12] measured and analyzed the change in glomerular capillary pressure produced by elevation of arterial pressure during tubuloglomerular feedback inhibition in Sprague–Dawley rats. The data indicated that intrinsic adjustments in renal vascular resistance could provide about 50% compensation for a rise in arterial pressure. The author suggested that this mechanism is probably an intrinsic myogenic reflex of the afferent vessels stimulated by changes in intravascular pressure. In a study by Casellas and Navar[13] of *in vitro* perfusion of juxtamedullary nephrons in rats, spontaneous cyclic vasomotion in the face of a constant perfusion pressure was observed. This was first detected visually as cyclic variations of glomerular tuft perfusion and could be quantitated as cyclic alterations in glomerular capillary and tubular pressure. Kreisberg *et al.*[14] demonstrated that the smooth muscle-like cells in the cultured glomerular mesangium appear to be contractile in nature and may play a role in regulating the surface area for ultrafiltration. In the last 2 years, experimental studies on the myogenic mechanism in relation to the regulation of renal blood flow have been few, and more work needs to be done to further investigate and clarify the possible importance of this phenomenon in autoregulation of renal hemodynamics.

1.2. Tubuloglomerular Feedback Mechanism

Tubuloglomerular feedback is a well-established mechanism thought to be of great importance in renal autoregulation. Many studies have demonstrated the existence of a distal tubuloglomerular mechanism that is responsive to changes in flow rate in distal tubules and serves as regulator of GFR.[15] This feedback mechanism may be divided into three components that take place sequentially in a series of events in response to flow-related alterations in the concentration of tubular fluid. First,

changes in the tubular fluid concentration of one or more components are detected as the flow is exposed to the macula densa cells of the distal tubule (detector component); second, the signal is transmitted from the macula densa cells to renal vascular elements (transmitter component); and third, the elicited response is manifested by vascular smooth muscle contraction or relaxation (effector component). The sensitivity of the tubuloglomerular feedback mechanism may be enhanced or reduced under different physiologic states.

1.2.1. Detector Component

It is now generally believed that the interaction that exists between the distal tubule and glomerular vascular structures during changes in flow rates may be triggered by flow-dependent alterations in the concentration of sodium and chloride at the macula densa cells. Studies by Briggs *et al.*[16] and Schnermann *et al.*[17] have shown that increases in sodium chloride concentration—perfused retrograde in the loop of Henle—of between 15 and 60 meq result in proportionate decreases in filtration rate. Wright and Persson[18] demonstrated that injection of electric current into the early distal tubule lumen made the lumen more negative and produced a reduction in stopflow pressures (SFP). These observations support the theory that ion transport, especially of chloride by the macula densa cells, plays an important role in the detector component of the feedback mechanism.

The proposal that tubuloglomerular feedback is initiated by transport of sodium chloride at the macula densa has been challenged by Bell and co-workers.[19,20] They propose that alterations in osmolality act as the luminal signal triggering feedback responses. This hypothesis is based on the demonstration that solutions containing low concentrations of chloride and other electrolytes (for example, sodium iothionate) produce changes in filtration rate when perfused retrograde through the loop of Henle.[21] Thus, the detector component of the tubuloglomerular feedback mechanism remains controversial. Some experiments show that the luminal signal is initiated by alterations in ion transport at the macula densa, but others support the proposal that changes in the distal tubular fluid osmolality constitute the initiating signal of tubuloglomerular feedback.

1.2.2. Transmitter Component

The mode of transmission of the signal of tubuloglomerular feedback has recently been studied. Bell[22] proposed that a cytosolic calcium system, probably in the cells of the macula densa, participates in the

transmission of the luminal signals to the glomerular vasculature. According to this theory, there is a mobilization of calcium from intracellular stores as the concentration of the distal tubular fluid increases from hyposmotic toward isosmotic values. This increase in cytosolic calcium concentration may help in the transmission of the signal to glomerular vascular elements, resulting in vasoconstriction and a reduction in filtration rate.

To examine the possible role of cytosolic calcium in the transmission of tubuloglomerular feedback signals, a series of micropuncture experiments utilizing agents that have been reported to elevate intracellular cyclic AMP (cAMP) level were performed by Bell.[22] These experiments are of interest since cAMP can modify calcium-mediated events. Retrograde microperfusion with isotonic Ringer's solution decreased SFP from an average of 37 mm Hg to 25 mm Hg. Addition of 3-isobutyl-1-methylxanthine (IBMX), a phosphodiesterase inhibitor, to the isotonic Ringer's solution, produced a dose-dependent decrease in the magnitude of SFP feedback responses. Therefore, it was concluded that IBMX is an inhibitor of tubuloglomerular feedback responses when perfused retrograde into the distal tubule. Similar inhibition in tubuloglomerular feedback responses to that of IBMX addition to Ringer's solution was obtained when forskolin, an agent that stimulates adenylate cyclase activity, was added to the perfusate. Also, it was demonstrated that administration of the dibutyryl form of cAMP markedly inhibited the SFP feedback response obtained with retrograde microperfusion of isotonic Ringer's solution. To evaluate the effects of increases of cytosolic calcium on the inhibition of tubuloglomerular feedback produced by IBMX, calcium ionophore (A23187) was added to the isotonic Ringer's solution containing IBMX. Results show that addition of 5 μm of A23187 in the presence of 250 μm IBMX significantly enhanced tubuloglomerular feedback response and restored it to near-control levels (Fig. 1). The author presented a possibility that an adenylate cyclase-activated cAMP system may exist and that this mechanism can influence the transmission of tubuloglomerular feedback signals by stimulation of calcium transport across the plasma membrane or endoplasmic reticulum. Hence, elevated intracellular cAMP may prevent the mobilization of intracellular calcium, thus directly lowering cytosolic calcium concentration and impairing the feedback responses.

1.2.3. Effector Component

The controversy that exists in explaining the detector and transmitter components also extends to the explanation of the effector side of tubuloglomerular feedback mechanism. The major thrust of this con-

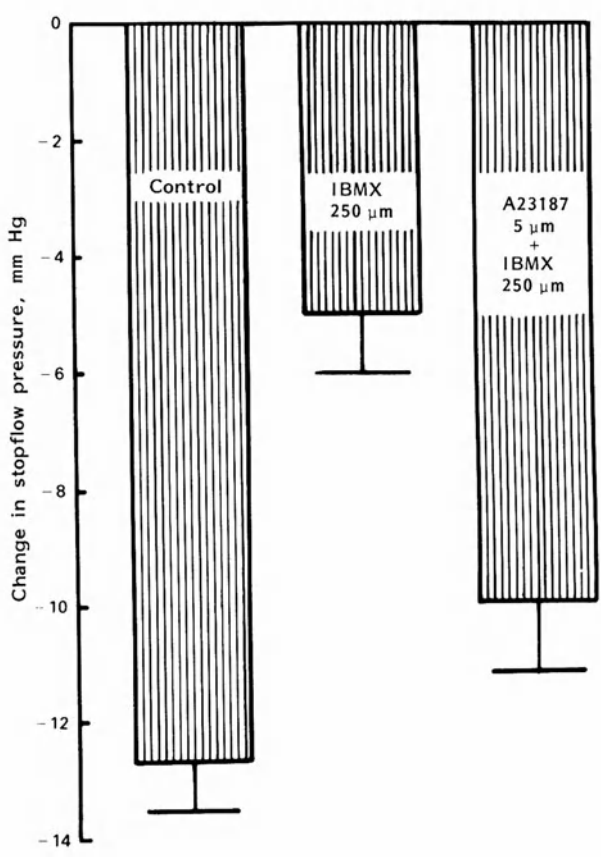

Fig. 1. Changes in stopflow pressure obtained during retrograde perfusion of the distal tubule using control isotonic Ringer's solution, Ringer's solution containing 250 μm IBMX, and Ringer's solution containing both 250 μm IBMX and 5 μm A23187 (n = 11).[22]

troversy is centered on whether the primary segment of resistance responsible for hemodynamic autoregulation is preglomerular or located at other sites. In a recent study by Bell *et al.*,[23] it was shown that increases in flow rate out of the late proximal tubule led to decreases in glomerular capillary pressure and single-nephron glomerular filtration rate (SNGFR). In these micropuncture experiments, glomerular capillary pressure was measured directly with a micropressure servo-null micropipette system. It was also demonstrated that significant decreases in glomerular capillary pressure were obtained at rates of infusion into the late proximal tubule as low as 10 nl/min (Fig. 2). The authors suggested that glomerular capillary pressure is responsive to changes in late proximal flow rates

that are within the normal range. The results of these experiments supported the hypothesis that increases in afferent arteriolar resistance are mainly responsible for feedback-mediated decreases in glomerular filtration rate. Even though it is generally accepted that glomerular pressure exhibits an autoregulatory behavior, still other studies suggested that other sites may be involved in the effector site of tubuloglomerular feedback mechanism.[24,25] In studies performed by Tucker *et al.*,[24] carbonic anhydrase inhibitor, when administered systemically, produced decreases in SNGFR, but no significant decrease in glomerular pressure. Also, Ichikawa[25] showed that tubuloglomerular feedback-induced changes in SNGFR were not coupled with alterations in glomerular hydrostatic pressure, and that the feedback regulation of glomerular filtration rate is mediated by changes in vasomotor tone of preglomerular, glomerular, and postglomerular vessel sites. It was suggested that these alterations in vasomotor tone may be mediated through mesangial cell contractility. In a recent study by Persson *et al.*[26] on angiotensin II-prostaglandin-blocked rats, glomerular capillary hydrostatic pressure and SFP feedback responses were completely eliminated, while SNGFR response persisted but to a lesser extent. The authors suggested that in angiotensin II-prostaglandin-blocked rats, tubuloglomerular feedback SNGFR responses can occur without changes in glomerular capillary pressure, possibly by parallel alterations in afferent and efferent arteriolar resistances. It remains to be seen whether there are other physiologic states where SNGFR and glomerular capillary pressure feedback responses can be dissociated.

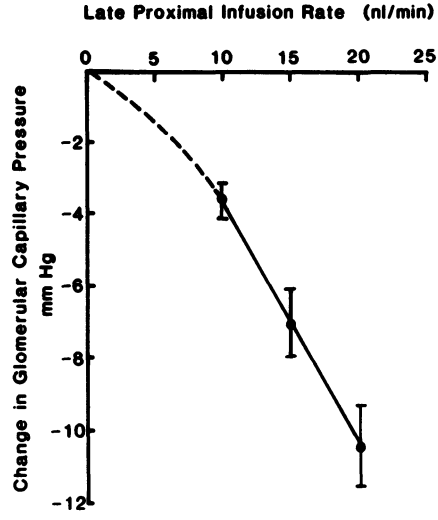

Fig. 2. Average glomerular capillary pressure feedback responses obtained during addition of perfusate at 10 (n = 8), 15 (n = 7), and 20 (n = 5) nl/min. Change in glomerular capillary pressure is calculated as difference in glomerular capillary pressure between no perfusion and perfusate addition at designated rate.[23]

1.3. Sensitivity of Tubuloglomerular Feedback Mechanism

The sensitivity or feedback gain of tubuloglomerular feedback has been measured under different physiologic conditions and has been found to be variable.[27–39] Göransson and Sjöquist[27] demonstrated that pressor doses of exogenous angiotensin II impaired autoregulation of SNGFR in rats. Opposing effects of captopril, a converting enzyme inhibitor, and aprotinin, a kallikrein inhibitor, on tubuloglomerular feedback responses have been shown by Schnermann *et al.*[28] The results of these experiments demonstrate that captopril produced an attenuation in the responsiveness of tubuloglomerular feedback mechanism, while aprotinin is capable of augmenting feedback responses. When administered together, aprotinin prevented to a large extent the attenuation effect of captopril on feedback responsiveness. The authors raised the possibility that kinins may modulate the magnitude of tubuloglomerular feedback responses and that the well-established effect of converting enzyme inhibitors on the mechanism may be, at least in part, related to their interference with kinin metabolism. It was thus concluded that the balance between vasodilators and vasoconstrictors may be a factor in determining the setting of feedback sensitivity. Prostacyclin (PGI_2) has been shown by Boberg *et al.*[29] to reduce the sensitivity of tubuloglomerular feedback. In this study two different intraarterial doses of PGI_2 were used in Sprague–Dawley rats, a low nonhypotensive dose of 100 and a high hypotensive dose of 500 ng/min per kg body weight. The results of these micropuncture experiments were interesting in that the high dose of PGI_2 caused a reduction in SFP and feedback sensitivity during the infusion. This attenuation in sensitivity is abolished immediately after termination of PGI_2 infusion. For the low PGI_2 dose, the reduction in feedback sensitivity started during the first 15 min of administration and persisted for at least 30 min after the infusion was stopped. The authors concluded that there are probably two different mechanisms for the reduction in feedback sensitivity as a result of high and low doses of PGI_2. The high does may inhibit tubuloglomerular feedback sensitivity through a direct vascular action, while the low nonhypotensive dose of PGI_2 may reduce the feedback sensitivity by affecting the renal interstitial pressure conditions. An increase in renal hydrostatic pressure and a decrease in oncotic pressure might lead to an alteration in interstitial pressure and an inhibition of tubuloglomerular feedback responsiveness.

In a recent study by Häberle and Davis,[30] a set of experiments was designed to investigate the possibility that a humoral factor in tubular fluid might be responsible for the resetting of tubuloglomerular feedback mechanism. The results showed that in salt-loaded rats, endogenous

tubular fluid produces an extremely attenuated feedback response, whereas exogenous tubular fluid from salt-depleted rats or Ringer's solution elicits normal feedback responses. In salt-depleted rats, endogenous tubular fluid and Ringer's solution elicit similar feedback responses, but exogenous tubular fluid from salt-loaded rats produces a small feedback response. These results led the authors to suggest that the resetting of the tubuloglomerular feedback mechanism is probably due to the presence of some inhibitory humoral factor in the tubular fluid of salt-loaded rats.

In two recent studies by Seney and Wright[31,32] and one by Wright[33] designed to determine whether tubuloglomerular feedback contributes to the increase in GFR that takes place when animals consume a high-protein diet, it was found that the feedback mechanism is less sensitive to the normal rate of flow through the loop of Henle in these rats. On the contrary, the feedback mechanism is more sensitive to loop-of-Henle flow in rats that were fed a low-protein diet. In further experiments[32] utilizing controlled microperfusion of the loop of Henle, it was shown that tubuloglomerular feedback response was activated at a lower threshold in rats fed the low-protein diet as compared with those that were placed on a high-protein diet. In the low-protein group, suppression of SNGFR and SFP began at a late proximal flow rate of 10 nl/min, while the suppression did not start until flow into the loop of Henle exceeded 20 nl/min in the high-protein group. It was concluded that the sensitivity of tubuloglomerular feedback mechanism increases by a low-protein and decreases by a high-protein diet.

Other factors that have been believed to influence the sensitivity of tubuloglomerular feedback control are renal interstitial hydrostatic and oncotic pressures. Boberg and Persson[34] showed that during volume expansion in rats, the net renal interstitial pressure (subcapsular interstitial hydrostatic pressure minus interstitial oncotic pressure) increased and the sensitivity of the feedback mechanism, as measured by SFP, declined. When renal venous pressure was increased from 2.3 to 20 mm Hg by clamping in volume-loaded rats, net renal interstitial pressure and the sensitivity of tubuloglomerular feedback were normalized. These findings indicate that both renal interstitial hydrostatic and oncotic pressures may be important in resetting the sensitivity of the tubuloglomerular feedback control mechanism. Single-nephron obstruction[35] for 1 day and release of 24-hr unilateral ureteral obstruction[35,36] have been shown to enhance the sensitivity of tubuloglomerular feedback.

Baylis and Blantz[37] studied the activity of tubuloglomerular feedback in virgin and 12-day-pregnant Munich–Wistar rats. The results showed that in spite of the increases in plasma volume and GFR during pregnancy, tubuloglomerular feedback activity is not suppressed at 12

days of pregnancy. The authors suggested that the kidney in pregnancy senses its volume-expanded status as normal. In a study by Dilley and Arendshorst,[38] it was demonstrated that 6-week-old Okamoto spontaneously hypertensive rats (SHR) exhibited a more sensitive and reactive tubuloglomerular feedback than age-matched Wistar–Kyoto rats. This hyperactivity of the feedback system seen in young SHR is less marked with normalization of GFR and filtration dynamics in adult SHR with established hypertension. In another study by Briggs *et al.*[39] performed on male Sprague–Dawley rats, evidence was provided to demonstrate that the feedback mechanism is most sensitive when tubule flow is close to the normal operating range. With growth, the mechanism is adjusted such that this relationship is maintained. The authors concluded that the slope, the maximum response, and the flow range of maximum sensitivity increase as GFR increases, but changes are approximately proportionate so that relative sensitivity is unchanged.

Sjöquist *et al.*[40] cautioned against predicting the effects of tubuloglomerular feedback on whole-kidney function from results based on micropuncture studies of superficial nephrons. In this study it was shown that under normal blood pressure range, the sensitivity of deep nephrons, as measured by SNGFR responses, to distal flow rates is greater in deep nephrons than in superficial nephrons.

1.4. Other Factors Controlling Renal Hemodynamics

1.4.1. Angiotensin II

The renin–angiotensin system has been shown to play an important role in controlling GFR, particularly during decreases in sodium intake or renal perfusion pressure or increases in renal venous pressure.[41,42] The renal site of action of angiotensin II on renal vasculature and the resulting changes in resistance have been widely studied, but are still controversial.

In a recent study by Kastner *et al.*,[2] the role of intrarenally formed angiotensin II in controlling GFR during reductions in renal perfusion pressure was investigated in dogs. In this experiment renin released by the kidney was prevented from entering the systemic circulation, and thus changes in circulating angiotensin II were prevented from affecting the control of GFR during short-term reductions in renal perfusion pressure. Reducing renal artery pressure to 70 mm Hg did not cause a significant change in GFR and renal blood flow in dogs with only a functional intrarenal renin–angiotensin system. However, after blockade of intrarenal angiotensin II formation, the same reduction in renal artery

pressure produced reductions of 24% and 41% in GFR and filtration fraction, respectively, and an increase of 29% in renal blood flow as compared with control. Calculated afferent and efferent arteriolar resistances decreased to 32% and 80% of control, respectively, as a result of reduction of renal arterial pressure. These calculations suggested that the intrarenal renin–angiotensin system controls GFR primarily by maintaining efferent arteriolar resistance, with little effect on the tone of afferent vessels. Similar results were reported by Textor *et al.*[3] in dogs with induced renal artery stenosis. In these dogs, intrarenal infusion of the angiotensin antagonist Sar-1-Ala-8-AII produced an abrupt decrease in GFR despite maintained renal blood flow. In the same study, the converting enzyme inhibitor captopril was administered orally to 14 patients with unilateral renovascular hypertension. Over a period of 1 hr blood pressure and GFR fell significantly, with no significant decrease in renal plasma flow. The differing effect on GFR and renal plasma flow reflected a significant reduction in filtration fraction. By contrast, similar blood pressure reduction with sodium nitroprusside in these patients produced no significant changes in GFR and renal plasma flow. Since GFR, following captopril administration, fell significantly below that during sodium nitroprusside infusion, the authors concluded that administration of converting enzyme inhibitor in subjects with renovascular hypertension produced selective reduction in the efferent arteriolar resistance.

Zimmerhackl *et al.*[43] studied the effect of systemically infused angiotensin II on microvascular parameters of the renal microcirculation. These experiments utilized *in vivo* preparation and, with the aid of fluorescence microscopy and a high-sensitivity video system, allowed observation of the passage of fluorescence-labeled erythrocytes through single glomerular capillaries on the surface of the rat kidney. The velocity and flux of the erythrocytes were measured from videotaped recordings using a modified dual-slit technique. The volume flow through the glomerular capillaries was calculated from the measured erythrocyte velocity and vessel diameter, while the hematocrit was considered to be the ratio of erythrocyte flux to volume flow. Intravenous infusion of angiotensin II produced a dose-dependent reduction in total renal blood flow. Also, volume flow through the glomerular capillaries decreased by 25% (from 3.2 to 2.4 nl/min) as a result of 0.4 μg/kg per min infusion of angiotensin II despite no changes in capillary diameter and hematocrit. The authors could not confirm the proposal that hypertensive doses of angiotensin II decrease the ultrafiltration coefficient by general vasoconstrictive mechanisms. Since the effect of angiotensin II on the hydraulic permeability of the capillary wall is still unclear, the authors hypothesized that the change in blood flow distribution that results from

a change in the efferent resistance might lead to a functional reduction in the surface area of filtration without the need for changes in the structure of the glomerulus. Schnermann *et al.*,[44] in a study of tubuloglomerular feedback and autoregulation of GFR, utilized saralasin to block the effects of angiotensin II. Their results showed that saralasin impaired autoregulation of SNGFR at the lower end of the autoregulatory range, 95–78 mm Hg. In this pressure range, it was estimated that the renin–angiotensin system contributes about 20% to the autoregulatory compensation. They suggested that saralasin influences autoregulation through a mechanism independent of tubuloglomerular feedback and that the effect of saralasin is probably due to blockade of the effects of angiotensin II on the efferent arteriole.

The density of angiotensin II receptors in the glomeruli may play a role in producing glomerular hemodynamic alterations. This density may vary under certain physiologic or pathophysiologic states. Bellucci and Wilkes[45] showed a strong negative correlation between plasma angiotensin II and glomerular angiotensin receptor density. In experiments that produced both high sodium intake and high plasma angiotensin, regulation of receptor density was inversely related to the circulating hormone and not to sodium intake. This study demonstrated that a major mechanism by which sodium intake regulates glomerular angiotensin receptor density is by changes in plasma angiotensin. Reduced glomerular angiotensin II receptor density has been found in early untreated diabetes mellitus in rats.[46] In normal, insulin-treated, and untreated diabetic rats the angiotensin II receptor density was inversely related to plasma renin concentration. However, untreated diabetic rats were found to have significantly lower glomerular angiotensin II receptor concentrations at all sodium intake levels despite the lower plasma renin concentration. The authors concluded that the decreased glomerular angiotensin II receptor density and the suppressed renin–angiotensin II system may contribute to the alteration in glomerular filtration dynamics and renal vascular responsiveness to angiotensin II seen in untreated diabetic rats.

The renin–angiotensin system may interact with other factors and play an important role in their renal hemodynamic responses. Such factors may include adenosine, prostaglandins, and renal nerves. Intrarenal infusion of adenosine leads to a decrease in renin release which can be dissociated from its hemodynamic effect.[47] This adenosine-induced decrease in renin release can be antagonized by theophylline.[48] Hall *et al.*[4] demonstrated that the renin–angiotensin system plays an important, time-dependent role in the renal hemodynamic responses to adenosine. These effects of intrarenal adenosine infusion in normal dogs and after blockade of angiotensin II formation and replacement of cir-

culating angiotensin II are shown in Figs. 3 and 4. The infusion of the converting enzyme inhibitor SQ 14225 (captopril) almost completely abolished the transient decrease in renal blood flow observed in normal dogs during adenosine infusion. Also, the secondary increase in renal blood flow seen after several minutes of adenosine infusion was greatly reduced in dogs given SQ 14225. In these dogs, the renal blood flow was elevated by only 10% (Fig. 4) as compared with a more than 22% increase in normal dogs after 10 min of adenosine infusion (Fig. 3). When circulating levels of angiotensin II were replaced after infusion of SQ 14225, the transient decrease in renal blood flow seen during adenosine infusion in normal dogs was restored. Maintenance of a constant level of circulating angiotensin II did not prevent, but magnified, the adenosine-mediated reduction in GFR and filtration fraction ob-

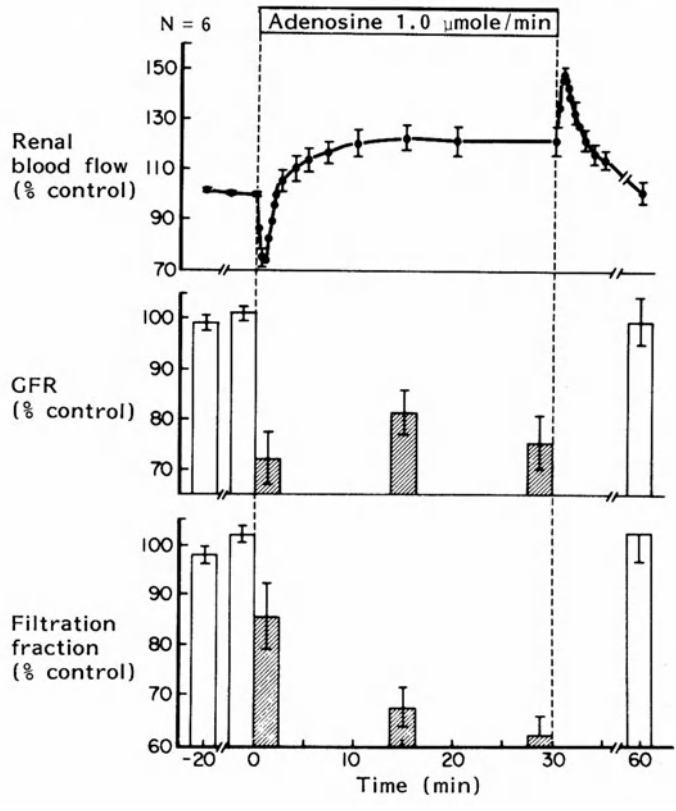

Fig. 3. Effects of intrarenal infusion of adenosine (1.0 µmole/min) on renal blood flow, glomerular filtration rate (GFR), and filtration fraction in six normal dogs. Values are means ± SE.[4]

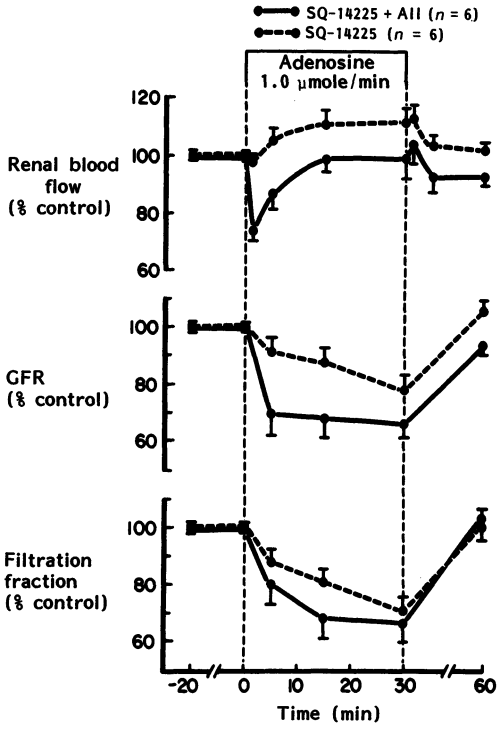

Fig. 4. Effects of intrarenal adenosine infusion (1.0 μmole/min) on renal blood flow, glomerular filtration rate (GFR), and filtration fraction in six dogs infused with SQ 14225 (dashed lines) and six dogs infused with SQ 14225 plus 20 ng/kg per min of angiotensin II (solid lines). Values are means ± SE.[4]

served during the infusion of SQ 14225 (Fig. 4). This study demonstrated that in normal animals the transient adenosine-mediated vasoconstriction appears to result mainly from an increase in the preglomerular resistance and depends on the presence of angiotensin II. The gradual waning of the preglomerular constrictor response to adenosine appears to be due in part to decreased angiotensin II formation.

Interaction between angiotensin II and prostaglandins has been shown to occur in isolated human glomeruli[49] and perfused rat kidney.[50] In both studies, administration of angiotensin II enhanced the formation of prostaglandin E_2 and 6-keto-$F_{1\alpha}$. Stahl *et al.*[49] suggested that this interrelationship might have physiologic importance in the regulation of glomerular hemodynamics.

Angiotensin II appears to be a critical factor for the full functional expression of renal nerve stimulation at the glomerulus.[51] Micropuncture measurements demonstrated that SNGFR was reduced by about 25% during moderate-frequency renal nerve stimulation. The reduction in SNGFR was the result of a decrease in nephron plasma flow and glomerular capillary hydrostatic pressure gradient. Under these circumstances, the glomerular ultrafiltration coefficient remained unchanged, while there was an increase of about 43% and 30% in the afferent and

efferent arteriolar resistances, respectively. When the activity of angiotensin II was inhibited by the intravenous infusion of Sar-1-Ala-8-AII (angiotensin antagonist) or MK 421 (angiotensin-converting enzyme inhibitor), renal nerve stimulation produced only a 7% decrease in SNGFR. This reduction in SNGFR was associated with a significant decrease of 7% in single-nephron plasma flow, but no significant increase in afferent or efferent arteriolar resistances. Generally, in this experiment the effects of renal nerve stimulation on glomerular hemodynamics during angiotensin II inhibition were of much less magnitude than those observed during renal nerve stimulation with the antiotensin II system intact.

1.4.2. Adenosine

Several investigators have hypothesized that intrarenal adenosine may play a role in the intrinsic regulation of GFR and renal blood flow.[52,53] However, the quantitative importance of adenosine in mediating renal hemodynamics is still unclear. In a recent study by Premen *et al.*[54] the role of adenosine in renal autoregulation was examined by comparing the hemodynamic responses of normal anesthetized dogs to step reductions in renal artery pressure with responses obtained after blockade of adenosine receptors with aminophylline or by flooding the kidney with exogenous adenosine. The results of this study showed that GFR and renal blood flow were well autoregulated (>90% of control) at renal arterial pressure of equal to or greater than 85 mm Hg before and after either aminophylline or adenosine intrarenal infusion in two separate groups of dogs. Even when the renal artery pressure was lowered to 75 mm Hg, the decreases in GFR and renal blood flow in both experimental protocols were comparable. Neither aminophylline nor adenosine attenuated the elevations in plasma renin activity observed with reductions in renal artery pressure. The results failed to provide evidence that adenosine plays an important role in autoregulation of GFR and renal blood flow during acute reductions in renal artery pressure within the autoregulatory range.

Arend *et al.*[55] provided evidence that intrarenal adenosine is important in mediating reduction in GFR associated with intrarenal infusion of dipyridamole, a nucleoside transport inhibitor, in anesthetized sodium-depleted dogs. In these dogs, GFR decreased by about 60% and renal vascular resistance did not change. GFR returned to control levels within 30 min after infusion of dipyridamole was stopped. In a separate group of sodium-depleted dogs, the dipyridamole-induced reduction in GFR was completely reversed or inhibited by theophylline, an adenosine receptor antagonist. In sodium-loaded dogs, dipyridamole infusion had

no effect on GFR or renal vascular resistance. This study demonstrates that the reduction in GFR observed during intrarenal dipyridamole infusion is mediated by increased endogenous adenosine.

1.4.3. Prostaglandins

Prostaglandins appear to be necessary for the maintenance of glomerular filtration, especially at the lower end of the autoregulatory range.[44] During indomethacin-induced inhibition of prostaglandin synthesis, SNGFR fell significantly in response to step reductions in arterial pressure from a normal value of 119 mm Hg to 95 and 78 mm Hg in Sprague–Dawley rats.[44] Indomethacin administration significantly increased the pressure dependency of the filtration rate. Whole-kidney GFR was reduced by half when arterial pressure decreased from 119 to 78 mm Hg. Analysis of renal autoregulatory components by the authors indicated that prostaglandins contribute about 30% to autoregulatory adjustments in the pressure interval of 115–95 mm Hg. In the lower pressure range (95–78 mm Hg), prostaglandin-dependent adjustments contribute 63% to the autoregulatory compensation. A part of the autoregulatory impairment observed during indomethacin administration may be related to its effect of reducing renin secretion, in addition to probable independent prostaglandin effects. The authors suggested that the existence of an intact prostaglandin system is critical in maintaining GFR at low pressures.

The prostaglandin system may have a compensatory role for the changes in glomerular hemodynamics in induced diabetes[56] and a protective role for the kidneys against the constrictor action of vasopressin.[57] Jensen *et al.*[56] demonstrated that indomethacin infusion causes no changes in glomerular hemodynamics in control, but produces remarkable effects in Munich–Wistar diabetic rats. Suppression of prostaglandin synthesis in diabetic rats produces a substantial increase in afferent arteriolar resistance, a moderate rise in efferent resistance, and thus a large decrease in single-nephron blood flow and glomerular capillary pressure. Results of a recent study by Yared *et al.*[57] indicate that the relative insensitivity of the renal vasculature to the vasoconstrictor effect of vasopressin may be due to vasopressin-induced release of a potent and indomethacin-sensitive renal vasodilator. The proposed intrarenal interaction between vasopressin and prostaglandin may yield an important mechanism for preservation of GFR and renal perfusion during acute extracellular fluid volume depletion.

In two-kidney, one-clip Goldblatt hypertensive rats, indomethacin infusion produced a significant reduction in mean arterial pressure, in addition to a significant reduction in the GFR of the clipped kidney.[58]

Indomethacin has no effect on the GFR of normal Wistar rats or on the untouched kidney in two-kidney, one-clip hypertensive models. Stahl *et al.*[58] concluded that glomerular vasodilatory prostaglandins (primarily PGE_2) may play an important role in maintaining GFR in the clipped kidney.

Other widely varied factors have been reported to cause changes in renal hemodynamics. Among these factors are calcium antagonists,[59,60] histamine,[61,62] amino acids and glucose,[63] leukotriene C_4,[64] parathyroid hormone,[65] pentobarbital anesthesia and hemorrhage,[66] changes in kidney anatomy and age,[67,68] development of spontaneous hypertension,[69] and Ringer's fluid infusion.[70]

2. Sodium Chloride Excretion

2.1. Sodium Balance and Its Regulation

It is well established that the normal kidney alters sodium excretion in response to changes in sodium intake. However, the renal adaptive alterations in response to such widely varying quantities of salt intake remain unclear, particularly in humans. In a recent study by Roos *et al.*,[71] renal sodium handling was investigated in normal humans at three levels of dietary sodium. Also, changes in extracellular fluid volume (ECFV, expressed per lean body mass), humoral factors, and blood pressure were measured after equilibration at the three levels of sodium intake (20, 200, and 1128 meq/day). Significant reductions in plasma renin activity and aldosterone were observed between successive levels of sodium intake, while blood pressure remained similar. Extracellular fluid volume increased significantly as the level of sodium intake was elevated, and this increase in ECFV was strongly correlated with fractional and absolute sodium excretion. Serum chloride increased significantly, but serum sodium was significantly increased only when comparison was made between the high and low sodium intake. GFR increased as the level of sodium intake was elevated. The results of this study demonstrate that in normal humans the maintenance of sodium balance during significant increases in sodium intake depends on renal adaptation of GFR, as well as proximal and distal tubular reabsorption. These changes in kidney function are associated with marked changes in neurohormonal factors and ECFV, whereas changes in blood pressure and serum sodium are only modest. The kidneys' precision in regulating sodium has been reaffirmed in Sprague–Dawley rats by Brensilver *et al.*[72] When sodium intake was less than the minimum daily requirement of 247 μeq/day, urinary sodium excretion was reduced to a minimum. When more than 247 μeq/day of sodium was ingested, the excess was

excreted quantitatively. The renin–angiotensin system appears to play an indispensable role in preventing sodium loss during low sodium intake.[73] Rats pretreated with captopril for 3 days and then maintained on a low-sodium diet for 5 days remained in a negative sodium balance state throughout the experimental period. Control rats did not receive captopril and achieved a new sodium balance within 3–5 days of sodium deprivation. Arterial pressure was markedly reduced (control, 109; captopril-treated, 65 mm Hg) and renal blood flow was significantly higher in rats treated acutely with captopril. Long-term inhibition of the renin–angiotensin system in chronically sodium-deprived rats (at least 4 weeks) produced a higher final arterial pressure than that observed in captopril-treated rats submitted to abrupt sodium restriction for 6 days. Similar observations were made by Tucker and Blantz.[74] In the former study,[73] renal blood flow in all groups with renin–angiotensin system inhibition was significantly higher than in rats that were only sodium deprived. Using the microsphere technique, the authors estimated that a selective increase in blood flow to inner cortical glomeruli took place in response to blockade of angiotensin. This finding suggests that redistribution of renal blood flow to inner cortical glomeruli may have resulted from the decrease in arterial pressure, renal vasodilatation, and inhibition of the intrarenal renin–angiotensin system.

The phenomenon of pressure-natriuresis and diuresis is well recognized by many investigators.[75–80] Recently, a new model was described by Roman and Cowley[81,82] for the study of pressure-natriuresis in rats. Neural and hormonal influences on the kidney were held constant by denervating the kidney and by maintaining constant high plasma levels of vasopressin, aldosterone, corticosterone, and norepinephrine by intravenous infusion. The results of these studies showed very clearly that increasing the renal perfusion pressure within the range of 90–160 mm Hg resulted in 5- to 20-fold increases in urine flow and sodium excretion with no significant changes in GFR, renal blood flow, or peritubular capillary pressure. When renal nerves remain intact, the pressure-diuresis and natriuresis relationship is shifted toward a higher level of renal perfusion pressure, with no change in the slope. The mechanism of the pressure-diuresis and natriuresis remains controversial. The proximal tubule,[77,78,83] thick ascending loop of Henle,[84] and collecting duct[85–87] have all been suggested as the site for tubular reabsorption changes. Recent studies by Haas *et al.*[88] show a selective effect of increased blood pressure on deep, but not superficial, proximal tubules.

Prostaglandins have been suggested to play an important role in the pressure-natriuresis mechanism.[89,90] Gleim *et al.*[89] studied the renal effects of changing perfusion pressure on control and indomethacin-treated isolated rat kidneys. In control kidneys, significant linear correlations

exist between renal artery pressure and GFR, filtration fraction, fractional sodium reabsorption, and sodium excretion. In kidneys treated with indomethacin, these correlations shift to the right. Therefore, prostaglandin-inhibited kidneys require higher renal perfusion pressures than control kidneys to maintain similar filtration rates and sodium excretion. It was suggested that prostaglandins promote pressure-natriuresis in isolated perfused rat kidney by an afferent arteriolar dilation mechanism. In prostaglandin-inhibited kidneys, afferent constriction may ensue, leading to an increase in renal vascular resistance and reductions in GFR, filtration fraction, and sodium excretion. In anesthetized sodium-replete dogs, prostaglandin synthesis inhibition dramatically impairs the pressure-natriuresis response.[90] In dogs treated with indomethacin, sodium excretion was reduced by 70% (Fig. 5), while GFR and autoregulation were not affected. These observations suggest that the renal prostaglandin system may have an important effect on the pressure-natriuresis mechanism.

An important role for renal pressure-induced natriuresis in the mechanism of escape from the sodium-retaining effects of aldosterone has recently been demonstrated by Hall *et al.*[91] In normal dogs in which renal artery pressure was permitted to increase during 7 days of aldo-

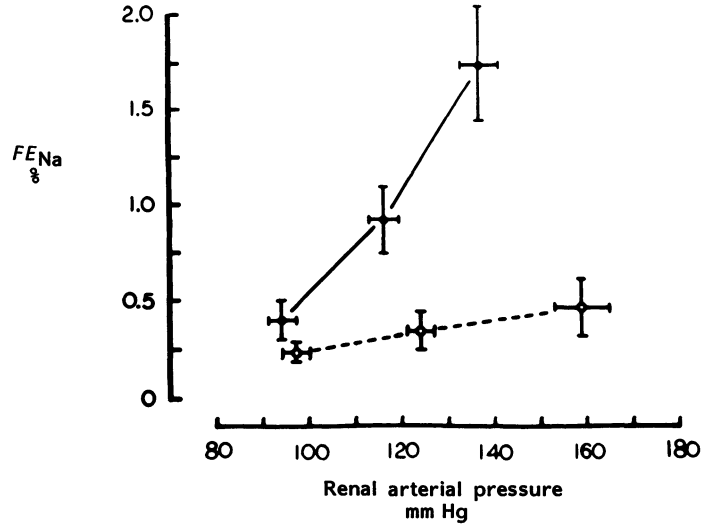

Fig. 5. Fractional sodium excretion (FE_{Na}) responses to changes in renal arterial pressure before and after indomethacin treatment. Indomethacin (open circles) significantly attenuated FE_{Na} to changes in perfusion pressure ($p < 0.01$).[90]

sterone infusion, sodium excretion decreased markedly on day 1 and then returned to control on days 2–4 (Fig. 6) as renal artery pressure and GFR increased 15–19 mm Hg and 20–25%, respectively. In another group of dogs where renal artery pressure was prevented from increasing with an electronically servocontrolled aortic occluder, escape from the sodium-retaining actions of aldosterone did not occur and the dogs continually retained sodium and water and developed severe edema (Fig. 6). Data from this study suggest that an increase in renal perfusion pressure is an essential factor in the mechanism of aldosterone escape.

2.2. Atrial Natriuretic Factor

The atrial natriuretic factor (ANF) is a peptide hormone that was discovered in 1981 by deBold *et al.*[92] Since then, a considerable body of evidence has been accumulating on the importance of ANF in the regulation of sodium excretion and extracellular fluid volume.

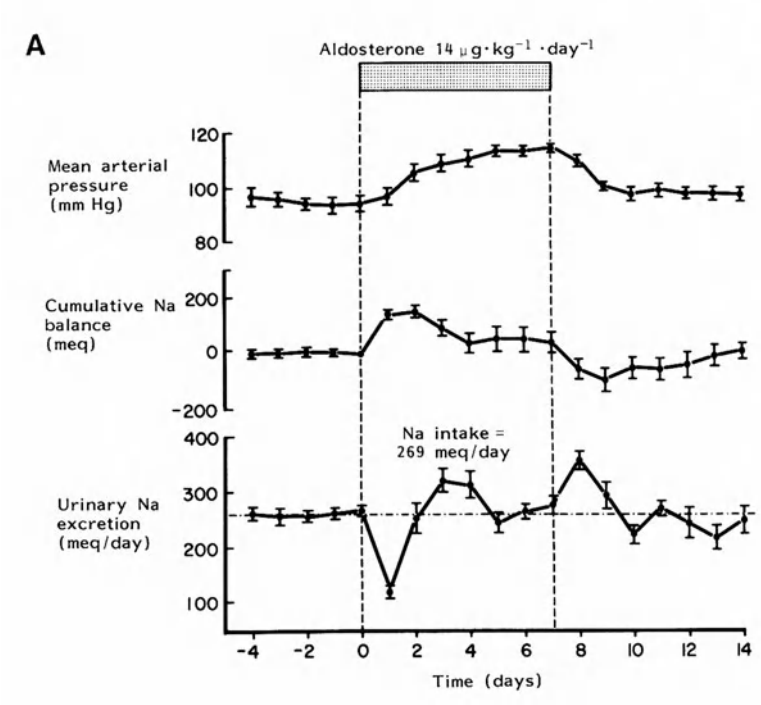

Fig. 6. Effects of aldosterone infusion on mean arterial pressure, cumulative sodium balance, and urinary sodium excretion in dogs when renal perfusion pressure was permitted to increase (A; $n = 5$) and when renal perfusion pressure was servocontrolled at the normal level (B; $n = 7$). Values are means ± SE.[91]

2.2.1. Synthesis and Release of ANF

ANF is a peptide that is synthesized and stored in atrial myocytes.[93] When released, it circulates[94] and has a potent effect on sodium excretion and a potential effect on mediating changes in body fluid composition, extracellular volume, systemic blood pressure, and vascular smooth muscle function.[95] It appears now, at least in rats, that the myocytes secrete a highly active low-molecular-weight natriuretic peptide, but store a less active form with large molecular weight.[96]

Anatomic and physiologic investigations have shown sites of low-pressure baroreceptors involved in plasma volume regulation in the atria. This observation is consistent with the proposal that the atrial myocytes are endocrine cells involved in the regulation of fluid and electrolyte balance.[93] Stimulation of the atrial volume receptors by distention causes diuresis and reductions in blood pressure, heart rate, and systemic vascular resistance.[97] It is reasonable to assume that increased plasma vol-

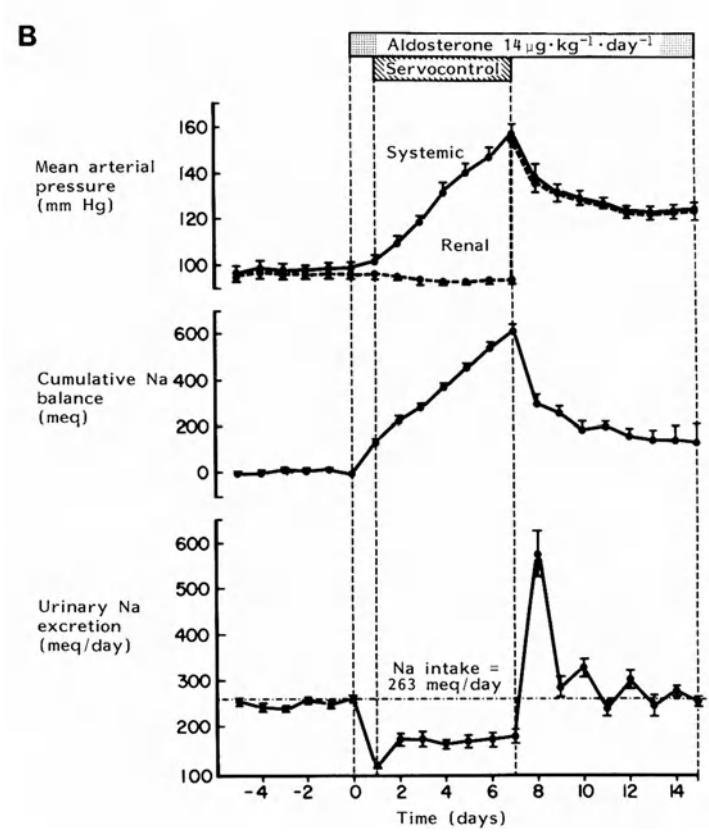

Fig. 6. *(Continued)*

ume can lead to stimulation of atrial receptors and the release of ANF into the circulation and subsequently activate target organs such as the kidneys to increase sodium excretion and reduce plasma volume.[98] Utilizing an isolated heart–lung preparation in rats, Dietz[99] demonstrated that increases in atrial perfusion pressure resulted in the release of a substance into the perfusate that induced a potent diuresis and natriuresis when infused into bioassay rats. Lang *et al.*[100] found a direct relationship between changes in right atrial pressure and the levels of immunoreactive ANF-like material in the perfusate in isolated rat hearts. *In vivo* studies have also shown that increased atrial distention may serve as a stimulus for ANF release from atrial myocytes in rats[101] and in dogs.[102] The effect of increasing left atrial pressure via mitral obstruction on plasma levels of ANF in control and bilateral cervical vagotomized dogs was studied by Ledsome *et al.*[102] Increasing the left atrial pressure by 11 cm H_2O caused a significant increase in plasma levels of ANF. Pulmonary vein distention increased heart rate, but had no significant effect on left atrial pressure or plasma levels of ANF. Mitral obstruction in vagotomized dogs provided similar elevations in left atrial pressure and plasma ANF levels as in control dogs. Administration of the beta blocker atenolol did not prevent the increase in plasma ANF caused by mitral obstruction. The results of this study suggest that the increase in plasma ANF levels in response to mitral obstruction is probably due to local stretch and atrial distention rather than activation of a neural reflex mechanism by stimulating atrial receptors.

In addition to atrial pressures, humoral substances have been suggested to play a part in stimulating ANF release.[103] Bolus injections of vasopressin, phenylephrine, angiotensin II, and oxytocin have been shown to cause a transient increase in circulating levels of ANF in rats.[103] In this study, treatment of rats with vasopressin and specific antagonist to the pressor effect of vasopressin abolished the transient increase in ANF release, suggesting that pressor agents may alter the release of ANF by a pressure-dependent mechanism like changes in atrial pressure. However, release of ANF from rat atrial tissue incubated *in vitro* when epinephrine, arginine vasopressin,[104] or acetylcholine[105] is added to the medium, suggests a direct effect of these agents independent of alterations in atrial pressure. More investigations are needed to clarify the importance of hormonal agents in the direct stimulation of ANF release under physiologic and pathophysiologic conditions.

2.2.2. Effects of ANF on Sodium Excretion

The most striking effect of ANF on renal function is its ability to produce enhanced sodium and water excretion. Figure 7 shows the ef-

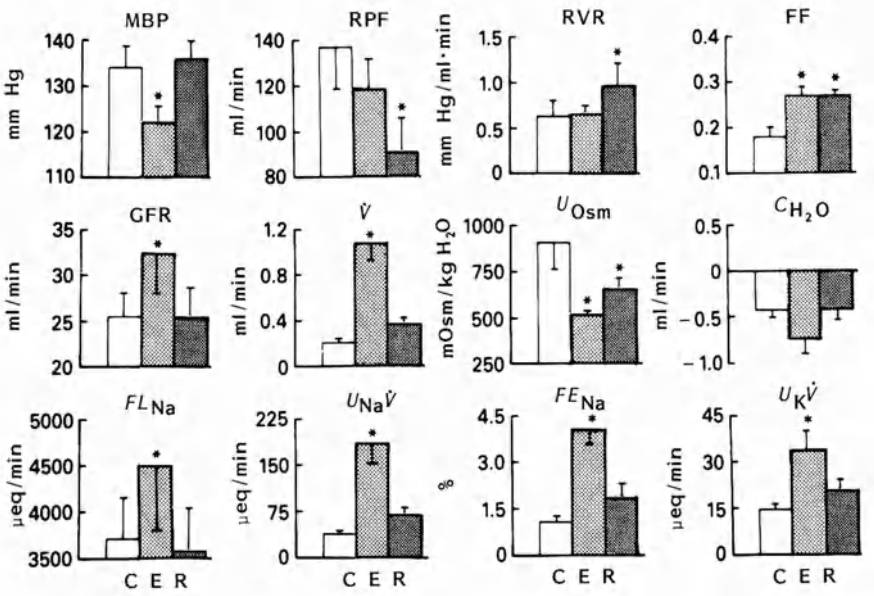

Fig. 7. Effects of synthetic ANF on renal functions in anesthetized dogs. After control periods (C), synthetic ANF was administered as a prime (1.0 μg/kg body weight) and a constant infusion (0.1 μg/min per kg body weight) for 1 hr. After steady-state experimental periods (E), the infusion was stopped and recovery periods (R) were performed. Results are mean ± SE (n = 5). MBP, mean arterial pressure; RPF, renal plasma flow; RVR, renal vascular resistance; FF, filtration fraction; GFR, glomerular filtration rate; $\dot{V}$, urine flow rate; U_{Osm}, urine osmolality; C_{H_2O}, free water clearance; FL_{Na}, filtered load of sodium; $U_{Na}\dot{V}$, urinary sodium excretion rate; FE_{Na}, fractional excretion of sodium; $U_K\dot{V}$, urinary potassium excretion rate; *$p < 0.05$.[106]

fects of continuous intravenous infusion of synthetic ANF on renal hemodynamic and excretory function in anesthetized dogs.[106,107] During ANF infusion, mean arterial blood pressure decreases, while GFR, urine flow rate, sodium, and potassium excretions increase. Similar results were obtained following continuous administration of ANF in conscious and anesthetized rats.[108]

Many studies have shown that ANF increases GFR significantly[106–108]; however, considerable controversy presently exists about whether ANF enhances sodium excretion primarily through an effect on increasing the filtered load of sodium by increasing filtration rate or by inhibition of tubular reabsorption, either directly or indirectly. Studies by Sosa *et al.*,[109] in which GFR was prevented from rising by aortic clamping, showed that the natriuretic effect of ANF is abolished when its renal hemodynamic actions are prevented. Also, the reduction in urine osmolality observed when ANF is infused in anesthetized dogs was prevented by aortic clamping. These results suggested to the authors that ANF-in-

duced natriuresis is mainly due to an increased filtered load of sodium into a washed-out inner medulla. Similar results were reported by Cogan[110] in rats where aortic clamping abolished 90% of the ANF-induced natriuresis and chloriuresis when GFR was normalized. The results of these studies suggest that ANF has no direct effect on reabsorption in the superficial proximal convoluted tubule except when GFR changes. In a recent study by Burnett *et al.*,[111] controlling GFR by aortic clamping did not abolish the natriuresis of ANF in anesthetized dogs. Despite no change in GFR and thus filtered load of sodium, there was a significant increase in fractional excretion of sodium and lithium, a marker for proximal reabsorption of sodium. The contrast between these studies is probably related to the aortic clamping. Reduction in renal perfusion pressure prevents the ANF-induced increase in GFR, but it can also have a direct effect on enhancing tubular reabsorption of sodium and water which could neutralize the proposed tubular action of ANF. Therefore, the degree of reduction in renal perfusion pressure observed during aortic clamping may be a deciding factor in whether one may or may not observe significant changes in tubular reabsorption of sodium and water during ANF infusion.

In support of a role for tubular action of ANF, Murray *et al.*[112] infused a low concentration of ANF (3 ng/ml) in rats. This dose produced natriuresis and diuresis without significantly changing GFR, suggesting a potentiating but not a prerequisite effect of GFR on sodium and water excretion in response to ANF. Other studies[113,114] have shown ANF-induced increases in GFR, while changes in urinary sodium excretion and GFR could not be correlated. These results appear to suggest that ANF diuresis and natriuresis may be attributed to increased GFR and tubular sodium reabsorption inhibition.

Several studies attempted to identify the possible nephron site at which ANF could be inhibiting sodium and water reabsorption. Some of these studies utilized whole-kidney clearance[115,117–120] and *in vitro* microperfusion methods,[116] others used micropuncture[108–117] and microcatheterization procedures.[117] Burnett *et al.*[120] showed that intrarenal infusion of ANF in anesthetized dogs results in an increase in fractional lithium and phosphate excretion, suggesting that this factor may have an effect on proximal tubule reabsorption. Infusion of ANF in thyroparathyroidectomized rats increases fractional excretion of sodium, phosphate, and bicarbonate.[115] Luminal brush border membrane vesicles from renal cortex of these rats have significantly decreased sodium-dependent phosphate transport as measured by rapid filtration techniques. Direct administration of ANF to brush border membrane vesicles had no effect on sodium-dependent phosphate transport, and direct application of this factor to isolated proximal tubules had no effect on

sodium transport. In another study by Baum and Toto,[116] *in vitro* microperfusion techniques were utilized to examine the effect of ANF on rabbit proximal tubule. The results of these studies showed that ANF does not have a direct inhibitory effect on transport in the proximal tubules. These results may lead to the conclusion that ANF does not directly inhibit sodium transport in the proximal tubules, but may induce changes in transport through an indirect mechanism.

A distal tubular action of ANF has been suggested by Sonnenberg *et al.*[117] Anesthetized rats were microcatheterized and tubular fluid was collected from end proximal and distal tubules as well as from outer medullary collecting ducts before and after intravenous injection of atrial tissue extract. Sodium excretion rose 17-fold after atrial extract injection, and tubular collection results showed a decrease of 16–20% in proximal sodium and fluid reabsorption. In the medullary collecting duct, sodium and chloride reabsorption did not rise in response to the increased filtered load after atrial extract injection. This decline in fractional reabsorption of the medullary collecting duct accounted for 80% of the natriuresis. The authors concluded that rat atrial extract may contain a factor that can cause natriuresis and chloriuresis by inhibiting transport in the medullary collecting duct.

There are many potential mechanisms by which ANF can have an effect on tubular sodium and water reabsorption. ANF could have a direct inhibitory effect on active tubular transport of sodium and water or indirectly inhibit this transport via changes in medullary blood flow and intrarenal hormones. Most studies failed to provide evidence for a direct effect of ANF to inhibit tubular sodium transport. Atrial extracts do not alter renal tubular sodium reabsorption by directly inhibiting the sodium, potassium-ATPase (Na, K-ATPase) enzyme system activity.[121] However, in a recent study by Cantiello and Ausiello,[121] the possible direct effect of ANF and cyclic 3',5'-guanosine monophosphate (cGMP) sodium transport of renal epithelial cells was investigated. Renal cell culture model LLC-PK_1, which contains an amiloride-sensitive conductive sodium transport pathway and a sodium–hydrogen exchanger, was used in these experiments. ANF (10^{-7} M) or exogenous cGMP (10^{-3} M) maximally inhibited the uptake of $^{22}Na^+$ through the amiloride-sensitive conductive pathway which represented up to 60% of the total $^{22}Na^+$ uptake. It was concluded that ANF can directly inhibit sodium transport in renal epithelial cells, probably through stimulation of cGMP.

ANF may decrease sodium reabsorption by dissipating the medullary tonicity via a medullary washout mechanism. Intravenous infusion of atrial extract produced an increase in medullary blood flow.[123] Continuous intravenous[105,106] and intrarenal[120] infusion of ANF in dogs caused a significant decrease in urine osmolality with maintained free-

water clearance. During recovery, urine osmolality returned to control values, suggesting that medullary washout did not occur.[120] In contrast, other studies have shown that urine osmolality increased during the recovery period as compared with that during ANF infusion, but was still significantly reduced as compared with that of the control period, suggesting a medullary washout. Further investigation is needed before the quantitative importance of medullary washout in renal effects of ANF is clearly determined.

Intrarenal infusion of ANF in anesthetized dogs significantly decreases renin secretion rate,[120] even under conditions of acute low-output heart failure which is a state of high renin secretion.[124] Intravenous infusion of ANF has a similar effect on renin secretion.[125] The mechanism by which ANF reduces renin secretion is not completely understood. Recent studies by Opgenorth *et al.*[126] support an important role for the macula densa in ANF inhibition of renin secretion. In the nonfiltering kidney, where the macula densa is nonfunctional, ANF was found to have no inhibitory effect on renin secretion. The macula densa may have been responding to an increased delivery of sodium chloride by signaling the juxtaglomerular cells to reduce renin secretion. Although these data provide strong support for a macula densa mechanism, the possibility that ANF has a direct inhibitory effect on juxtaglomerular cells cannot be ruled out. It is possible that part of the natriuretic effect of ANF may be mediated by the ability of this factor to suppress the renin–angiotensin system.

In addition to its inhibitory effect on renin release, ANF has been shown to significantly decrease plasma aldosterone levels[107,125] in anesthetized dogs. *In vitro* studies have also shown that ANF directly inhibits aldosterone production by suspensions of bovine adrenal glomerulosa cells[127] as well as the angiotensin II-stimulated aldosterone release in isolated rat adrenal glomerulosa cells.[128–130] It is possible that the reduced levels of circulating angiotensin II produced by the suppressed renin secretion observed during ANF infusion could be responsible for the decrease in aldosterone release. The suppression of aldosterone secretion may not play an important role in the acute natriuretic response to ANF. However, chronic alterations in circulating levels of this hormone by ANF could mediate the long-term regulation of sodium balance.

2.2.3. ANF and Regulation of Sodium Excretion

It is universally accepted that infusion of ANF has a potent effect on sodium excretion; however, the quantitative importance of this factor in regulating sodium balance is not as clear yet. Plasma levels have been shown to increase markedly in humans maintained on a high-sodium

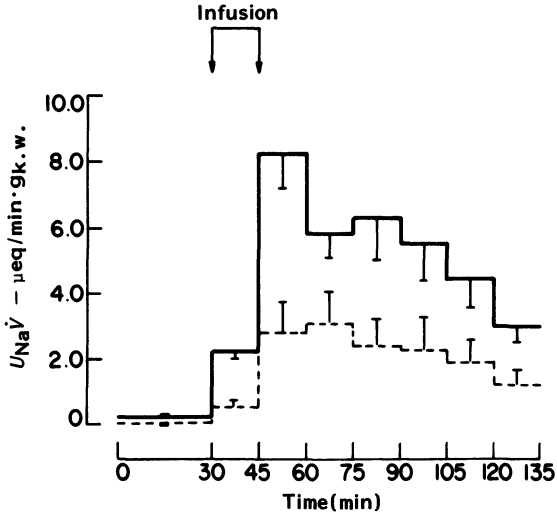

Fig. 8. Time course of sodium excretion in sham-operated (solid lines) and atrial appendectomy (broken lines) groups in response to acute blood volume expansion. Vertical bars, SE. $U_{Na}\dot{V}$, urinary sodium excretion.[134]

diet as compared with those on a low-sodium diet.[131,132] Pollock and Banks[133] have shown that atrial extracts from rats fed a low-sodium diet for 3 weeks contained significantly greater natriuretic activity than extracts from controls. These data suggest that changes in dietary sodium intake are associated with changes in atrial natriuretic activity. In a study by Veress and Sonnenberg,[134] acute right atrial appendectomy was shown to attenuate sodium excretion during volume expansion (Fig. 8). The attenuated renal response appears to be directly related to the atrial appendectomy and reduction in the circulating levels of ANF. The study provides strong evidence for an important role of ANF in the regulation of sodium excretion during acute volume loading. This conclusion is also supported by results from a study by Lang *et al.*[135] in which plasma concentration of ANF-like material increased rapidly in response to volume expansion. These results suggest that a chronic rise in ECFV is associated with elevated synthesis and metabolism of ANF.

3. Function of Discrete Nephron Segments

3.1. Proximal Tubule

In the proximal tubules, several transport mechanisms are utilized to transport sodium from the lumen to the proximal tubule epithelial cells. These include sodium-dependent organic solute cotransport, so-

dium–hydrogen exchange, directly coupled sodium chloride transport, and rheogenic sodium entry.[136] All forms of sodium chloride cotransport are examples of secondary active transport, and the primary active transport step is maintenance of the sodium gradient by the basolateral Na, K-ATPase. In the proximal straight tubules the active component is simple rheogenic sodium transport, with chloride absorption driven through the paracellular shunt pathway by the lumen negative potential difference. In the proximal convoluted tubules, sodium and chloride are transported in approximately equal amounts, thus resulting in a primarily neutral active transport component.[137]

Sodium uptake has been shown to be more sensitive to variations in membrane potential at high than at low sodium concentrations in brush border membrane vesicles taken from proximal tubular cells.[138] The results of these experiments suggested that the saturable sodium uptake occurs via an electroneutral sodium–hydrogen antiporter and that the diffusive flux occurs through a conductive pathway.

Gullans *et al.*[139] suggested that the interaction between gluconeogenesis and active sodium transport is not a simple competition for energy from ATP. Under normal conditions, the renal proximal tubule can provide enough energy for gluconeogenesis and sodium transport. Renal oxygen consumption can be dissociated from the calculated net rates of proximal tubular sodium, chloride, and bicarbonate reabsorption.[140] The data of Weinstein *et al.*[140] provided evidence that hydrogen ion secretion supporting bicarbonate reabsorption in the proximal tubule requires, at most, small amounts of oxidative energy. Benzolamide (carbonic anhydrase inhibitor) and hypertonic bicarbonate reduce proximal tubular fluid reabsorption while concomitantly reducing the transepithelial gradient for chloride. These data support the proposal that the chloride–bicarbonate transepithelial gradient appears to be an important passive driving force *in vivo* for proximal tubular reabsorption. Other data suggest that bicarbonate leaves the basolateral membrane in the proximal convoluted tubules by a rheogenic, chloride-independent mechanism.[141]

In isolated rabbit proximal tubules, when preferentially absorbed solutes are reduced or eliminated from the perfusate, volume absorption is significantly decreased in the convoluted tubules, while the absorbate sodium concentration is not significantly different from that in the lumen.[142] When preferentially absorbed solutes are present at normal concentrations in the perfusate, sodium concentration in the absorbate is significantly less than that in the lumen. This low concentration of sodium in the absorbate may be due to the dilutional effect of more rapid fluid reabsorption in the presence of preferentially absorbed solutes. Under conditions of reduced or eliminated preferentially absorbed solutes, sodium concentration of the absorbate in the proximal straight

tubules exceeds that in the lumen, and given the negligible measured transepithelial voltage, active transepithelial transport of sodium is demonstrated. Results of other experiments show that both segments of the proximal tubules can generate hyperosmotic absorbate when the peritubular volume is restricted.[143] Also, peritubular protein concentration appears to have an effect on fluid reabsorption in rabbit proximal convoluted tubule segments perfused *in vitro*.[144] This effect is dependent on some property of the tubular wall that is changed when distention occurs. Sodium transport was studied in microvillus membrane vesicles isolated from uninephrectomized or sham-operated rats fed a low-, normal-, or high-protein diet.[145] The results provided evidence for modification of the luminal membrane sodium–hydrogen exchange in response to changes in dietary protein content and nephron number. Bichara *et al.*[146] suggested that volume expansion-induced reduction in peritubular protein concentration contributes to the proximal expansion effect probably by inhibiting transcellular sodium chloride reabsorption. A mathematical model developed by Weinstein[147] predicts a decline in epithelial water permeability, salt reflection coefficient, and salt permeability in the proximal tubule, with the application of peritubular protein.

3.2. Loop of Henle

In the past few years the mechanisms and factors that control sodium chloride absorption by the thick ascending limb of the loop of Henle have been substantially modified. In a recent review by Hebert and Andreoli,[148] evidence was presented for a model of sodium chloride absorption in the thick ascending limb. According to this model (Fig. 9), net chloride absorption is rheogenic, involves a secondary active transport mechanism, and occurs via a furosemide-sensitive coupled electroneutral (1 sodium:2 chloride:1 potassium) apical chloride transport process. The apical chloride entry mechanism occurs in parallel with a large potassium conductance across the luminal membrane and a conductive chloride exit mechanism in basolateral plasma membranes. The metabolic energy needed for active transcellular sodium absorption is reduced in this model owing to the positive voltage in the lumen and the high paracellular conductance in the thick ascending limb that provides for 50% of net sodium absorption via the paracellular route. It can be postulated that in the medullary thick ascending limb of some mammalian species, antidiuretic hormone may elevate sodium chloride absorption by increasing the functional number of electroneutral cotransport units, enhancing the conductance of potassium across the luminal membrane, and indirectly increasing chloride conductance through the basolateral plasma membrane. Prostaglandin E_2 inhibits antidiuretic hormone stim-

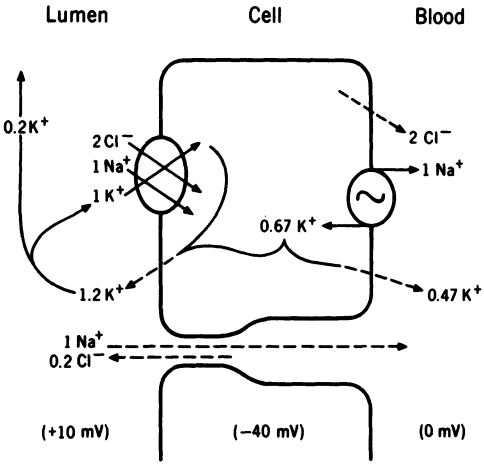

Fig. 9. Model for NaCl absorption in mouse medullary thick ascending limb of Henle (mTALH). Conductive pathways are denoted by dashed arrows. All flux values are normalized to an electroneutral (Na^+, K^+, Cl^-) cotransport mechanism in apical plasma membrane with a stoichiometry of 1:1:2. Stoichiometry of Na,K-ATPase is assumed to be $3Na^+$:$2K^+$. Depicted in the lower cell is ADH-dependent voltage profile across mouse mTALH.[148]

ulation of sodium chloride transport in the isolated microperfused mouse medullary thick ascending loop of Henle.[149] A similar physiologic response to that of antidiuretic hormone is elicited by human calcitonin *in vivo* in the thick ascending limb of Henle of homozygous DI Brattleboro rats.[150]

Scherzer *et al.*[151] studied the Na, K-ATPase activity in tubule segments from the cortex and medulla of rabbit kidneys after unilateral nephrectomy and after chronic salt loading. It was concluded that unilateral nephrectomy produces a general increase in Na, K-ATPase activity along the whole length of the nephron, while sodium chloride loading causes a selective increase in enzyme activity along the ascending limb of the loop of Henle and decrease in the distal segments. Acute renal denervation produces a significant decrease in net transtubular fluxes of sodium and water in perfused loop of Henle and distal tubule segments with delivery kept constant.[152] In anesthetized rats, renal denervation results in an increase in sodium, potassium, and urine excretion with no change in SNGFR, GFR, or arterial blood pressure. These results indicate that efferent renal sympathetic nerve activity may have a direct effect on sodium, potassium, and water reabsorption in the loop of Henle and distal tubules. DiBona and Swain[153] have demonstrated that low-frequency renal nerve simulation elevates sodium chloride absorption in the loop of Henle of hydropenic and isotonic volume-expanded rats.

3.3. The Collecting System

The apical cell membrane ionic conductive properties of rabbit isolated perfused cortical collecting tubule have been studied recently using

microelectrode techniques.[154] Stable cell membrane voltage recordings have been obtained by impaling cells from the bath side across the basolateral cell membrane. Addition of amiloride to the luminal perfusate produces a hyperpolarization in the voltage of the apical cell membrane, a reduction in the transepithelial conductance, and an increase in the fractional resistance as estimated by the ratio of the resistance of the apical cell membrane to the sum of the apical and basolateral cell membrane resistances. Increasing potassium in the luminal perfusate indicates a high potassium conductance at the cell apical membrane. This conductive pathway can be blocked by barium or by reducing luminal pH to 4.0. Addition of both amiloride and barium in the luminal perfusate results in a significant increase in fractional resistance. These results indicate that sodium and potassium conductances appear to be the dominant conductive pathways at the apical cell membrane and that this membrane contains an amiloride-sensitive sodium conductance and a barium-and-hydrogen-sensitive potassium conductance. The basolateral cell membrane appears to be predominantly chloride selective,[155] and this might be consistent with the role of this segment in electrogenic hydrogen secretion.

El Mernissi and Doucet[156] tested whether sodium availability controls the concentration of renal Na,K-ATPase. The effect of chronic alterations in apical membrane sodium permeability on the maximal Na,K-ATPase activity was studied in the collecting tubules. These nephron segments were microdissected from rats treated continuously for 3–8 days with either furosemide or amiloride. Na,K-ATPase pump activity was increased by both diuretics in the collecting tubule even in the presence of spironolactone, an aldosterone inhibitor. These results suggest that Na,K-ATPase maximal activity is not controlled by sodium availability or by aldosterone, and that furosemide or amiloride can produce an aldosterone-independent increase in Na,K-ATPase activity in the collecting tubule.

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3

Renal Metabolism

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1. Introduction

The amount of experimental work being done in the area of renal metabolism and biochemistry is growing at an increasing pace. As in previous editions, the authors of this chapter will not attempt to survey the literature on renal metabolism, but rather will discuss in depth several selected topics. Accordingly, the following topics have been selected for study: the use of cell culture in the study of renal metabolism and transport; the biochemistry of renal ischemia; a reevaluation of the importance of liver and kidney in acid–base hemeostasis; and the polyphosphoinositides and diacylglycerol as second messengers of cell function. Of particular interest is evidence for the continued narrowing of the gap between classical transport and biochemistry in the evaluation of renal function. More studies are being reported in which biochemical mechanisms are used to explain transport functions. Some of this will be apparent to the reader in perusing this chapter.

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2. Cultured Epithelia of Renal Origin: Characteristics and Applications to Physiological and Biochemical Studies

2.1. Introduction

Cultured cells of renal origin are becoming increasingly popular tools for investigators in nephrology and renal physiology. This discussion is intended as an introduction to the subject and to the literature for those who are not conversant with progress in the field. Reference to reviews as source material will be made when possible. The area of coverage will be confined to cells, presumably all of renal tubular origin, that form functional epithelia in culture. This chapter will not deal with glomerular or interstitial cells in culture which are also of major interest. Certain general characteristics of cultured epithelia and techniques available for study of transport in these preparations will be presented. Important characteristics of continuous cell lines of interest and certain primary culture systems will be summarized. Then, a more in-depth description of investigations of corticosteroid action in one continuous line, A6 epithelia, will be presented as an example of how such an epithelium may be utilized to approach a given problem. For additional information on epithelia in culture the reader is referred to text and other reviews.[1–4]

A major advantage of cultured cells of renal tubular origin is the large quantity of characterized and relatively homogeneous material that can be made available for transport or biochemical experiments. In contrast, *in vivo* dissected segments yield well-defined material but of limited quantity, and zonal preparations of kidney tubules produce large quantities of material that are heterogeneous in terms of segmental origin. Other important advantages are listed in Table I. Some of these potential

Table I. Potential Advantages of Kidney Cells in Culture

1. Decreased variability in cell preparations because of:
   a. Common genetic background
   b. Identical environmental influences, e.g., nutrition, hormones, growth factors, and age of culture
2. Homogeneity of cell type
3. Rigid and defined control of cell envionment over prolonged time periods, e.g., for studies of growth and differentiation, physiologic adaptation, metabolism
4. Production of large amounts of biologic material that can be harvested by subcellular fractionation or from the medium
5. Adaptable to large-scale screening of biologically active substances (e.g., hormones and nephrotoxic drugs) in terms of transport and metabolism effects
6. Ease of application of modern techniques in cell biology, including development of mutants or adapted cell lines for specific purposes

advantages have been exploited only to a limited degree but will undoubtedly be capitalized upon to a greater extent in the future. Most important, cultured renal cells are opening the door to use of modern techniques in cell biology for the disease-oriented nephrologist as well as the renal physiologist.

2.2. General Characteristics of Epithelia in Culture

2.2.1. Domes and Polarity

The essence of transporting epithelia in culture, as *in vivo,* is the formation of membranes of oriented cells with tight junctions between them. The basolateral side of these surface-adherent cells is in contact with the supporting structure, usually a plastic tissue culture dish. The apical surface is oriented toward the medium, and microvilli are often present. Polarity in various epithelia has been demonstrated by evidence for the presence of basolateral Na,K-ATPase and apical enzyme markers, a transepithelial potential difference (PD), and vectorial apical or transepithelial transport. A characteristic of many epithelia in culture is to form domes or hemicysts when grown on tissue culture dishes. These "blisters" are areas of cells of the epithelial layer lifted above the surface of the dish, presumably by transported fluid. Domes are quite characteristic in appearance and easily viewed by low-power light microscopy. Their presence and number have been used as a marker for the presence of apical to basolateral ion and water transport and even to quantitate transport. The hazards in interpretation of the latter approach have been emphasized.[3] Individual cell polarity seems to develop as subconfluent cells become confluent and form tight junctions. The signals and the mechanism of these processes are unknown, but epithelia in culture are an ideal system for such studies (cf. Ref. 5).

2.2.2. Permeable Attachment Surfaces

An important advance toward use of cultured renal epithelia for physiologic studies was the demonstration that transepithelial transport and electrical measurements can be made in epithelia grown on permeable supports, e.g., collagen or filters, in the fashion utilized for intact epithelia such as frog skin or toad bladder.[6,7] Some of these methods will be discussed later. Another advantage of growth of epithelia on permeable supports, in contrast to growth on tissue culture dishes, is to allow for direct access of nutrients and other factors in the growth medium to the basolateral surface, the nutrient surface of epithelia *in vivo.* Examples of the importance of basolateral feeding on differentiation in

epithelia of renal origin are the dependence of basal lamina formation in MDCK cells[8] and vasopressin responsiveness in A6 epithelia.[9] Exposure of the basolateral surface directly to medium may be less important in low-resistance or leaky epithelia than in tight epithelia with less permeable tight junctions. It is possible that nutrients, polypeptides, and even macromolecules may access the basolateral surface via tight junctions in leaky epithelia. A recent review discusses the importance of basolateral access to nutrients and other factors that influence development of maintenance of morphological and functional differentiation in epithelia.[9] The latter factors include age of the culture, the nature of the substratum, presence or absence of serum in the medium (and even the particular batch of serum), hormones, and certain other substances.

2.2.3. Growth and Defined Media

Cultured renal epithelia provide an excellent tool for study of growth and differentiation. Serum is used in most cell culture media for growth and maintenance of cell viability. However, the presence of this complex and ill-defined material not only may enhance fibroblast overgrowth (in primary cultures), but may hamper investigations of the regulation of growth and differentiation and indeed may reduce the degree of differentiation. It has been repeatedly emphasized that development of defined media, e.g., serum-free medium, will be quite valuable for such studies. Defined media have been developed for continuous cell lines [10,11] and primary culture systems.[12] Such media should also simplify study of hormonal and other regulating mechanisms.

A popular serum-free medium for use, at least as a starting point or framework for evaluating additives essential for growth and differentiation, in both primary culture and continuous lines is K-1 medium originally devised for MDCK cells. The basal nutrient medium contains insulin, transferrin, triiodothyronine, prostaglandin E, and hydrocortisone. However, serum may not always be an unwanted constituent of culture medium. Factors as yet unknown may be present which are necessary for the expression of desired functions in cells in culture. The reader is referred to reviews of the development of defined media and their usefulness in cell culture.[13,14]

2.3. Techniques for Study of Transport

2.3.1. Porous-Bottom Culture Dishes

Most studies of transport of ions and organic substances by cultured cells of renal origin have utilized cell uptake or, in some instances, wash-

out kinetics. The most significant advance in this area (*vide supra*) was to utilize permeable materials as the substratum or attachment surface. This allows one to place the support, and thus the intact epithelium, into appropriate chambers for measurement of transepithelial flux and for electrical measurement. However, epithelia grown on porous supports, such as collagen films and filters, can be difficult to mount conveniently in chambers or otherwise manipulate without damage, particularly edge damage in the former case.

Workers in the Laboratory of Kidney and Electrolyte Metabolism, NHLBI, NIH, have pioneered the development of tissue culture dishes with porous bottoms of various kinds for basolateral cell attachment. The reader is referred to recent reviews from this laboratory for technical details and discussion of usage.[15,16] These structures (porous-bottom cups) are much easier to manipulate without damage since they can be handled by grasping the side of the cup. The porous materials that have been used by these workers are cellulose acetate filters (Millipore), polycarbonate filters (Nucleopore), collagen membranes, and denuded placental amnion. The bottoms are attached to polycarbonate rings of an appropriate diameter and height to fit in a standard tissue culture dish, e.g., cluster.[6] The sides of the porous-bottom tissue culture dish are then the polycarbonate ring. The porous-bottom dishes are usually elevated by small "feet" from the bottom of the well of the receptacle tissue culture dish to allow access of medium to the basolateral surfaces of cells seeded in the "cup." Medium is also placed in the cup and thus exposed only to the apical surface. This arrangement allows polarity of exposure of cells to hormones, nutrients, ions, etc., and polarity of measurement of efflux of substances from the cells. Large rings and filters can be used for growth of large numbers of cells, e.g., as used for preparation of membrane vesicles and purification of the apical Na^+ channel.[17,18]

2.3.2. Transepithelial Electrical and Flux Measurements

PD can be easily and quickly measured by placing bridges in the basolateral solution. In epithelia that have a spontaneous PD, this measurement is useful to monitor viability and integrity as an epithelium and to match epithelia into subgroups for experimental comparison, e.g., for transport measurements. Ion or other transepithelial fluxes are very easily done simultaneously on large numbers of epithelia in the dishes used for growth by adding to or sampling apical and basolateral media. For instance, serial ion fluxes and PD are very stable in both control and hormone-pretreated A6 epithelia maintained in an incubator on a shaker for several hours (*vide infra*).

A convenient chamber system for instantaneous measurement of

PD, short-circuit current, and resistance under sterile conditions has been developed.[19] Filter-bottom cups* designed to fit into a cluster[6] (filter area 5 cm^2; polycarbonate ring O.D. 1.25 in. and height 0.5 in.) have been extensively used for electrical and ion flux measurements, e.g., in A6 epithelia (cf. Refs. 20,21). Again, large number of epithelia can be maintained in an incubator during experiments and removed temporarily for these measurements performed in a laminar flow hood and then returned to the incubator. Thus, serial determinations can be conducted on the same epithelia for hours, days, or even weeks. Ussing chambers for continuous voltage clamp can be designed so that the chamber seals to the ring of the filter-bottom cup to avoid edge damage.

Collagen membranes are the only substrata commonly used that allow visualization of cells without staining. Cells so grown have been used for apical cell impalements and patch clamp,[22] although this preparation is not essential for such studies.

2.4. Continuous Epithelial Cell Lines of Renal Origin

2.4.1. Summary of Continuous Lines

Most reports on the biology, transport properties, and hormonal responses of cultured kidney cells are from studies on continuous lines. Often they were established in the 1950s and 1960s and were used initially for viral culture. Many of these lines are listed in Table II.

This summary (Table II) emphasizes electrical and ion transport properties of the epithelia. Many epithelia are too permeable to ions (low resistance) to measure conveniently transepithelial ion fluxes or short-circuit current as indices of active transport. This does not mean that very "leaky" epithelia are not useful for ion transport studies. For instance, GRB-MAL1 epithelia do not have a PD and have a very low conductance in contrast to the medullary thick ascending limb of origin. However, the apical membrane is hyperpolarized by furosemide and barium, implying presence of the NaCl/KCl cotransporter and calcium-activated K^+ channels present *in vivo*.[22] Thus, these cells still express important differentiated properties of interest.

2.4.2. MDCK and LLC-PK_1 cells

The most popular continuous lines have been MDCK (Madin–Darby canine kidney) and LLC-PK_1 cells. MDCK cells have been used primarily

* Commercially produced filter-bottom cups for cluster[6] and cluster tissue[24] cell dishes can be obtained from Millipore Corporation to reduce time of preparation and sterilization.

Table II. Continuous Epithelial Lines of Renal Origin[a]

Designation	Source	PD[a] (mV)	R (ohm-cm^2)	Short-circuit current (μeq/cm^2)	Reference[b]
MDCK	Dog	6	4000	0.04[c]	See text (6,7,24,25)
Strain I (passages 60–70)					
Strain II (passages > 100)		1.4	80–100	0.11–0.62	
		—	200–300	—	
LLC-PK$_1$	Pig	2.5	200	0.5	See text (26)
A6	Toad	10	5000–10,000	0.1[d]	See text (20)
JCK-5	Dog (cortex)	1.7	350	0.25	(27)
GRB-PAP 1	Rat	—	100	—	(28)
GRB-MAL 1,2	(papilla)				
BSC-1	Monkey	—	13		(29,30)
LLC-MK$_2$	Monkey	—	—	—	(31,32)
JTC-12.P3	Monkey	—	—	—	(33)
OK	Possum	—	—	—	(34)
MDBK	Cow	—	80	—	(35)

[a] For those cases where electrical parameters are not shown values were not found or were trivial in magnitude.
[b] The references cited are not meant to be a comprehensive survey of the literature.
[c] Potential difference (PD) is apical negative. Active Na^+ transport indicates basolateral positive.
[d] Active Cl^- secretion (chloride current) demonstrable after stimulation with certain hormones and other substances (see Section 2.6.7).

for studies of the following: growth regulation,[36,37] electrical properties of the epithelium and cell membranes,[25] viruses as agents for evaluation of vectorial processing of membrane constituents,[38] development of tight (occluding) junctions,[39,40] regulation of dome formation,[41] and ion co-transport mechanisms.[42] LLC-PK$_1$ epithelia have been popular for studies of Na^+ coupled-PO_4,[43] hexose[44] and amino acid transport,[45] and vasopressin regulation of adenylate cyclase activation.[46,47] Of particular interest is the presence of transepithelial Na^+/coupled hexose transport as a function of time in culture[48] and apical medium glucose concentration[49] and glucose metabolism as the regulatory signal.[50]

Morphologic characteristics,[51] shared surface antigens with canine cortical collecting tubule,[52] and the specific hormones that stimulate adenylate cyclase have led to the conclusion that MDCK cells were derived from distal nephron cells. Similarly, it has been proposed that the LLC-PK$_1$ line resembles proximal tubular cells owing to the presence of apical Na^+/hexose coupled transport systems. However, LLC-PK$_1$ cells

have distal tubular adenylate cyclase responses (calcitonin and vasopressin) and lack a proximal tubular adenylate cyclase response (PTH).[53] One must be cautious in assuming too much similarity of a line, even in primary or early-passage culture, to specific *in vivo* segments or cells since marked changes may occur as they adapt or are selected by culture conditions. An example of an apparent spontaneous change in properties even after numerous passages is illustrated by the two MDCK strains (Table II). Indeed, Cereijido now finds a two- to threefold higher resistance in the low-resistance strain[25] than he originally described.[6] One must be content to study a cell for its properties of interest and not demand that a cell line behave in an identical manner to any nephron cell or segment. Indeed, the zone of the kidney from which the continuous lines arose is known for only two of the lines in Table II.

2.4.3. Mutants or Strains of Interest

Mutants or selected variants of kidney cells in culture, as noted in Table I, hold great promise for the future. Examples from MDCK cells and their use are amiloride-resistant clones,[54] ouabain-resistant mutants,[55] mutants with defects in NaCl/KCl cotransport activity,[56] variants that have lost the dome stimulation response to PGE_1 but retain the growth response to PGE_1,[57] and vice versa.[58] LLC-PC_1 cells, which require glucose for growth, lack the enzyme 1,6-biphosphatase, which is necessary for gluconeogenesis.[59] Gstraunthaler and Handler have isolated a strain that has the necessary enzyme and grows in the absence of glucose.[60] Burg *et al.*[28] have developed a continuous line from the rat papilla (GRB-PAP1). By growing these cells in hypertonic medium, they developed a strain (PAP-HT25) that maintains osmotic equilibrium in the hypertonic state by synthesizing sorbitol.[61]

2.4.4. Tight Epithelia

A6 cells, which are high-resistance or tight epithelia with easily measurable apical-to-basolateral active Na^+ transport (short-circuit; I_{sc}), have relatively large I_{sc} responses to corticosteroids[20,21,62] and have attracted interest for this reason.

Johnson and Handler have generated several continuous lines from toad bladder. These lines are of interest because of the large body of renal-oriented study done in intact toad bladder and turtle bladders and the fact that these lines form tight epithelia with I_{sc} (equivalent to net Na^+ flux) which is stimulated by aldosterone.[19,63] In contrast to A6 cell and other renal epithelia that grow in monolayer, these bladder lines are multilayered and contain more than one cell type similar to the

epithelium of origin. These lines and similar ones from acidifying toad and turtle bladders do not acidify the apical medium. Despite attempts to enrich them in mitochondria-rich cells to obtain a cell culture model for acidification studies, the enrichment is not retained during sequential passages (J.P. Johnson, personal communication).

2.5. Primary Culture Systems

Primary cultures of renal epithelia have attracted interest in recent years not only in attempts to establish continuous lines, but as a means of obtaining larger quantities of specific renal tubular cell types to circumvent the relatively small quantity of defined material obtained, e.g., with microdissection. Some of the problems encountered are fibroblast overgrowth, slow growth rate, and loss of differentiated properties. Fibroblast overgrowth has been reduced by low-serum or serum-free medium.

Microdissected segments in primary culture have been studied extensively by Horster and co-workers and others [64–66] including recent experience using human kidney.[67] A current review is recommended.[2] Most studies have dealt with factors that influence growth and differentiation. Generally these techniques have yielded loss of differentiated function prior to obtaining sufficient material for extensive biochemical and physiologic study (cf. Ref. 66)

The primary culture system of Chung *et al.*[12] yields relatively large quantities of cells from rabbit cortex with many of the properties of proximal tubule, including Na^+-coupled hexose and phosphate uptake, enzyme activity associated with proximal tubule brush border, and PTH-sensitive cyclic AMP synthesis. In this method of culture iron oxide is injected into the kidney prior to fragmentation of the tissue and collagenase treatment. Glomeruli containing iron are separated with a magnetic stirrer. Another method uses Percoll density grandient separation of canine renal cortex to achieve a proximal tubule-enriched preparation of cells.[68]

A relatively high yield of papillary epithelial cells in primary culture with differentiated function is obtained by the method of Grenier and Smith, which utilized hypotonic disruption.[69] These cells can be passaged many times. Immunodissection is an interesting method applied successfully to obtain larger quantities of material from defined segments, by several orders of magnitude more than can be obtained from microdissected segments. In essence, monoclonal antibodies are obtained which are directed against surface antigens of a specific cell type. Such an antibody can be used (e.g., coating the surface of a tissue culture dish) to separate the cell type of interest from a heterogeneous popu-

lation of cells and then the primary culture is initiated. This immunoaffinity method has been applied to primary culture of canine and rabbit cortical collecting tubule.[52,70] The technique of immunodissection has been recently reviewed.[71]

Use of primary culture systems has led to further insights into proper conditions for culture to obtain differentiated biochemical and hormonal responses and for determination of transport by cell uptake techniques. Unfortunately, reports of transepithelial transport studies have generally been limited, probably, in part because these epithelia often are too leaky for such experiments. Improved cell culture methodology in the future may yield better preparations for transepithelial transport studies. An interesting technique to this end was developed by Gross *et al.*[72] In this system the capsular membrane of the kidney overlying the renal cortex of the rat is stripped and yields a preparation of cortical collecting tubule cells enriched in principal but not intercalated cells. These cells grow in culture to an epithelial monolayer of high PD and resistance which allows transepithelial ion transport and electrophysiologic studies of principal cells. The preparation may be particularly adaptable to study of hormonal regulation since the principal cell is a major target site for aldosterone and vasopressin in the cortical collecting tubule.

2.6. Hormonal Regulation of Transepithelial Ion Transport in a Tight Epithelium (A6 Cells)

2.6.1. General Characteristics of A6 Epithelia

A6 cells, a continuous line derived from kidney of *Xenopus laevis* by Rafferty,[73] grow in a monolayer of cuboidal cells which appear homogeneous by electron microscopy and have tight junctions and apical microvilli.[20] When grown on permeable supports (collagen-coated Nucleopore filters), they behave as tight epithelia with resistances of 5,000–10,000 ohm-cm^2. Basal short-circuit current (I_{sc}) is approximately 2 μAmp/cm^2 (0.08 μeq/cm^2) and increases four- to sixfold after 24-hr exposure to aldosterone (A). Basal and A-stimulated I_{sc} is amiloride inhibitable and equivalent to net apical-to-basolateral Na^+ fluxes (J_{ab}) as measured in Ussing chambers. Aldosterone stimulation of I_{sc} has the time course typical of a steroid; i.e., it begins at 1–2hr and peaks at approximately 6 hr.[62] Other hormones, which also act on the distal mammalian nephron, stimulate active Na^+ transport in A6 cells (*vide infra*).

The ease of measuring active Na^+ transport (e.g., with the in-hood short-circuiting device) and the responsiveness of A6 cells to aldosterone

have created interest in this renal-derived epithelium as a convenient tool for study of corticosteroid mechanisms in ion transport regulation. Thus, this cell line has advantages of intact epithelia but also capitalizes on the advantages of cell culture.

2.6.2. Corticosteroid Binding and Na^+ Transport Stimulation in A6 Cells

A6 cells grown on tissue culture dishes and incubated *in situ* with radiolabeled A and corticosterone (B) were hypotonically disrupted and rapidly filtered to yield a nuclear-enriched fraction. This technique allowed correlation of nuclear binding with active Na^+ transport under conditions similar to those in which transport studies were done. Two corticosteroid-binding sites were found in the nuclear-enriched fraction of A6 cells.[21] They were cooccupied by A and B with apparent dissociation coefficients (K'_d) for B and A of approximately 1.0×10^{-10} M for the high-affinity sites (type I) and 3×10^{-9} M and 3×10^{-8} M, respectively, for the lower-affinity sites (type II). Occupancy of the latter sites correlated with approximately 80% of the I_{sc} stimulation for both B and A. Cold-analog competition for binding to the lower-affinity sites indicated a 10-fold higher affinity for dexamethasone (Dex) and B than A, relative affinities for the three steroids similar to mammalian renal glucocorticoid or type II receptors.[74] Approximately 20% of I_{sc} stimulation correlated with occupancy of the high-affinity site. The latter sites may be analogous to type I or mineralocorticoid receptors in the mammalian kidney since they have the steroid specificity now proposed for type I sites in the rat from cytosolic binding sites performed in the absence of corticosteroid-binding globulin contamination,[75] i.e., $A \cong B >> Dex$. Also, recent studies on cells grown on filter-bottom cups show a K_d for A binding to this site of 5×10^{-10} M (C. O. Watlington, unpublished), which is quite in keeping with values from mammalian studies for the type I site.

Studies of cytosolic receptors in toad bladder[76] and nuclear binding in toad bladder cells in culture[77] yielded similar results in terms of K'_d for the two receptors, except that in the former occupancy of type I and II sites correlated with 45 and 55% of the I_{sc} increase, respectively. Spironolactone inhibits 100% of A-induced I_{sc} in A6 cells (R. L. Duncan, and C. O. Watlington, unpublished), as it does in toad bladder, suggesting that it is a competitive inhibitor for both type I and II sites. These studies in A6 cells and the toad bladder experiments described earlier raise the possibility that type II receptors in the mammalian kidney, as well as type I receptors, may (at least under certain conditions) mediate corticosteroid-induced Na^+ reabsorption. Spironolactones may not dis-

tinguish between activation of renal mineralocorticoid (type I) and glucocorticoid (type II) receptors. This possibility merits more study *in vivo* using selective corticosteroid antagonists.

2.6.3. A Unique Glucocorticoid Mechanism of Active Na^+ Transport Stimulation

In contrast to the initial findings, subsequent studies demonstrated a greater stimulation of I_{sc} by B than A, possibly due to change in the cells or method of culture.[78] Recently this finding has been confirmed for B, Dex, and cortisol (F); i.e., the glucocorticoids induce enhanced stimulation of I_{sc}, which, after 24-hr exposure to the steroids, is twice that produced by the maximal effective concentration of A.[79] The effects on I_{sc} produced by the three glucocorticoids are not additive, suggesting a common mechanism for the enhanced stimulation, in addition to the receptor-mediated process previously shown to be shared with A (types I and II). As noted earlier, S completely inhibits A-induced I_{sc} increase. The enhanced effect of the glucocorticoids is not inhibited by S, is inhibited by a glucocorticoid antagonist RU 486, and develops relatively slowly since it is minimal at 6 hr (R. L. Duncan and C. O. Watlington, unpublished). The EC_{50} for the enhanced I_{sc} stimulation by glucocorticoids, determined in the presence of the maximal effective concentration of A, is in the range expected to be achieved by ACTH administration and stress in mammals. For Dex, B, and F the EC_{50} is in the range of 3×10^{-8} to 3×10^{-7} M.[79] Preliminary studies of [^{3}H]B binding to the nuclear-enriched fraction of A6 cells were performed in the presence of a 500 × concentration of nonradiolabeled A, which should produce 98% occupancy of shared sites. Modest numbers of specific [^{3}H]steroid-binding sites were found in the 10^{-8} to 10^{-7} range. The possibility that glucocorticoids, at relatively high concentration, induce unique glucocorticoid receptors is being examined to explain the slow development of the enhanced effect on I_{sc}. There is precedence for this phenomenon in rat liver.[80] Thus, glucocorticoids stimulate active Na^+ transport by an additional mechanism to that shared with A. This effect could be mediated by receptors not previously described in A6 cells, renal or other epithelia.

Two polar metabolite fractions of B were found on chromatography of ethanol extracts of the nuclear-enriched fraction of A6 cells incubated in [^{3}H]B. The nuclear content of these derivative peaks was a saturable function of the concentration of B in the medium, suggesting that they may be agonists.[21] The major corticosterone (B) metabolite produced by A6 cells has been identified as 6β-OH-B and as one of the nuclear-bound metabolites of B. The metabolite increases I_{sc} (which is equivalent to net Na^+ flux), and this effect is additive to that of maximal effective con-

centrations of A.[78] It was speculated that 6β-OH-B may contribute to the enhanced effect of B on I_{sc}. Dex and F, like B, have been reported to be metabolized to 6β-OH-derivatives *in vivo* in mammals. Although 6β-OH-B induces antinatriuresis without kaliuresis in the rat,[81] the role of 6β-OH-glucocorticoid derivatives in the effects of the parent compounds on ion transport is uncertain at present.

Also of interest is the finding that 6β-OH-corticosterone is preferentially released into the apical (luminal) medium of A6 cells (R. L. Duncan and C. O. Watlington, unpublished). Although steroid 6β-hydroxylase activity has not been described in mammalian kidney (it is found in liver, gonads, adrenal, and placenta[78]), there is evidence to support this notion based on published values of urine and blood 6β-OH-F in humans. The concentration of 6β-OH-F in serum is 1/100 the F concentration, and yet five times as much free 6β-OH-F is excreted in the urine compared to free F.[82] Thus, the mammalian renal tubule may metabolize glucocorticoids to 6β-OH derivatives and preferentially release them into the tubular lumen to account for the relatively large quantity of free 6β-OH cortisol in human urine.

2.6.4. Synergism of Aldosterone and Insulin

Insulin (I), which stimulates active Na^+ transport in other epithelia and induces Na^+ retention in humans and other mammals, also increased I_{sc} (equivalent to net Na^+ flux) in A6 epithelia.[83] Although previous studies of insulin action in epithelia suggested a primary effect on the basolateral membrane, intracellular potential measurements in A6 epithelia revealed a decrease in apical membrane resistance associated with I-induced I_{sc} increase.[84] Concomitant A + I exposure stimulated I_{sc} by twice the sum of the increments induced by A and I separately.[62] Time course of I_{sc} stimulation demonstrated that the synergism began 80 min postexposure to insulin, suggesting the necessity of induction of protein synthesis for the A + I interaction. These findings were also consistent with the hypothesis that insulin, in addition to its own effects on Na^+ transport, may cause an amplification of the mechanism of aldosterone. The synergistic effect had an EC_{50} approximating the K_d of the type II nuclear binding site(s).[62] The I amplification of A's effect on active Na^+ transport may be by a postreceptor mechanism since insulin does not increase [^{3}H]A nuclear binding (C. O. Watlington, unpublished).

It was also found, in A6 epithelia incubated under open-circuit conditions for 24 hr with K-free medium initially placed on the apical side, that A stimulates apical accumulation of K^+, i.e., basolateral-to-apical (b → a) K^+ transport.[62] I potentiated this response. Results of experiments in which the basolateral K^+ concentration was varied were

found to be most consistent with b → a transport being stimulated by hormonally induced change in membrane transport parameters, as well as by an effect of increased transepithelial PD produced by these hormones. The relative role of these factors in K^+ transport stimulation induced by A and A + I was evaluated using ^{86}Rb to measure unidirectional K^+ fluxes (J_{ab}, J_{ba}). Under short-circuit conditions in Ussing chambers there was significant net J_{ba} or active K^+ secretion in A and A + I treated epithelia. Other studies demonstrated ouabain inhibition and thus Na^+ pump dependence of the A-induced secretion. In addition, both J_{ab} and J_{ba} were increased over controls by hormonal exposure, indicating that A also increases transepithelial permeability (R. L. Duncan, M. F. Fidelman, and C. O. Watlington, unpublished). There was no evidence of A + I synergism on K^+ permeability or secretion, as found in earlier studies.

Experiments requiring large numbers of epithelia, e.g., dose–response analysis, are preferably performed simultaneously on cells from the same seeding to reduce variability between seeding. Therefore, ^{86}Rb fluxes were determined under open-circuit conditions with incubations done in the incubator in clusters[6] rather than the Ussing chamber (see Section 2.3.2.). K^+ secretion was easily detected for flux ratios (J_{ba}/J_{ab}) of A, and A + I treated epithelia (paired by PD) were approximately 2.5 times greater than predicted using the Ussing criteria.[85] Thus, determination of flux ratio in the open-circuit state will be particularly useful for future studies, e.g., to evaluate whether the A-induced b → a K^+ secretion correlates with occupancy of the type I or the type II site.

The morphologic and thus topologic simplicity of A6 epithelia have attracted Fidelman and Mikulecky to apply network thermodynamic modeling to I, A, and A + I stimulation of Na^+ transport.[86] This model predicts the necessity for a two- to threefold increase in shunt permeability to ions as active Na^+ transport is stimulated by A and A + I under open-circuit conditions. Preliminary observations of ^{22}Na and ^{36}Cl fluxes under open-circuit conditions support the validity of this prediction. A and A + I produced an increase in the apparent permeability of Cl^- and Na^+ (in the passive direction), as calculated from the Goldman equation, which approximated the percentage change in shunt conductance predicted by the models (M. L. Fidelman and C. O. Watlington, unpublished).

2.6.5. Aldosterone and Na^+ Transport across the Apical Membrane

A increases active Na^+ transport in part by altering apical membrane Na^+ permeability via increasing the number of amiloride sensitive Na^+

channels (cf. Ref. 87). Sariban-Sohraby *et al.* demonstrated amiloride inhibitable apical Na^+ uptake in A6 epithelia grown on filters which was stimulated by A.[88] This report again emphasizes the importance of basolateral feeding, at least in tight epithelia, for apical Na^+ uptake was not inhibited by amiloride in epithelia grown in plastic tissue culture dishes. These same investigators then developed a plasma membrane vesicle preparation from cells grown in filter-bottom cups which was enriched 7–10 times with apical membrane markers.[17] Na^+ uptake in these vesicles was stimulated by pretreatment of cells with A and exhibited a similar K_i for amiloride as found for apical Na^+ uptake in the intact epithelium. Wiesmann *et al.* found that A selectively stimulated incorporation of methyl groups into phosphatidylcholine (PC) and proteins in toad bladder cells in culture.[89] A methylation inhibitor completely but reversibly blocked the I_{sc} response to A and inhibited PC methylation. They suggested that conversion of phosphatidylethanolamine (PE) to PC and alteration of the PC/PE ratio might alter membrane permeability or membrane enzymes, as previously proposed,[90] to mediate A's effect on apical membrane permeability to Na^+.[91]

These two groups of workers collaborated to evaluate this hypothesis in apical-enriched A6 membrane vesicles.[92] They found that the methyl donor S-adenosylmethionine stimulated amiloride-inhibitable Na^+ uptake to the same degree as A pretreatment, and the two effects were not additive. Methylation inhibitors blocked the Na^+ uptake induced by both the methyl donor and A pretreatment. Phospholipid and protein methylation from radiolabeled S-adenosylmethionine was demonstrated in vesicles and was blocked by methylation inhibitors. Thus, the vesicle studies demonstrated a direct effect of methylation on Na^+ transport, suggesting that methylation of apical membrane lipids and/or proteins contributes to the increase in apical Na^+ transport or permeability induced by A.

The apical membrane of A6 cells has also been studied by patch-clamp technique. The first report of single-channel recordings described a high-conductance anion channel with a permeability ratio for Cl^- to Na^+ of 9:1.[93] This channel was inhibited by SITS (a disulfonic stilbene). These studies were done on cells grown on plastic dishes, and the relevance to cells grown on filters with basolateral feeding is unclear. Hamilton and Eaton characterized Na^+ channels in A6 epithelia.[94] They deliberately used cells grown on plastic dishes to reduce the density of amiloride-sensitive Na^+ channels and described voltage-dependent behavior of the channel and the kinetics of its amiloride inhibition. These channels have a Na^+/K^+ selectivity of 3–4:1. In more recent studies these two workers have described another Na^+ channel with a higher selectivity ratio for Na^+ of 20:1 or greater, which is found in much

higher density in cells grown on filters than on dishes.[95] A increases the number of these more selective channels and not the less selective class of channels (D. C. Eaton, personal communication). Apical channels with similar characteristics to the more selective Na^+ channels in A6 cells were observed in cortical collecting tubules of mineralocorticoid-treated rats.

Sariban-Sohraby and Benos *et al.* successfully inserted the amiloride-sensitive channels from A6 apical-membrane-enriched vesicles into thin lipid membranes.[96] They recently reported 900-fold purification of this channel, a significant step forward for future biochemical characterization.[18]

2.6.6. Regulation of Na,K-ATPase and Other Proteins by Aldosterone

Increase in the activity of the sodium pump or Na,K-ATPase is considered as another major effect of A to stimulate transepithelial Na^+ transport, in addition to its effect on apical membrane Na^+ permeability. Numerous studies have shown increase in Na,K-ATPase activity in kidney by administration of mineralocorticoids. Two of the questions that have been addressed are (1) whether increased Na,K-ATPase activity is the result of increased synthesis of new units or activation of existent units, and (2) whether the increase in activity of Na,K-ATPase is a primary effect of steroid action or secondary to the increase in Na^+ entry into the cell as a result of increase in apical membrane permeability. The second question was first addressed in A6 cells utilizing ouabain binding as an index of the number of Na^+ pump sites.[97] Specific ouabain binding to whole-cell homogenates was increased 40% by 18-hr exposure to A. The increase was inhibited by simultaneous exposure to amiloride. It was concluded that the increase in Na^+ pump units available for binding was dependent on Na^+ entry into the cell. Johnson *et al.*[98] have found that Na,K-ATPase activity in the microsomal fraction of A6 cells increased after 18-hr incubation with A but not at 3 or 6 hr. In contrast to the study of ouabain binding, amiloride did not inhibit the enzyme activity increase. It should be noted that I, which alone had no effect on Na,K-ATPase, enhanced the activity increase produced by A. This latter finding is in keeping with A + I synergism on active Na^+ transport described earlier.

Rossier and co-workers are currently quite active in studying the aldosterone effects on Na,K-ATPase in epithelia. The production of antibodies to both subunits of Na,K-ATPase has and will allow them to perform elegant and detailed studies on steroid-induced Na,K-ATPase, its cellular processing and ultimate cell surface expression (cf. Refs. 99,100).

They have found that A induces radiolabeled amino acid incorporation into immunoprecipitable Na,K-ATPase in A6 cells, indicating increased enzyme synthesis.[101] These workers have discussed the possibility that A may not only increase synthesis, but also activate an inactive pool or existent ATPase units that exist either in the basolateral membrane or intracellularly as vesicles.[99] Thus, aldosterone-induced increase in Na^+ entry may activate existent Na,K-ATPase units yielding increased ouabain binding sites to explain amiloride inhibition of this phenomenon. However, the newly synthesized Na,K-ATPase may not be dependent on Na^+ entry, may be detected by the microsomal enzyme assay[98] and by immunoreactivity,[101] but not have been processed to the point where it is accessible to ouabain binding. Thus, these parameters of Na,K-ATPase increase would not be inhibited by amiloride.

Citrate synthase activity and synthesis is induced by A in renal cortical collecting tubule and other mammalian target tissues.[102] It has been proposed that this enzyme is not only a marker for activation of mineralocorticoid receptors, but that it may be an important enzyme in supplying energy to the Na^+ pump to increase pump activity. Johnson and Green showed that activity of citrate synthase is not increased by A in A6 cells or toad bladder lines and concluded that activation of this enzyme is not necessary for A-induced increase in active Na^+ transport.[103] Rossier *et al.* have confirmed these findings in A6 cells.[104]

The two enzymes described here are the only aldosterone-induced proteins (AIPs) that have been identified. Numerous AIPs have been found in toad bladder by stimulation of amino acid incorporation into proteins separated on one- or two-dimensional SDS–polyacrylamide gel electrophoresis (SDS–PAGE) in toad bladder (cf. Ref. 105). Handler *et al.* reported the first studies in A6 cells using SDS-PAGE and identified two AIPs in the 200,000 g pellet and one in the supernatant.[97] Rossier *et al.* recently reported AIPs as well as proteins whose synthesis was suppressed by A in A6 cells.[104] There is little doubt that A6 cells will be an increasingly important tool for study of the mechanism of A's effects, primary and secondary, on the many enzymes and other proteins involved in the stimulation of ion transport.

2.6.7. Other Studies in A6 Cells

Vasopressin, PGE, adenosine, and isoproterenol stimulate active Na^+ transport in A6 cells.[9,106,107] Activators of protein kinase C such as phorbol esters inhibit Na^+ transport[108] and such as vasopressin[109] stimulate active Cl^- secretion. Active Cl^- secretion has also been induced by hormones in MDCK cells.[23]

The issue of regulation of plasma membrane polarity has been ad-

dressed in A6 cells in studies of mobility of fluorescent lipids incorporated into the membranes.[110] Monoclonal antibodies against apical membrane antigens have been used to assess polarity of processing of apical membrane constituents.[111]

2.7. The Future

It is clear from the information reviewed here that renal epithelia in culture have and will continue to produce many new insights using the variety of techniques previously applied to the kidney *in vivo* and to intact epithelia *in vitro*. However, application of approaches from the field of cell and molecular biology to cultured renal epithelia offers tremendous potential for progress in our knowledge in renal biochemistry and physiology and in the pathogenesis of renal disease.

3. Role of Liver and Kidney in Acid–Base Homeostasis

3.1. Introduction

The traditional approach to acid–base balance has been to assign a prominent role to the kidney for the generation of bicarbonate ions consumed in the metabolism of certain amino acids. The generation of these bicarbonate ions is linked to synthesis and excretion by the kidney of ammonium ions derived from glutamine. The process of ammonium synthesis and excretion is augmented markedly in states of acute and chronic metabolic acidosis. In contrast, the liver has not been viewed as an organ important in acid–base homeostasis. Ureagenesis, an important metabolic function of the liver, has been traditionally considered a means of disposing of nitrogen resulting from amino acid catabolism. Recently, however, these traditional views have been questioned with the suggestion that the liver, rather than the kidney, is more important with respect to acid–base balance. In the subsequent section, some of the data will be reviewed addressing the questions. Several reviews can be referred to for more detailed analysis.[112–117]

3.2. Traditional Views

As pointed out by Halperin and Jungas,[112] the traditional view with respect to the kidney's role in acid–base balance is only partially true. The partial truth relates to the view of ammonium excretion as being equated with hydrogen excretion and hence bicarbonate generation. It was held that ammonia formed from the deamidation of glutamine by glutaminase and deamination of glutamate by glutamate dehydrogenase

within renal cortical mitochondria passed out of cells and into the tubular lumen. There, NH_3 combined with secreted H^+, forming the poorly permeable ammonium ion (NH_4^+); the excreted ammonium represented hydrogen ions that had been titrated by NH_3.

Several investigators have recently agreed that this explanation, utilizing nonionic diffusion trapping as an explanation for proton excretion,[118,119] cannot be considered the correct explanation.[112,115,117] Since the pKa of ammonium/ammonia is 9.3, the product of the glutaminase and glutamate dehyrogenase reactions is virtually entirely NH_4^+ rather than NH_3. Thus, the transport of this NH_4^+ into the tubular lumen, even if it occurs by a process involving dissociation into NH_3 and H^+, does not result in the *net* excretion of a hydrogen ion. As Halperin and Jungas point out,[112] and as had previously been suggested by Oliver and Bourke,[120] two factors are necessary in order for hydrogen ions to be disposed of: (1) metabolic disposal of the carbon skeleton resulting from glutamine, namely 2-oxoglutarate, by either reduction or decarboxylation reactions within renal tubular cells, and (2) excretion of the ammonium resulting from glutamine degradation. Regardless of whether the end product of glutamine metabolism is CO_2 and H_2O, via metabolism through the TCA cycle, or glucose production, two protons are removed for each glutamine utilized. It is the metabolism of the 2-oxoglutarate, rather than the actual ionic trapping of NH_4^+, which results in the removal of hydrogen ions. The above-mentioned workers who have addressed this point seem to be in agreement that the metabolism of the resultant 2-oxoglutarate represents the critical step(s) in disposal of protons rather than the actual excretion of ammonium. Although the oxidation could occur in any organ,[117] there is no evidence for significant net release of glutamate or oxoglutarate by the kidney, indicating that oxoglutarate metabolism occurs in the kidney. Excretion of the ammonium ion is important, but primarily to prevent its recirculation to the liver where it may be in incorporated into urea, which is a bicarbonate-consuming process (see Section 3.3).

3.3. Amino Acid Metabolism—Net Generation of Acid or Base?

Traditionally, the metabolism of dietary amino acids has been considered to generate nonvolatile acids, such as H_2SO_4 and HCl. This acid load is disposed of by processes involving renal glutamine metabolism and ammonium excretion (see Refs. 112,113). Recently, this view has been questioned. Atkinson, Bourke, and co-workers [114,115,121] contend that since the oxidation of compounds containing carboxylate ions, such as many simple amino acids, yields an amount of HCO_3 equimolar with the carboxylate, over 1 mole of bicarbonate is generated from the me-

tabolism of a typical daily human intake of 100 g of protein. Thus, these workers view the need for disposal of bicarbonate as essential to prevent the development of progressive metabolic alkalosis. The mechanism by which the excess bicarbonate is disposed is by incorporation into urea, suggesting a prominent role for the liver in the maintenance of acid–base homeostasis.[115,116] In contrast to this large amount of bicarbonate, the generation of nonvolatile acids by metabolism of primarily sulfur-containing and cationic amino acids is viewed by these workers as relatively insignificant.[115]

It had been pointed out several years previously by Oliver and Bourke[120,122] that urea synthesis, involving the consumption of bicarbonate ions, may be altered under metabolic acid–base conditions. For example, when HCl acidosis was induced in the rat, a significant decrease in urea excretion resulted, with a simultaneous rise in ammonium excretion such that no change in total nitrogen balance was evident. Although, as will be discussed in Section 3.4, there is now a mounting body of evidence to indicate that alterations in hydrogen ion balance may affect hepatic metabolism, the controversy over whether bicarbonate is an end product of amino acid metabolism is in part a matter of semantics. Halperin and Jungas[112] and Walser[117] indicate that there is no controversy with respect to resultant bicarbonate and ammonium ions from amino acid metabolism. Halperin and Jungas maintain a different view of what constitutes a metabolic pathway. Utilizing the proposal of Newsholme and Crabtree[123] that a metabolic pathway is defined as the conversion of a substrate into its final end product, and is not confined to a specific organelle or organ, they argue that it is artificial to separate the resulting bicarbonate and ammonium from amino acid metabolism and the incorporation of these ions into the neutral urea molecule. Rather, these workers suggest, according to the traditional approach, that the pathway starting with amino acids and ending with neutral urea yields no net acid or base gain. Walser[117] also supports the traditional view by pointing out that existent data indicate that amonium ion concentration appears to be a major, if not the primary, regulator of urea synthesis rather than pH[117] (cf. Ref. 124, *vida infra*). Although he concedes that total cessation of urea synthesis may result in accumulation of bicarbonate ions in extracellular fluid, he views the hyperammonemia accompanying bicarbonate retention as the more hazardous and one that would lead to consequences prior to the development of significant alkalosis. This latter point seems to beg the question in that it does concede some role to the liver for acid–base balance. Similarly, Halperin and Jungas[112] point out the importance of ammonium excretion to prevent the utilization of bicarbonate, equivalent to proton generation, if ammonium returns to the liver and is incorporated into urea.

In an extensive recent analysis, Walser[117] approaches the problem by careful characterization in terms of ions "whose concentrations change at the same time as the change in bicarbonate concentration. There must be an accompanying change in the concentration of one or more other anions or cations." Walser concludes his analysis by stating that a significant role of the liver in regulation of acid–base balance has not yet been clearly demonstrated. However, it must be emphasized that a mounting body of evidence has accumulated to suggest a possible important role for the liver in acid–base homeostasis; this role would nicely complement, rather than exclude, a role for the kidney according to the traditional views summarized earlier.

3.4. Effect of Acid–Base Balance on Hepatic Metabolism

Data have been obtained from several laboratories suggesting that hepatic glutamine metabolism is altered by changes in acid–base balance. In 1970, Lueck and Miller[125] demonstrated that perfused rat livers utilized only half as much glutamine at pH 7.15 as at pH 7.45. The reduction in glutamine utilization at acid pH correlated with a decreased production of urea and of $^{14}CO_2$ from labeled glutamine. As mentioned earlier, Bourke and associates demonstrated a reduction in urea excretion in HCl acidosis in the rat[120] and a decreased urea production with increased net glutamine synthesis in the isolated perfused rat liver preparation at acid perfusate pH values.[122] Bean and Atkinson[121] have also demonstrated a reduction in urea synthesis at reduced ECF pH.

The most comprehensive analysis has resulted from a series of studies reported from the laboratory of Haussinger and associates. Haussinger *et al.* had shown, utilizing the isolated perfused rat liver preparation, that a decrease in perfusate pH reduced the flux through glutaminase with glutamine as substrate.[126] In subsequent studies, these workers demonstrated a reciprocal alteration in glutamine synthesis and glutamine degradation plus urea synthesis. That is, at reduced pH there was a decrease in glutaminase flux and urea synthesis with an increase in net glutamine release, consequent to an increase in flux through glutamine synthetase.[127] Glutaminase flux was also increased by ammonium ion and glucagon. The simultaneous activity of glutaminase and glutamine synthetase fluxes were subsequently explained by experiments demonstrating an intercellular glutamine cycle in rat liver[128,129] (see Ref. 116). In brief, these studies, also performed with the isolated rat liver perfused in both the antegrade and retrograde directions, demonstrated geographic separation within the liver parenchyma of glutaminase and ureagenesis from glutamine synthetase. The data indicated that the cells in the periportal region contained glutaminase and enzymes of the urea

cycle plus carbamoylphosphate synthetase, while cells in the perivenous region contained glutamine synthetase. These workers have investigated a variety of effectors of the reactions in these two separate areas within the liver, but of particular interest are the studies involving effects of pH. Their studies are compatible with the observation that glutaminase flux is reduced by acid pH, which Verhoeven *et al.*[130] have demonstrated is due to a decreased affinity of glutaminase for ammonia.

More recently, utilizing the carbonic anhydrase inhibitor acetazolamide, Haussinger and Gerok[131] have provided evidence for both carbonic anhydrase-dependent and -independent urea synthesis within the rat liver. They have demonstrated a carbonic anhydrase dependence of urea synthesis which is strongly affected by ECF pH. In the absence of carbonic anhydrase activity, urea synthesis was a function of the supply of HCO_3 and CO_2, but not pH. These workers utilized these findings to provide an explanation for the reduction in ureagenesis in metabolic acidosis, also observed by others,[132] while only a small effect is seen in respiratory acidosis; under the latter circumstances, at least in the chronic state, an increase in total HCO_3 plus CO_2 addition to portal blood may maintain urea synthesis at an elevated level despite the reduction in systemic pH. Further work by Meijer and colleagues[133–135] has demonstrated that the urea cycle enzymes themselves are not pH sensitive. Rather, at least under conditions in which ammonium supply is constant, much of the regulation appears to occur at the level of carbamoyl phosphate synthetase. This, in turn, may relate to the sensitivity of this enzyme for HCO_3 plus CO_2 and the influence of carbonic anhydrase, which in the liver has significant activity within the mitochondrial matrix.[136,137]

3.5. Summary and Conclusions

A recent report by Cohen *et al.*[124] has convincingly demonstrated that NH_3 rather than NH_4^+ is the substrate of the enzyme carbamoyl phosphate synthetase. Moreover, based on data obtained by these workers and evaluation of the literature, the steady-state concentration of NH_3 in liver is likely to be well below the apparent K_m.[124] Since at a given total ammonium/ammonia content NH_3 would be expected to decrease in acidosis, decreased flux through carbamoyl phosphate synthetase could result and explain the observed reduction in urea production.

These composite data indicate that the extreme views of the kidney or liver alone being primarily important in acid–base homeostasis are no longer tenable. Rather, the data suggest that the two organs function in a complementary fashion with regard to acid–base balance. Future

studies that will further elucidate the detailed mechanisms and interactions are eagerly awaited.

4. Polyphosphoinositides and Diacylglycerol as Second Messengers

In an extremely short period of time, a large body of evidence has accumulated to provide a firm foundation for the role of polyphosphoinositides and diacylglycerol as second messengers in hormone, neurotransmitter, and other agonist functions at the level of the cell membrane. The reader is referred to several excellent and comprehensive reviews on this topic,[138–140] which will be covered only briefly at this time. As reviewed in these articles, a bifurcating signaling system at the cell membrane has been defined which results in a diverse network of intracellular second messengers, acting via changes in intracellular calcium as well as pH, to control a wide variety of cellular processes. The extensive data indicate that hormones and other agonists activate a cellular cascade by action on its receptor to stimulate the hydrolysis of polyphosphoinositides by phosphodiesterase, yielding diacylglycerol (DG) and inositol trisphosphate. The latter appears to result in an increase in cytosolic free-calcium levels by mobilization of calcium from intracellular reservoirs, probably from the endoplasmic reticulum. Subsequently it is believed that the increased cytosolic calcium acting via calcium/calmodulin results in protein phosphorylation, which in turn elicits a cellular response. The other arm of the agonist stimulation of phosphodiesterase hydrolysis yields diacylglycerol, which in turn has been shown to activate protein kinase C. Protein kinase C, which is also activated by the tumor-promoting phorbol esters as experimental probes, elicits cellular responses via protein phosphorylation. The second-messenger action of diacylglycerol is terminated by either phosphorylation of DG to phosphatidic acid or hydrolysis by a diacylglycerol lipase to monoacylglycerol and, often, arachidonic acid. The latter has been implicated in affecting guanylate cyclase-catalyzed conversion of GTP to cyclic GTP with subsequent effects on hormonal action.

Of particular interest has been evidence to suggest that diacylglycerol via protein kinase C activates the Na^+/H^+ exchanger in plasma membranes. This appears to be particularly important with respect to various growth factors such as epidermal growth factor, platelet-derived growth factor, and other mitogens (see Ref. 139). A growing body of evidence suggests that various mitogens affect growth by virtue of a stimulation of Na^+/H^+ exchange, leading to intracellular alkaliniza-

tion.[141–151] Most studies reported to date have been performed with fibroblasts or tissue from other organs than the kidney. However, in a recent report evidence was presented in support of stimulation of the Na^+/H^+ antiport in hypertrophy of renal proximal tubular cells. The extensive studies mentioned previously evaluated the role of this antiport, stimulated through activation of protein kinase C, and mitogenesis. However, this represents the first report in which stimulation of the antiport, and possibly intracellular alkalinization or enhanced cytosolic sodium content, stimulate hypertrophy as opposed to hyperplasia.[152] Rogers *et al.* have demonstrated the use of phorbol esters in evaluating the effect of intracellular alkalinization on gluconeogenesis[153]; phorbol esters inhibited gluconeogenesis. Additional recent reports indicate that more work will be done to establish the important role of inositol triphosphate and diacylglycerol as second messengers in a variety of cellular functions involving the kidney.[154–159]

5. Renal Ischemia and Anoxia

5.1. Introduction

The kidney is very susceptible to ischemic insult.[160–162] Postischemic acute renal failure, the syndrome resulting from persistent ischemia, has been extensively investigated. This section will focus on the energy metabolism of the cell and how various perturbations caused by ischemia and anoxia interfere with this metabolism and lead to cellular dysfunction. Although acute renal failure (ARF) can also be caused by various nephrotoxins, drug-induced ARF will not be addressed here. However, the similarities in the biochemical and morphologic lesions in these two models are striking.[163,164]

A variety of perturbations in the biochemistry of the ischemic cell has been described. Of particular interest is the limited oxygen and substrate availability to the ischemic cell and its influence on the regulation of cellular ATP and adenine nucleotide content. An enhanced entry of calcium into the ischemic and postischemic cell is also a well-recognized event which leads to membrane disruption due to calcium-activated phospholipases. Membrane disruption also occurs through free-radical reactions. The role of these events in the pathogenesis associated with renal ischemia and anoxia will be discussed. Since the tripeptide glutathione is an important defense mechanism of the cell against free-radical-mediated membrane damage, a discussion of the role of glutathione in ischemia is also included. When possible, specific references will be made to studies of renal ischemia. However, since much of our current understanding of the events during ischemia have come from

studies of the heart and liver, a limited discussion of these studies is also included. Many good reviews have appeared that detail other aspects of renal ischemia and ARF.[161,163,165–167]

5.2. Renal Oxygenation

The primary insult to the kidney during ischemia and hypoperfusion is usually considered to be due to inadequate tissue oxygenation.[160,161,168] This is initially surprising considering that the kidney's requirement for oxygen decreases with a decreasing flow rate. However, ARF is a common complication of various conditions associated with renal hypoperfusion.[161]

Despite the normal oxygen supply and perfusion conditions of the *in vivo* kidney, PO_2 measurements in the inner medulla range from 1 to 10 mm Hg.[169,170] This oxygen tension is considerably lower than that measured in renal venous blood.[169] The low medullary PO_2 arises from A-V shunting of oxygen,[171] which presumably occurs across the limbs of the vasa recta. The low oxygen tension does not allow for a complete reduction of cytochrome aa_3 of the respiratory chain, which could limit respiration in certain areas of the kidney. In studies of the isolated perfused kidney (IPK) of the rat, the reduction state of aa_3 is even lower.[172] Such studies of the IPK model have been instrumental to the present understanding of the delicate relationship between renal tissue oxygenation and function.

The IPK is most often perfused in the absence of any substance to increase the oxygen content of the buffer. The GFR and fractional sodium reabsorption are characteristically lower than that measured in the *in vivo* kidney.[173] This deficiency, in part, can be reduced by the inclusion of glucose[174] and a mixture of amino acids[175] in the perfusate. However, early studies by Franke and co-workers[176,177] suggested that poor tissue oxygenation was also involved. They observed that perfusion with a perfluorocarbon-supplemented media, which increases the perfusate oxygen content, enhanced renal function and reduced the extent of tissue damage. Alcorn *et al.*[178] identified a morphologic lesion in the medullary thick ascending limb (mTAL) that occurs upon isolated perfusion of the rat kidney. More recently, Brezis and associates[179,180] and subsequently Schurek and Kriz[181] demonstrated that the occurrence of the mTAL lesion was related to the balance between the oxygen delivery to the IPK and the mTAL requirement for oxygen (i.e., its metabolic demand). The lesion is completely absent in kidneys perfused with ouabain and in kidneys perfused with hyperoncotic media to suppress GFR.[180] Other studies have shown that the loop diuretic furosemide increases the reduction state of aa_3 in the IPK[172] and attenuates the mTAL lesion.[180]

The incidence of the lesion is also reduced if the oxygen content of the perfusate is increased with inclusion of either erythrocytes, hemoglobin, or perfluorocarbon emulsions.[179,181] Brezis and associates have proposed that the mTAL region is highly susceptible to damage because it normally operates on the brink of anoxia due to the A-V shunting of oxygen.[161,182] The abundance of mitochondria[183] in this region of the nephron is testimony to its high rate of aerobic metabolism. Furthermore, because NaCl reabsorption in the mTAL occurs against an osmotic gradient, it requires a greater expenditure of energy than in the "leaky" proximal tubule[183,184] (but see Ref. 185).

Anoxic lesions to the mTAL have only recently been implicated in the etiology of postischemic ARF.[161] More extensive damage probably occurs in the proximal tubule,[186] but this region does not appear to be as susceptible to anoxic injury as is the mTAL.[161] Studies of the unilateral clamp model of ARF by Venkatachalam *et al.*[187] describe the sequence of morphologic changes in the proximal tubule during the reflow period of postischemic ARF. Briefly, upon reflow there is a rapid loss of brush border microvilli integrity. Segments are both interiorized into the cell cytosol and shed into the nephron lumen.[161,186–188] Nephron obstruction[187,188] and elevated proximal tubule pressures[189] are well-documented manifestations of renal ischemia. The S_3 segment was found to be most susceptible.[187,188] It is of interest that approximately half this portion of both cortical and juxtamedullary nephrons extends into the medulla[188] (see also Ref. 181).

One of the first morphologic changes seen after ischemia in the proximal tubule and mTAL cells is swelling and discription of mitochondria.[160,178,187] Biochemically, the first change noted is a rapid decrease in the tissue ATP content.[160,168] This effect is undoubtedly due to the rapid cessation of oxygen and, to a lesser extent, substrate delivery to the tissue causing a suppression of oxidative phosphorylation. In the rat, within 30 sec of renal ischemia, ATP content drops to 31% of control values and AMP increases about threefold.[168] The dramatic rise in the tissue AMP content is due to the shift in the equilibria of the adenylate kinase reaction caused by the drop in the ATP/ADP ratio.[190] The accompanying increase in the level of inorganic phosphate leads to activation of AMP deaminase and 5′-nucleotidase. This causes a lowering of the total adenine nucleotide pool (ATP + ADP + AMP) and dramatic increases in the tissue content of adenosine, inosine, and hypoxanthine. After 30 sec of ischemia, the tissue contents of each of these intermediates increase about threefold. After 10 min, they are increased by 7.4-, 31-, and 90-fold, respectively, above control levels.[191] At this point, mitochondrial and cellular damage are completely restored if reflow occurs.[192] Only after about 1 hr of warm ischemia is the kidney unable to

recover.[186,193] Despite extensive catabolism of the adenine nucleotides,[191] complete recovery of the kidney occurs if reflow is permitted prior to 1 hr of ischemia. Therefore, the drop in the tissue ATP and total adenine nucleotide content *per se* is thought not to be the primary cause of ischemic tissue damage.[194]

Although this argument seems to be well founded, it is doubtful that ARF would develop if the kidney possessed a mechanism for maintenance of tissue ATP levels during ischemia. The kidney, unlike heart and liver, contains a poor supply of glycogen.[168,195] This, in part, accounts for the rapid fall in kidney ATP levels in ischemia.[168] It is of interest that severe ischemic tissue damage in the heart only occurs after depletion of its glycogen stores and cessation of lactate production.[196,197] Moreover, the decrease in function of the isolated perfused rat kidney is attenuated if substrates are added to facilitate substrate-linked phosphorylation.[198] More likely, the decreased ATP content of the ischemic kidney allows for a host of secondary deleterious events (see Section 5.2) that are slower in onset.

In an attempt to reestablish tissue ATP levels following hemorrhagic shock, Chaudry *et al.* discovered that intravenous infusion of ATP together with magnesium chloride ($ATP.MgCl_2$) significantly increased the animal survival rate[199] and the ATP content of the liver and kidney.[200] Administration of either ADP or AMP, alone or with $MgCl_2$, was without effect.[199,200] The authors suggested that the mechanism was not due to vasodilatation since the magnesium salts of ADP and AMP are more potent dilators than that of ATP.[199] Furthermore, administration of either $MgCl_2$ or ATP alone is without effect, although both agents are vasodilators.[201] The beneficial effects of $ATP.MgCl_2$ on the recovery of the postischemic kidney[202–205] and liver[206] have now been demonstrated in many laboratories. The treatment results in a faster recovery of tissue ATP content[202–206] and blood flow[203,205,206] in both organs. In the rat IPK model, this treatment was shown to result in a more rapid recovery of intracellular pH as determined by the shift in the orthophosphate resonance peak using nuclear magnetic resonance techniques.[203] The degradation rate of perfusate ATP by the IPK has been determined by Sumpio *et al.*[205] Their data indicate a catabolic rate of about 1.4 μmoles/min per kidney. Interestingly, the same rate was obtained in postischemic and control kidneys.

Although the mechanism responsible for the $ATP.MgCl_2$ effect is not known, two theories have been proposed. The first is that ATP is able to gain access to the cell cytosol and directly contribute its energy of hydrolysis to the work functions of the cell.[207,208] Although this has not been convincingly demonstrated, Chaudry and co-workers cite evidence obtained in other studies for the ability of ATP to cross the plasma

membrane.[207] Alternatively, ATP, or one or more of its hydrolysis products, provide the necessary carbon backbone to "spark" the resynthesis of cellular adenine nucleotides.[209] Regardless, the treatment appears to be clinically relevant. In a randomized trial of patients who developed ARF due to ischemic insult, treatment with $ATP.MgCl_2$ resulted in a significant increase (100% versus 73.3%) in patient survival versus non-treated controls.[210] Further research into the mechanism of this effect should provide additional information regarding adenine nucleotide metabolism in the postischemic kidney.

5.3. Calcium

Calcium is known to be involved in a wide range of metabolic events. However, this discussion will be limited to a survey of the pathogenic processes that are initiated by calcium during ischemia which cause a disruption of cellular function.

Calcium is compartmentalized almost exclusively to the extracellular space. The extracellular-to-cytosolic calcium concentration gradient is about three to four orders of magnitude, and thus there exists a large driving force for calcium entry into the cell. The cytosolic free-calcium content of rabbit proximal tubules has been estimated in two studies by Murphy and Mandel. They obtained values of 0.45[211]; and 0.32 μm[212] using the null-point method. More recently, Bonventre and Cheung[213] estimated a concentration of 0.10 μm in cultured LLC-PK_1 cells using both the null-point procedure and the trapped dye method with Quin-2. This low value is maintained by a calcium-pumping ATPase and by an electrogenic calcium-sodium exchange system in the plasma membrane.[214,215] Both systems are thus linked, either directly or indirectly via the Na,K-ATPase, to the hydrolysis of ATP. How the cells responsible for reabsorption of luminal calcium maintain a low cytosolic calcium content is not known.

It is not clear whether calcium leaks into the cytosol during renal ischemia. Even in heart and liver, where calcium movements during ischemia have been more extensively investigated, controversy exists. In the ischemic rat heart, Shen and Jennings[216] found no accumulation of calcium until reflow was permitted. Reflow after 40 min of ischemia led to calcium accumulation that was primarily localized within mitochondria. Accumulation of calcium during reflow was also observed by Jennings and Ganote.[217] However, Cheung *et al.*[218] did not observe calcium accumulation by cultured myocytes when submitted to an ischemic model. In this model, myocytes were incubated with no substrates under anoxi conditions. Reoxygenation was also not associated with increased cell

calcium despite significant cell injury. In a similar model of renal anoxia using isolated rabbit proximal tubules, hypoxic conditions caused a two-fold accumulation of calcium.[219]

Several studies have shown an increased calcium content in mitochondria isolated at various time points after reflow to the ischemic kidney. This occurs because of the decreased ability of the cell to extrude calcium, owing to the lowered cell ATP content and a disruption of plasma membrane integrity, and to the tendency of mitochondria to concentrate available calcium within the matrix space. Each of these will be discussed in turn.

Mitochondria possess separate influx and efflux pathways for calcium movement (for review, see Refs. 220,221). Influx is through an electrophoretic carrier that displays a low K_m and high V_{max} for calcium, whereas efflux occurs through an exchange with sodium that display a low K_m and low V_{max} for calcium. If available at concentrations greater than when the rates of influx and efflux are equal (the so-called set-point, approximately 1 μm), influx greatly exceeds efflux, and rapid accumulation occurs. It has long been known that mitochondria will accumulate in preference to phosphorylation of ADP.[222] This, in effect, causes a temporary uncoupling of respiration and phosphorylation. In the absence of ATP, excessive calcium accumulation (> 80–100 nmoles/mg) leads to rapid and irreversible inner mitochondrial membrane damage in which coupled phosphorylation is not possible.[223]

Calcium accumulation and the loss of plasma and inner mitochondrial membrane integrity are related through a positive feedback cycle. The accumulation of calcium leads to activation of phospholipases, the most thoroughly studied being phospholipase A.[176,224,225] This enzyme is located in mitochondria, and the products of the reaction, free fatty acids and acyl lysophospholipids, are known to be deleterious to lipid membranes. Low concentrations of these agents cause a marked increase in membrane permeability to calcium.[226] Changes in the phospholipid and lysophospholipid content of the ischemic and postischemic rat kidney [227,228] and dog heart[229] are consistent with an enhanced phospholipase activity. These mitochondrial events are associated with a collapse of the membrane potential, uptake of hydrogen ion, large amplitude swelling, and release of accumulated calcium.[223–225]

Several investigators[193,230–232] have examined the functional integrity of mitochondria after isolation from tissue subjected to ischemia of varying duration and after postischemic reflow. Integrity was assessed by measurement of their ability to perform coupled respiration, by estimation of the acceptor control ratio (ACR), or by their ability to accumulate calcium. Mergner *et al.*[193] have shown that the ACR drops

almost linearly, with respect to time, during the first 30 min of ischemia from about 3.5 to 1.2. These changes are correlated with an increased swelling rate[231] and a decreased ability of the isolated mitochondria to accumulate calcium.[232] Mitochondria isolated after 45 min of ischemia followed by 1 hr of reflow display a higher ACR than mitochondria isolated without reflow[230] and a modest 20% increase in mitochondrial calcium. However, after 24 hr of reflow, mitochondrial calcium was increased to about threefold above the levels obtained without reflow, and this was associated with a dramatic fall of the ACR to values similar to those obtained without reflow.[230]

Weinberg and Humes[233] demonstrated that isolated cortical mitochondria display a more rapid deterioration of respiratory function if allowed to accumulate calcium during the isolation procedure. Recently, Arnold *et al.*[234] estimated that approximately half of the respiratory dysfunction of mitochondria isolated after renal ischemia is due to the calcium accumulated during the isolation procedure. These and other related studies[223,235,236] strongly suggest that the accumulation of calcium by mitochondria is directly related to their degree of functional impairment.

Several studies have tested the ability of verapamil to improve postischemic renal function. This agent blocks the voltage-dependent calcium channel in the plasma membrane and has no effect on the calcium-pumping ATPase.[237] When infused into the renal artery of the dog following 1 hr of ischemia, verapamil caused an increase in GFR and urine flow rate.[238] In a rat model where 45 min of ischemia was used after contralateral nephrectomy, verapamil improved the animal survival rate and lowered the plasma creatinine level.[200] It has also been shown to delay the decrease in respiration rates (state 3) and prevent the lower rates of calcium uptake by mitochondria isolated after 30 min of renal ischemia in the guinea pig.[192] Similar effects on mitochondrial respiration rates have been noted in a canine ischemic model produced by intrarenal norepinephrine infusion,[239] although the use of verapamil in this model has been questioned.[192,237]

Verapamil blocks the constrictor effect of angiotensin II.[240] Since other methods of angiotensin II blockade attenuate nephrotoxic ARF,[241] angiotensin II blockade has been suggested as one possible mechanism of verapamil action in the postischemic kidney.[241] A second mechanism could be by limiting calcium availability to mitochondria. In this regard, Widener and Mela-Riker[192] found no effect of verapamil on the calcium content of the postischemic kidney, although the function of isolated mitochondria was preserved. Of interest, this study noted a decrease in the tissue magnesium content that was prevented by verapamil treatment.

5.4. Free Radicals

Oxygen-derived free radicals are potent mediators of tissue injury and are continually generated in the body. However, they have only recently been shown to be involved in the pathogenesis of tissue injury due to ischemia. Their role in ischemia was initially dismissed, largely because it was believed that the hypoxic conditions of ischemia and hypoperfusion would preclude their role in tissue damage.[242] However, evidence for their role in ischemia has steadily accumulated since the demonstration of their involvement in the ischemic rat heart[243–245] and intestine.[246,247]

Several reviews have recently appeared that detail the chemistry of oxygen-derived free-radical production.[248–251] Briefly, about 98% of cellular oxygen consumption is linked to the mitochondrial respiratory transport system in which oxygen is completely reduced (i.e., tetravalent) to water.[252] In this system, only minor amounts of the intermediates, superoxide (monovalent) and hydrogen peroxide (divalent), are released into the surrounding media.[250] However, there are now known to be about a dozen oxidases in the cell that utilize oxygen directly with the concomitant production of either the superoxide radical or hydrogen peroxide.

The superoxide radical is a good reductant and a fair oxidant that can readily attack cellular components.[242] Its removal is normally through a spontaneous dismutation to hydrogen peroxide and water. This reaction is also catalyzed in the cytosol, the mitochondrial intramembranous space, and the mitochondrial matrix by the enzyme superoxide dismutase (SOD).[253] Hydrogen peroxide, which in itself is not very deleterious to the cell,[251] is removed by intramolecular dismutation to oxygen and water by the enzymes catalase and glutathione peroxidase. Catalase is located in the cytosol and in high concentrations in the peroxisomes. Glutathione peroxidase also catalyzes the reduction of hydrogen peroxide as well as many other organic peroxides to their corresponding alcohol using glutathione as a reductant. The enzyme is located in the cytosol and mitochondrial matrix.[254]

The detrimental feature of hydrogen peroxide is its ability to form the highly reactive hydroxyl radical either directly, through catalysis by iron or copper salts, or by reaction with the superoxide radical via an iron-catalyzed Haber–Weiss reaction.[251,255] The hydroxyl radical is a powerful oxidant that will react with everything in its immediate environment.[250] The reactions of these radicals in the cell, some of which are considered below, are numerous.

The unsaturated fatty-acid moieties of membrane phospholipids are particularly vulnerable to peroxidation through attack by hydrogen per-

oxide and the hydroxyl radical.[256] This process proceeds through a chain of free-radical reactions producing various intermediates of lipid peroxide radicals, lipid hydroperoxides, and other lipid fragments which are themselves active oxidants.[257,258] Many studies illustrating the disruptive nature of the reactions of free radicals with lipids and lipid membranes have been performed *in vitro* using artificial liposomes or erythrocyte membranes.[257,259,260] They have shown that free-radical-mediated lipid peroxidation leads to an accumulation of a few characteristic lipid-derived products, most notably malondialdehyde, fatty-acid diene conjugates, and lipid hydroperoxides. Evidence that these products result from the presence of specific free radicals or hydrogen peroxide comes indirectly from the ability of added free-radical scavengers, SOD, or catalase to inhibit the formation of these products. Studies using the hamster cheek pouch model, where the microvasculature is visible, have demonstrated marked alterations of microvascular permeability due to free radicals.[256]

Mitochondria are known to undergo a loss of functional integrity if exposed to a superoxide radical generating system[261] or to hydrogen peroxide.[262,263] This loss is associated with the production of conjugated dienes and is prevented with the addition of catalase and SOD.[261] In mitochondria, hydrogen peroxide is readily formed through dismutation of the superoxide radical because of the high matrix SOD activity.[263] The resulting hydrogen peroxide is reduced via the mitochondrial glutathione peroxidase and glutathione reductase system (see Section 5.4), as evidenced by the rapid oxidation of the pyridine nucleotides upon addition of hydrogen peroxide.[262,264] Since mitochondria lack catalase,[248] a rapid and irreversible loss of mitochondrial function occurs if the glutathione peroxidase system is overwhelmed.[261] Apparently, there is an interaction between mitochondrial calcium and mitochondrial tolerance for free radicals since both isolated mitochondria[262] and hepatocytes[235] are more susceptible to damage if they are allowed to accumulate calcium.

Evidence for the involvement of free radicals in ischemic tissue damage first emerged from studies of the isolated perfused rat heart. In this tissue, as in the kidney, considerable tissue damage occurs during the early phase of postischemic reflow.[243,244,265] The severity of reflow damage in the heart was found to be proportional to both the length of the hypoxic period and the PO_2 of the reflow media.[243] The addition of α-tocopherol (vitamin E), a free-radical scavenger, to the perfusate diminished the damage due to reoxygenation,[266] and the extent of damage was proportional to the production of malondialdehyde.[244] During the hypoxic period, the tissue activity of SOD and catalase progressively decreased, suggesting that the heart becomes increasingly susceptible to

oxidative stress during hypoxia.[244] In 1982 Meerson *et al.* proposed a scheme of events to account for the membrane damage of the postischemic heart.[245] Their "lipid triad" scheme consisted of the combined influence of lipid peroxidation, activation of phospholipases (see Section 5.2), and the detergentlike action of the excessive free fatty acids and lysophospholipids on membrane integrity and cardiac function.

One explanation for why the reoxygenation period was especially deleterious to the heart and kidney came with the discovery of the role of xanthine oxidase by McCord and associates.[246] Using a cat intestine model, these workers observed that significant tissue damage occurred during the early phase of reflow that was largely prevented by pretreatment with allopurinol,[246] pterin aldehyde,[247] other inhibitors of xanthine oxidase, or SOD.[246] They proposed that the adenine nucleotides are catabolized to hypoxanthine during the ischemic period. This has been demonstrated in the ischemic rat kidney[246] (see Section 5.1). Upon reoxygenation, this hypoxanthine is further catabolized to uric acid, causing a burst of superoxide formation via xanthine dehydrogenase activity which initiates tissue damage. The lesions due to reoxygenation and their attenuation by these treatments have been correlated with measured changes in vascular permeability.[247]

McCord and associates previously demonstrated that xanthine oxidase, called type O, normally exists in tissues as an NAD-linked xanthine dehydrogenase, type D. Ischemia causes the conversion of type D to type O in a variety of tissues.[267] The D-to-O conversion is nearly complete within 10 sec of ischemia in the rat ileum. In the heart, the type O content doubles after 8 min of ischemia, whereas the same increase in the kidney and liver requires about 30 min.[267] It is of interest that the muscle enzyme does not convert during ischemia and that muscle does not display significant ischemic damage.

The D-to-O conversion is believed to be due to proteolysis since the conversion is irreversible and is prevented in the presence of soybean trypsin inhibitor.[268,269] Calcium appears to be involved in the conversion since pretreatment with fluoperazine, a calmodulin inhibitor, appreciably slows the conversion in ischemic intestine.[267] Furthermore, in the isolated rat heart, perfusion with a media devoid of calcium followed by its sudden restoration (the "calcium paradox") leads to an influx of calcium into the myocytes, massive tissue damage, and the D-to-O conversion in the absence of ischemia or anoxia.[270]

Allopurinol treatment is known to protect the kidney from ischemic damage.[271,272] This was first proposed to be due to preventing the loss of purine bases from the cell.[271,272] McCord and co-workers later interpreted this effect to be a result of the inhibition of a source of superoxide radicals.[246] The latter view has subsequently received considerable ex-

perimental support. Hansson *et al.*[273] verified the accumulation of hypoxanthine in the rabbit kidney subjected to 30 min of ischemia. Upon reflow, they observed a rapid twofold increase in the renal venous effluent hypoxanthine concentration, suggesting that reflow does cause a loss of purine bases from the tissue. Pretreatment with allopurinol dramatically reduced the xanthine content of the venous effluent both before and after the ischemic period.

The beneficial effects of allopurinol treatment have been well documented in studies employing unilateral ischemia after contralateral nephrectomy in the rat. Pre-[274] and postischemic[265] treatment has been found to lower plasma creatinine levels. Postischemic treatment increased the animal survival rate and prevented the extensive tubule damage.[265] Treatment with SOD causes the same protective effects.[265,274,275] SOD has also been shown to lower the malondialdehyde content of mitochondria isolated from the postischemic rat kidney.[274] In rabbit and dog ischemic models, SOD treatment caused a more rapid reestablishment of renal blood flow, urine flow rate, and GFR.[276,277] Pretreatment with catalase was not observed to affect plasma creatinine levels in the rat,[274] although it was shown to improve the recovery of renal blood flow in the rabbit.[276]

5.5. Glutathione

The study of renal glutathione metabolism is a rapidly expanding area of research. This is due, in part, to the recognition of the numerous roles glutathione performs in the cell and to the availability of methods to selectively perturb the cell glutathione status (for review, see Refs. 278–280).

Glutathione (GSH) plays an important role in preventing peroxide and free-radical-mediated tissue damage because it serves as a multipurpose reductant and scavenger of hydrogen peroxide and free radicals. GSH reacts readily with hydrogen peroxide, superoxide, and hydroxyl radicals, producing the glutathione radical.[281,282] The glutathione radical, however, is not very reactive or deleterious to the cell and is only capable of reacting with a second glutathione radical to produce the disulfide (GSSG). The GSH concentration in the rat kidney is about 2.5 mm.[283] Its high cellular content and its mobility in the cell help to assure that the glutathione radical will encounter a second glutathione radical before it can accumulate. Thus, this action serves to quench free-radical chain reactions.[282,283]

GSH also serves as an important reductant of sulfhydryl groups, hydrogen peroxide, and other organic peroxides. Sulfhydryl group reduction occurs both nonenzymatically and through enzymic catalysis by various glutathione transhydrogenases.[280] The reduction of hydrogen

and organic peroxides occurs in the cytosol and in the mitochondrial matrix through catalysis by gluathione peroxidase. The resulting oxidized glutathione (GSSG) is reduced by glutathione reductase, which also occurs in both cellular compartments, using NADPH as a source of reducing potential. In this manner, peroxide reduction is linked to the energy derived from respiration (for review, see Refs. 264,278,283).

Although the role of glutathione in renal ischemia has not been directly investigated to date, many studies have linked the perturbations caused by free-radical and peroxide-mediated oxidative stress to the functional impairment of the glutathione peroxidase/reductase system.[223,284–286] Isolated liver mitochondria have been used as a model because they contain a complete glutathione peroxidase/reductase system[287] and membrane integrity is easily assessed. Stimulation of the mitochondrial system by the addition of an organic peroxide, such as *t*-butyl hydroperoxide (TBH), is associated with an immediate inhibition of respiration if pyruvate or 2-oxoglutarate is the substrate.[286] Since this effect is reversed by dithioerythritol, it was proposed to be due to oxidation of mitochondrial lipoamide and coenzyme A. Evidence for this effect has also been obtained in the perfused rat liver.[288] Addition of TBH leads to a decrease in the mitochondrial NAD(P)H/NAD(P) ratio,[264,284,286,289] consistent with an increase in glutathione reductase activity, followed by mitochondrial swelling, calcium loss, and a loss of membrane integrity.[284,285,289,290] This process is inhibited by dithioerythritol[286] and dibucaine,[285] the latter being an inhibitor of phospholipase A. Added ATP also inhibits the process, probably through stimulation of NADPH formation via the energy-linked transhydrogenase or through inhibition of NAD catabolism.[289] Evidence has also been obtained for a peroxide-mediated loss of mitochondrial integrity in isolated hepatocytes.[291] Beatrice *et al.*[284] have postulated that it is the decreased thiol redox state which accelerates the membrane damage due to phospholipase activation, since the removal of lysophospholipids by lysophospholipid acyltransferase requires reduced coenzyme A.

Glutathione and the thiol redox rate have also been shown to be important factors in the membrane integrity of the perfused rat liver and isolated hepatocytes subjected to oxidative stress. Addition of TBH to the perfused rat liver causes an oxidation of GSH.[288] The oxidation is followed by an increased consumption of oxygen, and both processes are inhibited by (+)-cyanidanol-3, a free-radical scavenger, illustrating the coupling of the GSH peroxidase/reductase system to respiration. TBH addition also causes an increased rate of $^{14}CO_2$ release from [1-^{14}C]glucose by the liver, consistent with an activation of the pentose phosphate shunt.[292]

In isolated hepatocytes, ADP.FE^{3+} addition stimulates lipid peroxidation, as evidenced by the accumulation of malondialdehyde and

conjugated dienes.[293] Addition of this agent causes a decreased GSH content. Moreover, prior depletion of tissue GSH increases the cells' sensitivity to ADP.Fe^{3+}-mediated lipid peroxidation. Hepatocytes that have been allowed to accumulate calcium are also more sensitive to oxidative stress.[236]

As mentioned earlier, the role of GSH in the prevention of renal ischemic damage has yet to be investigated. However, recent reports have appeared that suggest this role in ischemic heart and liver. In the ischemic rat liver, the GSH/GSSG ratio steadily declines, and the rate of decline is decreased by pretreatment with either formate, a free-radical scavenger, or allopurinol.[294] This ratio also drops in the ischemic rat heart, and a further decrease occurs upon reperfusion,[295] suggesting enhanced glutathione peroxidase activity. Treatment of the perfused heart, specifically to deplete tissue GSH, stimulates malondialdehyde production and increases the tissue chemiluminescence emission.[296] Furthermore, maneuvers to decrease or increase cell GSH levels alter the susceptibility of endothelial cells to hydrogen peroxide in a predictable fashion.[297]

Depletion of GSH in kidney slices causes an inhibition of the Na,K-ATPase activity and an increased tissue sodium content.[298] However, the experimental evidence to define the role of GSH in renal ischemia is lacking.

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4

Renal Prostaglandins

Michael J. Dunn

1. Prostaglandin Synthesis Degradation and Excretion by the Kidney

Recent studies have not altered our basic belief that major sites of renal eicosanoid synthesis are the vasculature, glomeruli, cortical and medullary collecting tubules, and renal medullary interstitial cells. Some studies have demonstrated new products of eicosanoid oxygenation, but proof that the kidneys can synthesize leukotrienes has remained elusive. Stimuli of prostaglandin synthesis have been well studied, and our understanding of the cellular mechanisms of action of stimuli, such as angiotensin and vasopressin, has improved substantially. Several reviews of renal arachidonic acid metabolism can be recommended.[1,2]

1.1. Renal Synthesis of Eicosanoids and Stimuli of Arachidonic Acid Metabolism

Studies of isolated glomeruli have reinforced previous reports that a lipoxygenase pathway converts arachidonic acid, primarily to 12-hydroxyeicosatetraenoic acid (12-HETE), and coincubation of glomeruli with macrophages had the interesting additive effect of augmenting prostaglandin synthesis. There appeared to be glomerulus–macrophage

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interaction as an extract of macrophages inhibited 12-HETE synthesis by glomeruli, whereas a glomerular lipid extract actually stimulated macrophage 12-HETE synthesis. These interactions may be important in glomerular immune injury[3] (see Section 6.1). Adherence of rat macrophages to rat glomeruli depended on glomerular lipoxygenase activity, and adherence was inhibited by lipoxygenase enzyme inhibitors. The stimulatory effects of glomerular lipid extracts on rat macrophages may be transmitted by 12-hydroperoxyeicosatetraenoic acid, the immediate precursor of 12-HETE, since direct addition of this substance stimulated macrophage prostaglandin synthesis.[4] No one has succeeded in demonstrating glomerular leukotriene synthesis either with whole glomeruli or with isolated glomerular mesangial cells in culture. Nonetheless, glomeruli do have leukotriene C_4 (LTC_4) receptors which are specific for LTC_4 and show poor affinity for the other slow-reacting substances of anaphylaxis, namely, LTD_4 and LTE_4.[5] Although leukotrienes may have specific hemodynamic actions within the kidney, which will be discussed subsequently, it is noteworthy that Baud and co-workers showed glomerular epithelial receptors for LTC_4 and a stimulatory effect of both LTC_4 and LTD_4 on glomerular epithelial cellular proliferation.[6] Although leukotriene synthesis has not been found with renal cell cultures and glomerular preparations, Pirotzky and co-workers have demonstrated, using the isolated perfused rat kidney, that calcium ionophore stimulates renal release of a biologically active lipid with the characteristics of LTC_4–LTD_4.[7]

Using whole glomeruli or glomerular cells in culture, substantial evidence has been accumulated about various stimuli of prostaglandin synthesis. It has been difficult to document angiotensin II (ANGII) stimulation of whole glomeruli, perhaps related to glomerular injury during preparation. Nonetheless, Stahl *et al.*, using isolated human glomeruli, have shown selective stimulation of PGI_2 synthesis by ANGII. They also confirmed prior reports that human glomeruli synthesize PGI_2 in greater amounts than PGE_2, a situation different from other species.[8] It seems clear that ANGII, as well as other vasoactive stimuli, augment prostaglandin synthesis in glomeruli through stimulation of the turnover of phosphatidylinositol by phospholipase C and of phosphatidylcholine by phospholipase A2.[9] Folkert and co-workers, using cultured rat mesangial cells, found that ANGII released arachidonic acid from phosphatidylinositol, resulting in significant increments in free arachidonic acid as well as phosphatidic acid and diacylglyceride.[9] Human mesangial cells show similar responses to ANGII with stimulation of PGI_2 synthesis rather than PGE_2, as in the rat glomerular mesangial cell. Arginine vasopressin (AVP) and platelet-activating factor (PAF) also stimulate human mesangial prostaglandin synthesis.[10]

The effects of AVP on glomerular mesangial and epithelial cells have been well studied.[11–14] AVP, like ANGII, stimulates phospholipase C and thereby enhances phosphoinositide turnover. The preferred substrate for phospholipase C, often referred to as a phosphodiesterase or an acyl hydrolase, is phosphatidylinositol 4,5-bisphosphate resulting in the rapid release of inositol phosphates, 1,2-diacylglycerol, and phosphatidic acid.[11,13] The subsequent action of diglyceride lipase on diacylglycerol releases arachidonic acid. Figure 1 summarizes the pathways through which vasoconstrictor peptides activate a receptor on glomerular mesangial cells which is linked to an acyl hydrolase resulting in phosphoinositide turnover.[15] The subsequent mobilization of intracellular cytosolic calcium, a result of the action of inositol triphosphate (IP_3) on calcium efflux from endoplasmic reticulum, may partially account for mesangial contraction and/or PG stimulation which is seen with these

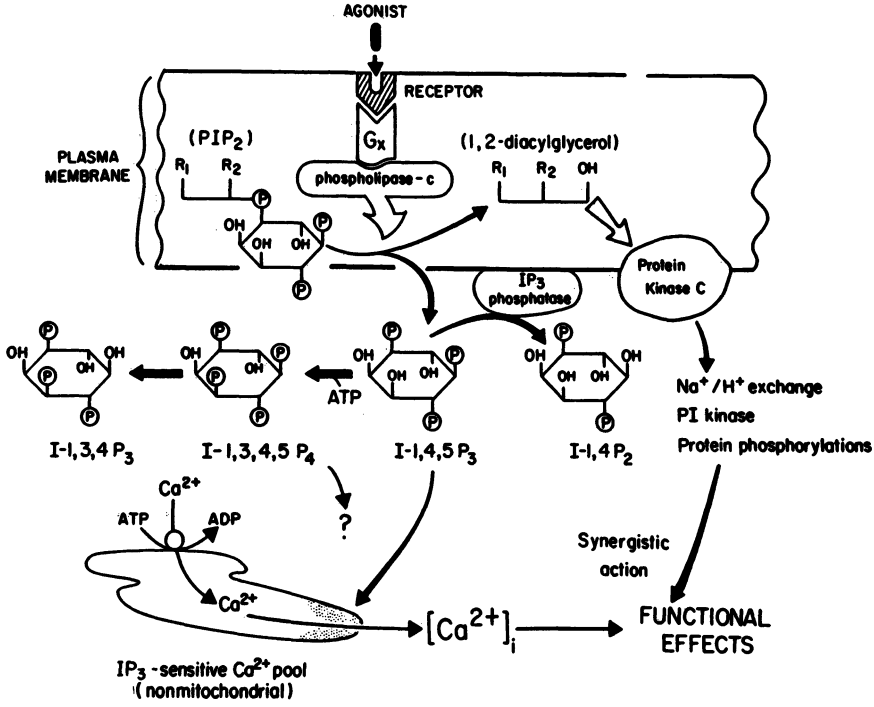

Fig. 1. Summary of the reactions linking an agonist, such as the peptides ANGII and AVP, with their receptors and the subsequent activation of phospholipase C to release inositol-1,4,5-triphosphate (IP_3), thereby mobilizing calcium and leading to the functional effects of ANGII or AVP. Arachidonic acid is released from 1,2-diacylglycerol, and the subsequent synthesis of prostaglandins downregulates the actions of the agonist, probably through multiple interactions within the cell. This schema applies to many cells and seems quite relevant to the glomerular mesangial cell.[15]

vasoactive peptides.[12] PAF, in a manner similar to ANGII and AVP, contracted glomerular mesangial cells and augmented PGE_2 synthesis.[10] It is quite likely that the mesangial cellular synthesis of PGE_2 counteracts the contractile effect of ANGII, AVP, and PAF through activation of adenylate cyclase and increased intracellular cyclic AMP (cAMP).

As noted earlier, the primary tubular epithelial sites of eicosanoid synthesis are the cortical and medullary collecting duct. Garcia-Perez and Smith, using an immunodissection technique to isolate canine cortical collecting tubular cells, have studied these cells in a confluent monolayer grown on millipore filters. These cells are AVP-responsive and release PGE_2 in response to either AVP or bradykinin.[16] Schlondorff *et al.*, using microdissected cortical and medullary rabbit collecting tubules, have obtained similar results with predominant synthesis of PGE_2 and stimulation of PGE_2 synthesis with either bradykinin or AVP.[17] Shayman and Morrison, studying cultured rabbit renal papillary collecting tubular cells, confirmed prior observations that PGE_2 was the prostaglandin synthesized in greatest amounts. When the cells were prelabeled with tritiated myoinositol and stimulated by bradykinin, there was a rapid phosphoinositide turnover with the rapid appearance of IP_3.[18] Bradykinin also stimulates an increase in cytosolic calcium in cultured renal epithelial cells from the canine kidney, which is consistent with rapid phosphoinositide turnover and the release of IP_3.[19] Craven and DeRubertis, using medullary tissue slices, studied AVP-stimulated PGE_2 and concluded that mobilization of intracellular calcium was critical since AVP augmented PGE_2 synthesis in the absence of extracellular calcium whereas TMB8 blocked the actions of AVP, presumably as an inhibitor of intracellular calcium mobilization.[20] Apparently, the collecting tubule epithelial cells respond to peptide stimuli in a fashion analogous to the mesangial cell (see Section 1.1). Although the rapid release of IP_3 would account for a calcium transient (i.e., increment of cytosolic calcium) within seconds of the addition of peptide hormones, it is important to emphasize that renal cells also respond to vasoactive peptides with augmented calcium entry from the extracellular fluid. Scharschmidt and Dunn have previously shown this to be the case with mesangial cells,[21] and Ausiello and Zusman have recently reported that cultured rabbit renal medullary interstitial cells respond to AVP with an influx of extracellular calcium followed by activation of a calcium-calmodulin-stimulated phospholipase.[22]

Schwartzman and her colleagues have described two novel oxygenated products of arachidonic acid which are synthesized by the medullary thick ascending limb of Henle cells from the rabbit kidney. These products are apparently synthesized by a cytochrome P-450 monooxygenase enzyme which yielded two separate monooxygenase derivatives of uncertain structure.[23–25] The biologic significance of these products is un-

clear, although one of them inhibits sodium–potassium adenosine triphosphatase.[25]

1.2. Prostaglandin Degradation

The kidney not only has substantial PG synthetic capacity, with medulla showing greater synthesis than cortex, but also prostaglandin degradative enzymes with greater amounts in cortex than in medulla. The major pathways for the metabolic inactivation of prostaglandins include dehydrogenation of the 15-hydroxyl to 15-keto (15-hydroxy prostaglandin dehydrogenase) and reduction of the 9-keto to 9-hydroxyl (9-keto reductase). These enzymatic activities result in the formation of metabolites with reduced or absent biologic activity. Uchida and co-workers, using microdissected nephron segments in the rat kidney, have localized the NAD-dependent 15-PGDH.[26] They confirmed earlier reports that the newborn rat kidney had 30- to 40-fold higher activity of PGDH than the adult kidney. PGDH activity was greater in midcortical and juxtamedullary layers than in the superficial cortex, and little activity was present in the medulla. The highest activity was found in the proximal convoluted and proximal straight tubules, thereby providing an enzymatic basis for the rapid degradation of filtered PGE_2 and $PGF_{2\alpha}$. Presently, it is unknown whether alterations of prostaglandin degradative capacity in the kidney account for changes in the biochemical balance between synthesis and inactivation in a physiologically significant way.

Administration of estradiol to rats reduced the maximum capacity of 15-PGDH to inactivate prostaglandins,[27] thereby possibly explaining enhanced excretion of PGE_2 in pregnancy. Cagen and co-workers found similar effects of estradiol to inhibit rat renal prostaglandin dehydrogenase activity, thereby supporting the belief that the greater 15-PGDH activity in male rat kidneys accounted for the lower urinary excretory rates of PGE_2 and $PGF_{2\alpha}$ in male compared to female rats.[28] Prostaglandin excretion does not differ between men and women, and hence, there may be some species variation in the response to normal amounts of estradiol; nonetheless, the excretory rates of PGE_2 and $PGF_{2\alpha}$ increase substantially during normal human pregnancy.

1.3. The Effects of Dietary Fatty Acids on Renal Prostaglandin Synthesis

Arachidonic acid (C20:4), the substrate for prostaglandin synthesis, is ingested as such and also can be formed from linoleic acid (C18:2), which is elongated by two carbons and unsaturated to yield arachidonic acid with 20 carbons and four unsaturated bonds. Diets deficient in linoleic acid reduce renal prostaglandin synthesis and excretion, whereas

diets supplemented with linoleic acid, such as with safflower oil, have the opposite effect.[29] Adam and Wolfram, studying 24 adults with varied linoleic acid intake, from 0% to 20% of total calories, found not only increased urinary excretion of PGE_2, but concomitant increments of sodium excretion and of the glomerular filtration rate (GFR) measured as creatinine clearance.[30] Croft, Codde, and co-workers have also shown that dietary supplementation with fish oil, rich in eicosapentanoic acid, reduced renal phospholipid content of arachidonic acid and renal excretion of both PGE_2 and a PGI_2 metabolite.[31,32] This work has recently been confirmed in humans with a dietary supplementation with cod liver oil and subsequent reduction of urinary dienoic prostaglandins and increment in trienoic prostaglandins.[33] These studies have stimulated interest since dietary supplementation with eicosapentanoic acid or fish oils has a beneficial effect in some forms of glomerular immune renal injury, especially murine models of systemic lupus erythematosus.

1.4. Renal Excretion of Prostaglandins

The urinary excretion of the major eicosanoids has been reasonably documented as an adequate measure of renal synthesis except under extraordinary circumstances, such as the systemic infusion of PGE_2 or PGI_2. This is not to say that all prostaglandins synthesized in the kidney appear in the urine, since renal venous plasma and lymph have significantly higher PG concentrations than renal arterial plasma, indicating renal synthesis. Vexing questions remain, such as the relative contribution of cortical versus medullary prostaglandin synthesis to the urinary excretion of prostaglandins. The prevailing belief is that prostaglandins filtered by the glomerulus do not appear in the urine but are degraded by enzymes prevalent in the proximal tubule. Prostaglandins are added to the lumen of the nephron via a secretory step which has been studied using rabbit renal basolateral membrane vesicles. Hydrogen influx into the vesicles augmented prostaglandin E_2 accumulation, and there was no evidence of either a sodium cotransport or potassium antiport pathway for prostaglandin E_2 flux. These workers concluded that there was an electrically neutral tubular secretory pathway for PGE_2, undoubtedly based on hydrogen ion–PGE_2 cotransport in the proximal tubule.[34] Total excretion of PGE_2 may be a variable fraction of total renal PGE_2 synthesis, introducing some interpretive difficulties using urinary PGE_2 as a measure of renal PGE_2 synthesis. Sejersted *et al.* increased renal prostaglandin synthesis either by changes in urine flow or by infusion of arachidonic acid and found that urinary excretion of PGE_2 was generally less than 50% of total renal synthesis and occasionally was less than 10%.[35]

Miller *et al.* have proposed that the kidney has different compartmental effects in response to stimuli such as ANGII and AVP when

prostaglandin release is measured in urine and venous effluent from the isolated perfused rabbit kidney. Surprisingly, ANGII stimulated urine PGE_2 far greater than did AVP, whereas both peptides stimulated the venous effluent levels of PGE_2 and 6-keto-$PGF_{1\alpha}$.[36] The complexity of renal prostaglandin compartmentalization was also demonstrated by Boyd and co-workers, who studied renal vascular and tubular compartments of prostaglandin synthesis and secretion in anesthetized dogs.[37] They concluded that 6-keto-$PGF_{1\alpha}$ could only enter the urine through glomerular filtration, but not tubular secretion, and hence, PGI_2 synthesis in the kidney is best measured by renal venous and not urinary levels of 6-keto-$PGF_{1\alpha}$. PGE_2 behaved differently, and PGE_2 synthesized within the kidney entered both the renal vascular, i.e., renal venous, and renal tubular, i.e., urinary, compartments equally after stimulation of renal synthesis with arachidonic acid or bradykinin.[37] It is fair to conclude that urinary excretion generally changes when renal synthesis, especially medullary synthesis, is augmented, but that the total renal output of prostaglandins would be more reliably measured with a combination of renal venous measurements combined with urinary excretion.

Evidence is lacking that alterations in prostaglandin synthesis at a specific cortical site, such as the glomerulus, will alter urinary excretion of PGE_2 or PGI_2. Chemical medullectomy in rats, induced with bromethylamine, reduced PGE_2 excretion from 457 ng/day in controls to 168 ng/day in rats with severe papillary necrosis.[38] These studies confirm the belief that the majority of urinary PGE_2 has its origin in the renal medulla, presumably the collecting duct and medullary interstitial cells. Additional studies have also reinforced the earlier observations that increasing urine volume, after water loading or diuretic administration, was accompanied by increased PGE_2 excretion. In the studies of Kaojarern *et al.*, urinary PGE_2 correlated better with urinary volume than with urinary sodium excretion.[39] Lifschitz *et al.* also reported a positive relationship between urine flow rate and urine PGE_2 excretion in dehydrated and hydrated subjects subjected to water immersion. They also concluded that factors in addition to urine flow rate, such as the extent of volume expansion, also regulated renal synthesis of excretion of PGE_2.[40] Haylor *et al.*, studying normal volunteers, documented that PGE_2 excretion increased not only as a function of urine flow rate, but also as a function of urinary alkalinity, with higher urinary concentration and excretion rate of PGE_2 after sodium bicarbonate loading.[41] Roberts *et al.*, in a careful study, ruled out methodologic artifact as an explanation for these findings since gas chromatography–mass spectometry measurements confirmed radioimmunoassay measurements of PGE_2 during water diuresis in seven normal women.[42] After the onset of water diuresis, there was a rapid increase in PGE_2 excretion for the first 3 hr,

which then returned to baseline by 5–6 hr. This is not a "washout phenomenon," because of high urine flows through the medulla, since urinary urea excretion showed a different pattern than did PGE_2.[42] These results may be explained by *in vitro* experiments using rat renal papillary collecting tubular cells in culture which responded to abrupt decrements in media osmolality with significant increments of PGE_2 synthesis.[43]

1.5. Summary

Renal eicosanoid synthesis is restricted to selected sites which include the renal vasculature, glomeruli, cortical and medullary collecting tubules, and medullary interstitial cells. The renal medulla synthesizes larger amounts of eicosanoids than does the cortex. Prostaglandin degradation follows the opposite pattern, with cortical degradation exceeding medullary. The major cortical site of prostaglandin metabolism is the late proximal tubule. Renal prostaglandin excretion provides an indirect measure of renal synthesis and is an accurate reflection of whether synthesis is increasing or decreasing. Nonetheless, renal excretion accounts for less than 50% of total prostaglandin synthesis, with greater than 50% appearing in renal venous plasma and lymph (not accounting for prostaglandins synthesized and degraded in the kidney before excretion or secretion). Stimuli, including ANGII, AVP, and bradykinin, trigger phosphoinositide breakdown through stimulation of phospholipase C, which releases inositol triphosphate and diacylglycerol from its substrate, phosphatidylinositol-4,5-bisphosphate. The diacylglycerol releases arachidonic acid through the action of diglyceride lipase. This mechanism allows for simultaneous prostaglandin synthesis and intracellular signal transduction mediated by increments of cytosolic calcium and diacylglycerol, which activates protein kinase C. If dietary intake of arachidonic acid or linoleic acid is reduced and fish oil or eicosapentaenoic acid is substituted, substantial changes in membrane phospholipids occur with reduced arachidonic acid (C20:4) and increased eicosapentaenoic acid (C20:5) in the membrane phospholipids. The ultimate result of fish oil supplementation is to reduce dienoic prostaglandin synthesis by the kidney, as well as in other organs and cells, and to increase the synthesis of the trienoic derivatives of eicosapentaenoic acid. These synthetic changes may partially explain the beneficial effects of fish oil supplementation in various inflammatory conditions.

2. Interrelationships of Prostaglandins and Vasopressin in the Control of Water Excretion by the Kidney

Previous work has amply documented that prostaglandins, especially PGE_2, in some way reduce the antidiuretic actions of AVP and that

AVP stimulates renal prostaglandin synthesis. There are two types of receptors for AVP, designated V1 for the vasoconstrictor receptor on smooth muscle cells and mesangial cells, and V2 for the antidiuretic receptor located on the collecting tubule. Stimulation of renal PGE_2 synthesis through the V1 receptor will be covered in the section on renal blood flow and glomerular filtration rate. Stimulation of prostaglandin synthesis by AVP through either the V1 or the V2 receptor thereby activates a negative feedback pathway through which the vasorelaxant prostaglandin (PGE_2 and PGI_2) or the diuretic–natriuretic prostaglandin (PGE_2) can exert a modulating or inhibitory effect on the action of AVP.

2.1. *In Vitro* Studies Using Cultured Collecting Tubular Cells in Isolated Nephron Segments

Garcia-Perez and Smith have studied canine cultured cortical, collecting tubule cells, isolated through an immunodissection technique, based on the selective capacity of monoclonal antibodies, raised against MDCK cells, to select out the cortical collecting tubule cells of the dog.[16] These cells, grown on millipore filters, demonstrate polarity and a small electrical potential difference. When AVP is added to the basolateral but not the apical surface of the monolayer, cAMP is stimulated. Furthermore, AVP augmented PGE_2 synthesis from either the apical (luminal) or the basolateral surface, suggesting different receptors on the basolateral and apical surface for these two actions. Bradykinin stimulated PGE_2 release only from the apical surface, and PGE_2 in high concentrations stimulated collecting tubular cAMP synthesis. Lower concentrations of PGE_2, 1×10^{-10} to 1×10^{-12} M, inhibited AVP-stimulated cAMP.[16] Our work with the rat renal papillary collecting tubular cell (RPCT) in culture is in agreement with the aforementioned work insofar as AVP stimulated cellular cAMP and PGE_2 was also a potent stimulus of adenylate cyclase augmenting intracellular cAMP. However, we could not confirm that the addition of PGE_2, or its substrate arachidonic acid, inhibited AVP-stimulated cAMP; furthermore, inhibition of cyclooxygenase with aspirin did not potentiate AVP-stimulated intracellular cAMP.[44] These differences between the findings of our group and those of Garcia-Perez and Smith may be related to the specific nephron site, with the cortical collecting tubule showing PGE_2 interaction with AVP-stimulated adenylate cyclase and the papillary collecting tubule lacking this response. Ishikawa *et al.* have shown that the stimulation of cAMP in cultured rat renal papillary collecting tubule cells is regulated by calcium–calmodulin. AVP, PGE_2, and forskolin stimulate renal papillary collecting tubule synthesis of cAMP, and inhibitors of calmodulin progressively reduce these responses in a dose-related manner.[45]

Schlondorff and his co-workers have measured PGE_2 synthesis in isolated collecting tubules from neonatal and adult rabbits, showing that the neonatal rabbit collecting tubule, whether cortical or medullary, produced less PGE_2 than the adult counterpart. Bradykinin and antidiuretic hormone stimulated PGE_2 synthesis in both cortical and medullary collecting tubule segments.[17] Schlondorff and Satriano have also examined the effects of vasopressin on PGE_2 synthesis and phosphoinositide turnover in the toad urinary bladder. AVP stimulated PGE_2 synthesis and cAMP formation, and cAMP inhibited phospholipase-mediated arachidonate release from phospholipids.[46] This model fits nicely with observations in other cell types, both renal and nonrenal, so that agonist-stimulated prostaglandin production downregulates prostaglandin production through the generation of cAMP, which in turn inhibits phospholipase-mediated arachidonate deacylation from phospholipids.[47] Schuster *et al.* have evaluated the interactions of bradykinin, prostaglandins, and antidiuretic hormone using microperfused cortical collecting tubules in the rabbit.[48] Bradykinin, added from the basolateral but not the apical or luminal surface, reduced the antidiuretic hormone-dependent water transport. This bradykinin-dependent inhibition of the action of vasopressin was blocked by indomethacin, indicating that PGE_2 was responsible for the bradykinin effects. However, arachidonic acid did not antagonize the actions of vasopressin on the tubule.[48] These results are puzzling because of the apparent contradiction between negative arachidonate effects and positive PGE_2 effects, but also because bradykinin worked only from the basolateral but not the apical surface, whereas others have shown that bradykinin effectively stimulates PGE_2 from either surface.

Carvounis *et al.* have examined the kinin prostaglandin interactions using toad hemibladders.[49] They have concluded that the inhibitory effects of prostaglandins and of kinins on vasopressin-stimulated water flow are independent of one another since inhibition of kallikrein with aprotinin in prostaglandin-inhibited bladders was accompanied by increased AVP-stimulated water flow. Inhibition of prostaglandin synthesis in aprotinin-pretreated bladders had a similar potentiating effect on water flow in response to AVP.[49] Although these results show independent effects of kinins and prostaglandins to inhibit the action of AVP, they do not rule out a collaborative interaction, as suggested by Schuster *et al.*[48] Stokes has extensively examined the question of prostaglandin inhibition of AVP-stimulated water flux in the perfused cortical collecting duct of the rabbit.[50] Both PGE_2 and $PGF_{2\alpha}$, added to the bath solution, inhibited the osmotic water permeability induced by vasopressin, whereas luminal PGE_2 had no effect. It is puzzling that inhibition of cyclooxygenase with meclofenamate or stimulation of prostaglandin for-

mation with arachidonic acid had no effects on AVP-stimulated water permeability. Stokes also studied endoperoxide analogs since others have shown an AVP-like action of thromboxane and endoperoxide analogs in the toad bladder; however, in the cortical collecting tubule endoperoxide analogs did not mimic AVP.[50] Torikai and Kurokawa have suggested a new site of action of PGE_2 in the medulla of the kidney, namely, the thin descending limb of Henle's loop.[51] Microdissected segments of the thin descending limb of Henle showed PGE_2 stimulation of cellular cAMP in both rabbit and rat segments. PGE_2 was substantially more potent than $PGF_{2\alpha}$ and PGI_2 as an agonist of cAMP synthesis in thin descending limbs of Henle. The cortical collecting tubule, as might be expected from the aforementioned studies, also responded to PGE_2 with increments of cellular cAMP. Other nephron segments that were examined included proximal convoluted tubules, cortical and medullary thick ascending limbs of Henle, and medullary collecting tubules, and no changes of cAMP in response to PGE_2 were seen in these segments.[51] These negative results are puzzling, since we and others have shown that PGE_2 is a potent stimulus of cAMP in papillary collecting tubular cells, and prior work has also shown an effect of PGE_2 and of nonsteroidal antiinflammatory drugs on vasopressin-stimulated electrolyte transport in the medullary thick ascending limb of Henle's loop. Finally, it is possible and perhaps likely that PGE_2 mediates its biologic actions in diverse ways, some of which are independent of cAMP. Therefore, the biochemical assessment of PGE_2-stimulated cAMP in various cells or tissues may overlook cAMP-independent physiologic effects perhaps mediated by IP_3 and changes of cytosolic calcium. Figure 2 summarizes many of these PGE_2–AVP interactions.[46]

2.2. *In Vivo* Experiments Evaluating AVP and PGE_2 in Animals and Humans

In addition to the conventional explanation for the inhibitory effects of PGE_2 on the actions of antidiuretic hormone (decreased cellular cAMP, inhibition of sodium chloride extraction from luminal fluid in the ascending limb of loop of Henle and in the collecting duct, and decreased urea permeability in the collecting tubule), Lemley and co-workers have offered *in vivo* evidence that acute inhibition of renal prostaglandin synthesis reduces blood flow through the vasa recta.[52] Employing videophotometric tracking techniques, Lemley *et al.* measured red-blood-cell velocity in ascending and descending vasa recta in both diuretic and antidiuretic rats and observed between 29% and 52% lower red-cell velocities after acute inhibition of prostaglandin synthesis with indo-

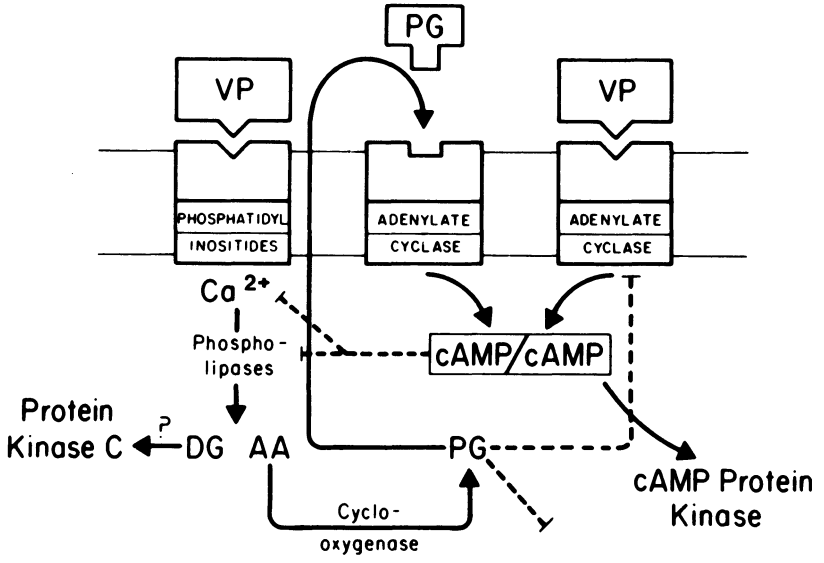

Fig. 2. Scheme, developed from experiments in the toad urinary bladder, applicable to the mammalian collecting duct. Vasopressin (VP) directly stimulates adenylate cyclase to release cAMP and also can trigger phosphatidylinositide-mediated cytosolic calcium increments. Prostaglandin, which is released in this reaction, directly stimulates PGE_2 receptors linked to adenylate cyclase, and cAMP increases in a separate compartment. Solid lines, stimulation; dotted lines, inhibition.[46]

methacin or meclofenamate.[52] These experiments point out the multifactorial nature of the negative feedback action of PGE_2 on the capacity of AVP to stimulate water reabsorption in the medulla. Moses and coworkers have confirmed the work of others as well as extended observations on the interrelationships among AVP, DDAVP, and the renal synthesis of PGE_2.[53] In confirmation of previous work, they have shown that both AVP and DDAVP stimulated renal excretion of PGE_2 in patients with central diabetes insipidus. AVP was a more potent stimulus, perhaps because of simultaneous stimulation of both V1 and V2 receptors, compared to DDAVP, which only stimulates the V2 antidiuretic receptor. Patients with nephrogenic diabetes insipidus did not concentrate their urine in response to AVP, but renal synthesis of PGE_2, measured as PGE_2 excretion, was augmented. These studies point to a selective defect in nephrogenic diabetes insipidus, which is specifically linked to the V2 receptor–adenylate cyclase–cAMP response and not the capacity of AVP to elicit other cellular responses, including enhancement of renal PGE_2 synthesis.[53] Monnens *et al.* have treated four children with nephrogenic diabetes insipidus with a combination of hydrochlorothiazide and indomethacin and concluded that this combination of therapy

was better than either drug alone to reduce urine volume and enhance urine osmolality.[54] Usberti *et al.* explored the interrelationships of ANGII, AVP, and PGE_2 in human subjects who underwent an ANGII infusion before and after administration of aspirin.[55] Angiotensin, as expected, augmented plasma AVP concentration and increased urinary excretion of PGE_2 and 6-keto-$PGF_{1\alpha}$. Aspirin reduced the augmented renal prostaglandin excretion and enhanced the hydroosmotic effect of antidiuretic hormone, as shown by increased urinary osmolality when angiotensin was infused after aspirin.[55] Perez-Ayuso *et al.* have emphasized the homeostatic importance of renal prostaglandin synthesis in the control of water excretion in patients with cirrhosis and ascites.[56] Patients with severe hepatic disease, who could not excrete a water load (failure to achieve a positive free-water clearance), had lower urinary PGE_2 and higher plasma AVP than patients with liver disease who had a positive free-water clearance or normal control subjects. Furthermore, acute administration of intravenous aspirin in the form of lysine acetyl salicylate caused a significant reduction of urinary renal prostaglandin synthesis, without altering plasma AVP, and acutely reduced free-water clearance. The authors concluded that the impaired ability to dilute the urine in cirrhosis with ascites may be secondary not only to the well-known renal hemodynamic alterations and the nonosmotic stimulation of AVP, but also to spontaneous reductions of renal PGE_2 synthesis.[56]

Several reports have documented the diuretic efficacy of prostaglandin analogs. Intravenous infusions of the stable prostaglandin analog 9-deoxo-16,16-dimethyl-9-methylene-PGE_2 in conscious sheep, receiving exogenous AVP, demonstrated antagonism of the antidiuretic action of AVP. In overhydrated sheep, the syndrome of inappropriate antidiuretic hormone release was created, and the PGE_2 analog effectively blocked the hydroosmotic effect of vasopressin, thereby suggesting possible therapeutic utility in the treatment of hyponatremia in humans.[57] Figure 3 summarizes these experiments. Using the same PGE_2 analog, Leksell and colleagues have obtained similar results in conscious humans. 9-Methylene-PGE_2 acutely induced a water diuresis with four- to fivefold increment in urine volume and a 75% reduction in urine osmolality.[58] These studies set the stage for therapeutic trials of PGE_2 analogs in the treatment of severe hyponatremia in patients, particularly those with the syndrome of inappropriate antidiuretic hormone release.

2.3. Summary

Prostaglandins, especially PGE_2, reduce the hydroosmotic or antidiuretic action of AVP at multiple steps. PGE_2, in some experimental situations, reduces AVP-stimulated cAMP in the collecting duct. Addi-

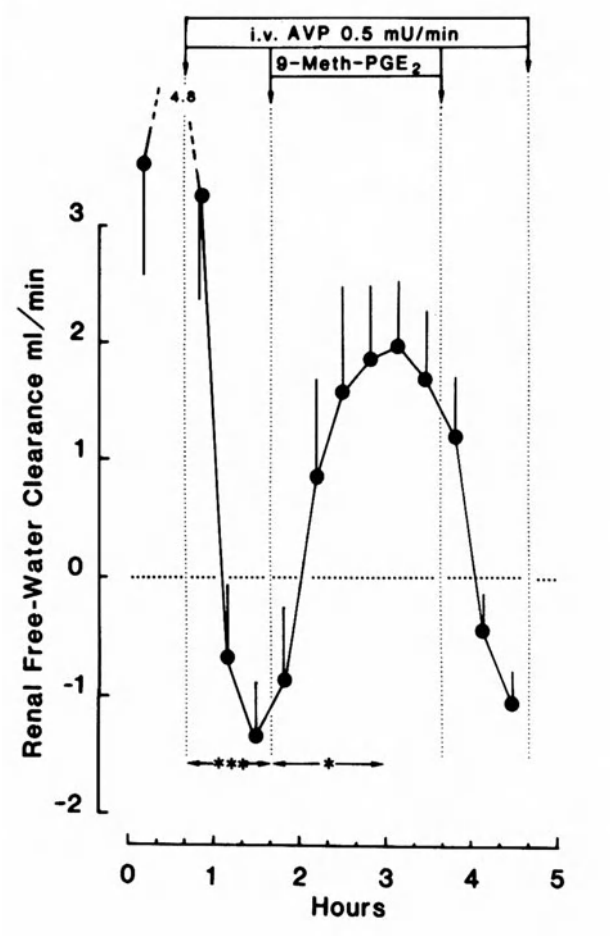

Fig. 3. Results of experiments conducted in five hydrated sheep infused initially with arginine vasopressin (AVP), followed by an intravenous infusion of 9-methylene-PGE_2. The reduction of free-water clearance due to the antidiuretic effect of AVP was rapidly antagonized by the PGE_2 analog in a reversible fashion, since cessation of the PGE_2 infusion was followed by an abrupt decrease of free-water clearance.[57]

tionally, the capacity to generate medullary interstitial hypertonicity by reabsorption of sodium chloride and urea from the luminal fluid is inhibited by PGE_2. Vasa recta blood flow is also partially regulated by medullary prostaglandins, and acute inhibition of prostaglandin synthesis reduces descending and ascending vasa recta flow. The diuretic action of bradykinin is at least partially mediated by bradykinin-stimulated PGE_2, with the resultant antagonism of AVP in humans. Intranasal DDAVP or intravenous AVP augments renal PGE_2 synthesis in normal subjects and in diabetes insipidus patients, whether of central or nephrogenic

origin. The biologic importance of PGE_2 as an antagonist of the action of AVP is highlighted in cirrhosis with ascites since acute administration of aspirin to these patients significantly impairs water diuresis. Stable analogs of PGE_2 may have therapeutic efficacy in dilutional hyponatremia, as the administration of a PGE_2 analog in sheep and in humans effectively antagonized water reabsorption and induced water diuresis.

3. Sodium Excretion, Diuretics, and Renal Prostaglandins

PGE_2 is natriuretic and chloruretic whether injected into the renal artery or synthesized, *in situ,* in the kidney. Conversely nonsteroidal antiinflammatory drugs that inhibit renal PGE_2 synthesis often reduce sodium excretion. The dominant intrarenal sites of action, to explain PGE_2-induced sodium chloride diuresis, are the medullary thick ascending limb of Henle's loop and the medullary collecting duct. Recent studies have partially elucidated the mechanisms of PGE_2-induced natriuresis as well as the *in vivo* risks of nonsteroidal antiinflammatory drugs.[59]

3.1. Mechanisms of Prostaglandin-Induced Natriuresis and Chloruresis

Cuthbert and co-workers have used pig renal papillary collecting tubule cells in culture (similar in preparation and in responsiveness to rabbit and rat renal papillary collecting tubule cells in culture) and have studied monolayers grown on collagen-coated millipore filters.[60] In general, their results confirm and extend conclusions based on rabbit and rat papillary collecting tubule cells in culture with documentation of short-circuit current with the basolateral side positive and a polarity or sidedness to the responses induced by various hormones and autacoids. AVP receptor-mediated changes in short-circuit current were present only on the basolateral surface, whereas kinins and PGE_2 altered short-circuit current when added to either the apical or basolateral side of the tissue culture. The kinin-induced stimulation of chloride secretion was prostaglandin-dependent and inhibitable by either indomethacin or piroxicam.[60] Similar conclusions have been reached by other investigators studying cultured toad kidney cells or canine cortical epithelial cells. Keeler and Wong described a PGE_2-stimulated chloride secretion and short-circuit current in cultured toad kidney cells (A6) in a high-resistance monolayer.[61] Lifschitz, using canine cortical epithelial cells (MDCK), noted a chloride gradient between the basolateral and apical surface, which was inhibitable by indomethacin and stimulable by PGI_2, sug-

gesting a chloride secretory step from basolateral to apical surface which was prostaglandin-dependent.[62] If these data are applicable *in vivo,* it suggests that PGE_2 may induce sodium chloride diuresis, not only by inhibiting sodium and chloride reabsorption from apical to basolateral surface, but also by enhancing chloride secretion from basolateral to apical surfaces.

Culpepper and Andreoli have examined the mechanism by which PGE_2 inhibits sodium chloride transport in the medullary thick ascending limb of Henle in the mouse.[63] They have elaborated on their previously reported observations about PGE_2 inhibition of AVP-stimulated chloride absorption in the isolated, microperfused, mouse medullary thick ascending limb of Henle by showing that PGE_2 competitively antagonized cholera toxin-stimulated as well as AVP-stimulated chloride reabsorption. As PGE_2 had no effect on forskolin-stimulated chloride flux or transepithelial voltage, these authors concluded that PGE_2 inhibits sodium chloride transport at a site distal to the AVP–receptor interaction but proximal to the catalytic subunit of adenylate cyclase, since the actions of forskolin were not affected.[63] Experiments by Luke and co-workers have reinforced the belief that prostaglandins alter chloride transport in the thick ascending limb.[64] *In vivo* microperfusion of the superficial nephron loop, from late proximal tubule to early distal tubule, showed an acute effect of indomethacin to enhance loop chloride reabsorption as measured by lower chloride concentration in fluid collected distally. This response was seen in normal rats as well as potassium-depleted rats, although the chloride reabsorptive defect in the thick ascending limb of potassium-depleted rats was only partially corrected by indomethacin.[64] Besseghir used the Sperber technique, an interesting preparation in the nonanesthetized chicken involving injection of PGE_2 into the venous portal circulation.[65] This technique allows the delivery of injected substances to the peritubular circulation of the kidney with no exposure of glomeruli or preglomerular vasculature to the injected prostaglandin. PGE_2 induced dose-dependent increments of urinary flow, sodium chloride and potassium excretion, and free-water clearance. There were no changes in RPF or GFR. Tubular injection of radiolabeled PGE_2 showed extensive metabolism of the ligand prior to tubular secretion and urinary excretion. Blockade of organic ion transport, which acutely reduced tubular transport or secretion of PGE_2, did not alter the effects of PGE_2 on electrolyte and water excretion, pointing to a dominant peritubular effect on the basolateral membrane of the responsive tubular epithelial cells.[65]

The mechanisms of pressure-natriuresis have been explored using *in vivo,* canine preparations and *ex vivo,* isolated, perfused rat kidneys. Pressure-natriuresis, induced in dogs by increased systemic arterial pressure secondary to carotid artery constriction, stimulated sodium excre-

tion and urinary PGE_2 excretion, which were highly correlated.[66] Indomethacin significantly blunted the sodium excretion in response to increased perfusion pressure, whereas the autoregulatory capacity of the kidney to moderate GFR and RBF was unaffected. Figure 4 summarizes these data. These results are consistent with prior publications which concluded that renal autoregulation is prostaglandin-independent, whereas the data are consistent with an integral role of PGE_2 in pressure-natriuresis. Pressure-natriuresis in the isolated perfused rat kidney was also prostaglandin-dependent, and regression lines relating pressure to urinary sodium were shifted to the right in prostaglandin-inhibited kidneys.[67] In the isolated perfused rat kidney studies, total renal vascular resistance was also higher after prostaglandin inhibition, and the authors concluded that afferent arteriolar dilation, mediated by prostaglandins, was important in mediating pressure-natriuresis, resulting in both an increased filtered load of sodium and decreased tubular reabsorption of salt.[67]

Haas *et al.* advanced the hypothesis that the natriuretic effect of prostaglandins is linked to an increase in renal interstitial pressure.[68] They studied a prostaglandin analog that increases renal blood flow but

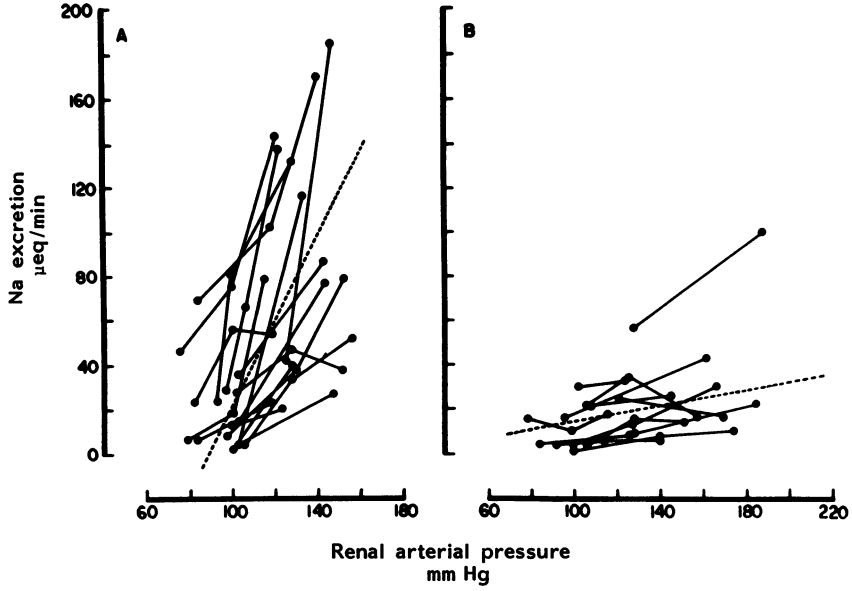

Fig. 4. Renal arterial pressure was altered in dogs through changes in carotid artery baroreceptor pressures, and sodium excretion was measured before (A) and after (B) indomethacin administration. Prostaglandin inhibition with indomethacin dramatically blunted the pressure natriuresis response and also reduced urine volume without affecting potassium excretion.[66]

does not cause natriuresis, and they compared this prostaglandin analog to PGE_2. Only PGE_2 increased renal interstitial hydrostatic pressure and resulted in natriuresis. If renal interstitial pressure was held constant, PGE_2 was no longer natriuretic.[68] It is puzzling that both prostaglandins increased renal blood flow but only PGE_2 increased renal interstitial pressure. Experiments by Hebert and co-workers may be relevant to this issue.[69] They infused PGE_2 and leukotriene B_4 (LTB_4) into the renal artery of dogs. PGE_2 had the expected natriuretic effect, whereas LTB_4 did not alter RBF or sodium excretion. LTB_4 had a substantial potentiating action when combined with PGE_2, resulting in augmented natriuresis, urine volume, and free-water clearance. There were no parallel changes of GFR and RBF, suggesting a direct action of LTB_4 and PGE_2 on tubular reabsorption.[69] As LTB_4 enhances capillary permeability, one wonders whether the synergistic effects of LTB_4 and PGE_2 are related to alterations of peritubular vascular permeability and renal interstitial hydrostatic pressure. It would be interesting to know whether the nonnatriuretic PGE_2 analog would become natriuretic if combined with LTB_4.

3.2. *In Vivo* Studies of Sodium Excretion and Prostaglandins in Humans

Kramer *et al.* studied six normal volunteers on high and low sodium intake to evaluate the roles of angiotensin and urinary catecholamines and their interactions with PGE_2 in salt-loaded and salt-restricted normal subjects.[70] Low sodium intake (35 mmoles/day), when compared to high sodium intake (350 mmoles/day), significantly increased urinary excretion of PGE_2, as well as plasma noradrenalin and renin activity. When prostaglandin synthesis was inhibited with indomethacin, there were no significant effects during high sodium intake, but subjects on low sodium intake receiving indomethacin showed significant decrements of sodium excretion as well as GFR, RBF, and plasma renin activity. Since urinary noradrenalin excretion remained increased after indomethacin, the authors concluded that the decrements in renal hemodynamics and sodium excretion after PG inhibition may be catecholamine-dependent.[70]

The effects of nonsteroidal antiinflammatory drugs on furosemide-induced natriuresis have continued to attract attention. Mackay *et al.* studied eight male volunteers who received intravenous furosemide before and after indomethacin. Indomethacin inhibited the furosemide-dependent increments in RBF and GFR and blunted the natriuresis. As expected, furosemide enhanced the urinary excretion of PGE_2 but did not alter a PGI_2 metabolite used as a marker of systemic or nonrenal PGI_2 synthesis.[71] This contrasts with prior publications which have concluded that the immediate, venodilatory effect of intravenous furosem-

ide is PGI_2-mediated. Trimarco *et al.* evaluated the importance of renal prostaglandin synthesis in normotensive and hypertensive subjects who received a salt load of 480 mmoles/day.[72] Indomethacin and ibuprofen reduced the natriuretic response to increased sodium intake in the hypertensive, but not in the normotensive subjects. Sulindac, which exerts negligible inhibition of renal prostaglandins, showed no interference with the renal natriuretic responses to sodium loading in either normotensive or hypertensive subjects. The authors concluded that the importance of renal prostaglandin synthesis for handling a sodium load was more critical in hypertensive situations.[72] Brater and his colleagues have reached contrasting conclusions regarding the renal-sparing effect of sulindac in normal volunteers receiving furosemide.[73] These authors compared placebo, ibuprofen, naproxen, and sulindac in a randomized, double-blind study and found that all the nonsteroidal antiinflammatory drugs reduced the natriuretic effects of 40 mg of furosemide and that sulindac acutely reduced urinary PGE_2 similar to ibuprofen. Although these studies reinforce the importance of renal PGE_2 for natriuresis after diuretics, especially in sodium-avid situations, the comparability of sulindac to other nonsteroidal antiinflammatory drugs is an important and contradictory issue (see Section 5.3).

The therapeutic use of prostaglandins as natriuretic agents was evaluated in nine patients with cirrhosis and ascites.[74] Continuous infusion of intravenous PGE_1 over 72 hr increased the low basal excretion of urinary kallikrein and resulted in substantial increments of urine volume, sodium excretion, and water excretion. These results, which are consistent with experiments described earlier showing enhancement of water diuresis with a PGE_2 analog in human subjects, reinforce the possible therapeutic potential of short-term administration of PGE_1 or PGE_2 in clinical situations with salt and/or water excess.

3.3. Summary

Renal prostaglandins are natriuretic and chloruretic, and PGE_2 exerts the dominant effect. This is primarily a direct tubular action to reduce sodium chloride reabsorption in the medullary thick ascending limb of Henle's loop and in the medullary collecting tubule. The possibility exists that chloride secretion in the papillary collecting tubule could be enhanced by PGE_2. The action of PGE_2 is exerted on the basolateral membranes of the tubular epithelial cells, probably through actions affecting intracellular cAMP and also IP_3 and cytosolic calcium. It is unknown how changes of cAMP, mediated by PGE_2, would have an opposite action to those mediated by AVP in the control of sodium chloride excretion. The capacity of the kidney to respond to increasing

renal arterial perfusion pressure with natriuresis is partially dependent on renal prostaglandin synthesis. Autoregulation of GFR and RPF, in responses to changes of renal arterial pressure, is independent of renal prostaglandin synthesis. In humans, the evidence is clear that PGE_2 is an important factor in the regulation of sodium excretion, particularly in conditions with avid sodium retention, such as sodium depletion or edema, and in conditions where plasma catecholamines and angiotensin are present in increased concentrations. Inhibition of renal prostaglandin synthesis augments the sodium-retaining and vasoconstrictor effects of catecholamines in sodium depletion and reduces the renal natriuretic responses to sodium-loading and loop diuretics. Infusions of PGE_1, PGE_2, or PGE_2 analogs merit clinical evaluation as therapeutic agents for sodium and water retention in edematous conditions, such as cirrhosis with ascites or congestive heart failure.

4. Prostaglandins, Renin Secretion, and Bartter's Syndrome

Prostaglandins, particularly PGE_2 and PGI_2, stimulate renin release *in vivo*, after renal arterial infusion, and *in vitro*, after addition to renin-secreting renal slices or other preparations such as isolated glomeruli. Although prostaglandin stimulation of renin secretion is thought to be mediated by cAMP, this has not been proved unequivocally. Renin secretion in the intact kidney can be stimulated by adrenergic stimulation, baroreceptor mechanisms, and changes of electrolyte reabsorption at the macula densa. Prostaglandins do not appear to exert a critical, mediatory role in any of these mechanisms of renin release. Nonetheless, indomethacin and related nonsteroidal antiinflammatory drugs suppress renin secretion in humans, are effective agents in the treatment of hyperreninism (Bartter's syndrome), and may cause hyporeninemic hypoaldosteronism with hyperkalemia.

4.1. Prostaglandin–Renin Interrelationships Studied *in Vitro*

Indirect evidence has been adduced linking prostaglandin stimulation of adenylate cyclase, the subsequent increments in cellular cAMP, and increased renin secretion by renal slices.[75,76] This evidence is indirect since the renin-secretory tissue is presumably limited to the juxtaglomerular apparatus and perhaps some glomerular mesangial cells, and the increments of renal, cortical-slice cAMP after the addition of PGE_2 or PGI_2 probably reflect increases of cAMP in other sites, such as vasculature, glomeruli, and cortical collecting tubules. Lopez *et al.* have shown that phosphodiesterase inhibition with theophylline potentiated

PGE_2-stimulated renin secretion in rat renal cortical slices. Prior dietary sodium deficiency potentiated the renin responses to added PGE_2.[75] Barchowsky and co-workers, using rabbit renal cortical slices, found that direct stimulation of renin secretion with forskolin, a direct stimulus of the adenylate cyclase, or by the β-adrenergic agonist isoproterenol was prostaglandin-independent and, hence, unaffected by cyclooxygenase inhibitors. They concluded that prostaglandins do not play a role in the stimulation of renin secretion mediated either by β-adrenergic stimuli or direct increments of intracellular cAMP.[76] Studies with canine renal cortical slices reinforce these conclusions, as indomethacin did not reduce isoproterenol-dependent renin release or renin secretion stimulated by dibutyryl cAMP. It is noteworthy that isoproterenol also did not stimulate the synthesis of PGI_2.[77] Henrich and Campbell also concluded, based on their studies of β-adrenergic-mediated renin secretion by rat cortical slices, that PGE_2 and PGI_2 do not subserve an essential function as mediators of isoproterenol-stimulated renin release.[78] These experiments with renal cortical slices from rat, rabbit, and dog are consistent with results and conclusions drawn from *in vivo* studies summarized in Section 4.2.

Itoh and Carretero have employed a novel approach with microdissected afferent arterioles, with or without attached macula densa, obtained from the rabbit kidney.[79] The attached macula densa inhibited renin release by the afferent arteriole, and furosemide stimulated renin secretion only if the macula densa was attached to the arteriole. Furthermore, indomethacin reduced basal renin release but did not decrease the percentage stimulation of renin secretion after the addition of furosemide, thereby indicating that the integrity of prostaglandin synthesis is not essential for stimulation of the macula densa and subsequent alterations of renin secretion by the afferent arteriole.[79] Renin and prostaglandin production by isolated superficial and juxtamedullary dog glomeruli were compared by Schryver *et al.* using glomeruli superfused in glass chambers.[80] Arachidonic acid augmented renin and prostaglandin synthesis and release by the glomeruli, and the renin secretion correlated best with PGI_2, but not PGE_2, synthesis. Although PGI_2 synthesis was similar in superficial and juxtamedullary glomeruli, renin secretion was significantly higher, as has been previously reported, in glomeruli from the superficial or outer cortex.[80] These results may be quite relevant to humans since human glomeruli synthesize predominantly PGI_2.

4.2. Prostaglandin Regulation of Renin Secretion *in Vivo*

Vikse and co-workers, using anesthetized dogs, have shown that afferent arteriolar dilatation, induced by ureteral obstruction or renal

artery constriction, augments renin release independent of prostaglandins, but these maneuvers potentiate prostaglandin-stimulated renin release after the infusion of arachidonic acid.[81,82] Additional studies of the mechanism by which β-adrenergic stimulation augments renin release have led to the conclusion that renal prostaglandins do not mediate this response.[81] Villarreal *et al.* have examined baroreceptor-regulated renin release and the role of prostaglandins in the anesthetized rat. Using either intact kidneys or denervated nonfiltering kidneys, the authors concluded that prostaglandins are not essential as mediators of the renin responses regulated by the baroreceptor mechanism.[83] Osborn and his co-workers reached different conclusions in anesthetized dogs, as their experiments showed substantial reductions of renin secretion after aortic constriction in dogs receiving indomethacin or meclofenamate. They also showed that low-frequency renal nerve stimulation enhances both renin and PGE_2 secretion, and prostaglandin inhibition blunted this response.[84] Less attention has been paid to the α-adrenergic control of renin and prostaglandin release. Experiments with selective α-I and α-II agonists led Takahashi *et al.* to the conclusion that α-I receptors may increase renin release independent of prostaglandin synthesis, whereas α-II adrenoreceptors in the kidney will increase PGE_2 secretion without affecting renin release.[85] These studies point to the experimental difficulty of using the intact kidney since the interpretation of such experiments is confused by the multiple sites of α-I and α-II adrenoreceptors and the uncertainty whether α-II stimulation of prostaglandin synthesis is within the vicinity of the juxtaglomerular apparatus.

4.3. Bartter's Syndrome: Renin Prostaglandins

There has been continuing debate as to whether Bartter's syndrome is strictly a disorder of renal electrolyte transport, with secondary changes of renin, aldosterone, and prostaglandins, or whether it is a systemic disease, with changes in extrarenal (vascular?) PGI_2 synthesis. A patient with Bartter's syndrome has been reported who developed renal insufficiency due to focal segmental glomerulosclerosis, while receiving continuous indomethacin therapy to control hypokalemia, and required renal transplantation. After renal transplantation, the manifestations of Bartter's syndrome disappeared.[86] My belief, that renal overproduction of PGE_2 is probably a secondary phenomenon in Bartter's syndrome, was reinforced by Senba *et al.*, who showed that potassium repletion with oral potassium chloride substantially reduced urinary excretion of PGE_2 and $PGF_{2\alpha}$, despite increments of plasma renin activity and plasma aldosterone.[87] Houser *et al.* have reported an unusual case, which resembles Bartter's syndrome, with the additional features of hypercalciuria as well as increased urinary PGE_2 excretion. Both aspirin and indo-

methacin simultaneously reduced prostaglandin and calcium excretion.[88] The capacity of PGE_2 to inhibit tubular calcium reabsorption, and conversely of indomethacin to reduce calcium excretion, has been experimentally documented in rats and will be discussed in Section 8.2.[89,90] Favre *et al.* have measured distal fractional delivery and reabsorption of chloride and sodium in eight normal volunteers and seven patients with hypokalemia, including four patients with Bartter's syndrome.[91] Acute blockade of prostaglandin synthesis by indomethacin in the normal subjects decreased distal delivery of sodium chloride without altering distal reabsorption, suggesting a site of prostaglandin action in the ascending limb of Henle. The sodium chloride reabsorptive defect in Bartter's syndrome was unaffected by prostaglandin inhibition, whereas patients hypokalemic from other causes showed enhanced distal reabsorption of chloride and sodium after indomethacin. These investigators concluded that the tubular reabsorptive defects for sodium and chloride in Bartter's syndrome are independent of prostaglandins.[91]

4.4. Summary

PGE_2 and PGI_2, whether infused into the kidney or added to renal cortical slices, increase renin secretion probably secondary to stimulation of adenylate cyclase and increases of intracellular cAMP in renin-secreting cells, especially juxtaglomerular arteriolar cells. These events are shown in Fig. 5. Renin synthesis and secretion, under the control of baroreceptors, adrenergic receptors, and the macula densa, is basically independent of prostaglandin synthesis, and these mechanisms for the control of renin secretion function adequately in the presence of prostaglandin inhibition. The clinical administration of nonsteroidal antiinflammatory drugs can diminish plasma renin activity and precipitate hyperkalemia, particularly in patients susceptible to hyporeninemic hypoaldosteronism. The enhanced renal prostaglandin synthesis seen in Bartter's syndrome appears to be secondary to alterations in tubular handling of sodium chloride and potassium, and not a primary, genetically controlled event. Nonetheless, the hypokalemia of Bartter's syndrome can be improved, but not entirely corrected, by indomethacin therapy.

5. Renal Blood Flow, Glomerular Filtration Rate, and Renal Eicosanoids

The beneficial vasodilatory actions of PGE_2 and PGI_2 within the kidney are amply proven. The vasoconstrictor actions of thromboxane A_2 and leukotrienes, although not important under normal conditions,

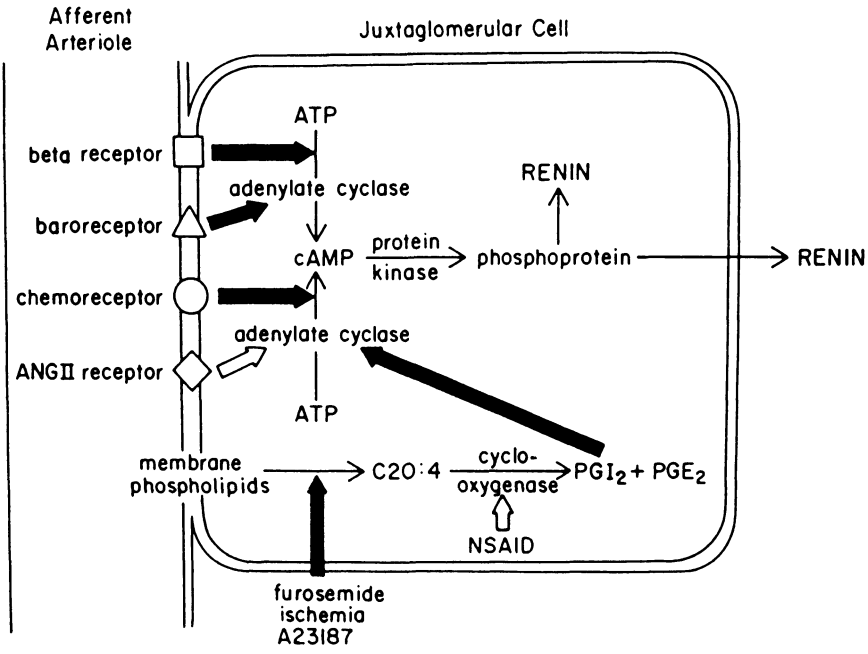

Fig. 5. Hypothetical schema of the interrelations of stimuli of renin secretion and prostaglandin synthesis in the juxtaglomerular cells of the afferent arteriole. Baroreceptor, adrenergic receptor, and chemoreceptor as well as macula densa control of renin release are independent of prostaglandin synthesis; however, prostaglandins that stimulate adenylate cyclase and hence increase cAMP in the JG cell will augment renin synthesis and release. Nonsteroidal antiinflammatory drugs generally reduce renin secretion unless the stimulus to renin secretion is intense. Hence, prostaglandins play a nonintegral, but facilitory role in renin secretion. Solid arrows, stimulation; open arrows, inhibition.[92]

undoubtedly play an important pathophysiologic role after renal injury. Clearly, catecholamines and the vasoconstrictor hormones ANGII and AVP stimulate renal synthesis and release of vasodilatory eicosanoids, especially PGE_2 and PGI_2. The clinical nephrotoxicity of nonsteroidal antiinflammatory drugs undoubtedly is a consequence of the inhibition of PGE_2 and PGI_2 synthesis.[93,94]

5.1. The Role of Eicosanoids in the Control of Renal Blood Flow and Glomerular Filtration Rate

It is logical that leukotrienes would be tested for their effects on RBF as the sulfidopeptide leukotrienes LTC_4 and LTD_4 vasoconstrict other vascular beds. Rosenthal and Pace-Asciak have shown that leukotrienes vasoconstrict the isolated perfused rat kidney, with a descending order of potency of LTC_4 greater than D_4 greater than E_4.[95] These

effects were not blocked by indomethacin and, hence, appear independent of secondary stimulation of TxA_2 synthesis. Intravenous infusion of LTD_4 in anesthetized rats increased vascular resistance less in the kidney than in mesenteric and hindquarter vascular beds.[96] Piper *et al.* used an *in situ*, blood-perfused, pig kidney preparation and injected LTC_4, LTD_4, or norepinephrine directly into the kidney. LTC_4 and LTD_4 were more potent than norepinephrine to increase renal vascular resistance, and this constrictor response was potentiated by inhibition of prostaglandin synthesis with indomethacin.[97] It is unknown whether the sulfidopeptide leukotrienes will augment renal prostaglandin synthesis. LTC_4 and LTD_4 do not increase prostaglandin synthesis in rat glomerular mesangial cell culture (unpublished observations). Badr *et al.* evaluated the effects of systemic infusion of LTC_4 in the rat and found a two-phase response with an initial renal vasoconstriction and hypertension as a consequence of the direct constrictor action of LTC_4.[98] Subsequently, LTC_4 caused vascular leak with loss of plasma volume, augmentation of plasma ANGII, and resultant elevations of systemic vascular resistance and renal vascular resistance mediated by ANGII. Chapnick[99] has obtained divergent results in the dog, with no changes in renal vascular resistance after intrarenal arterial administration of the leukotrienes, but with mesenteric vasoconstriction after intraarterial infusion of leukotrienes (LTC_4 greater than LTD_4 greater than LTE_4). It is unknown whether this reflects a species difference among dog, rat, and pig, or whether the failure to vasoconstrict was a result of some differences in experimental protocol. It is puzzling that *in vitro* incubation of either canine superior mesenteric or renal arterial rings showed dose-dependent relaxation of both preparations in response to LTD_4.[100] These relaxation responses depended on the vascular endothelium and were eliminated after removal of the endothelial surface of the isolated vascular ring, suggesting an endothelial-derived relaxing factor.

Edwards has employed a unique preparation, microdissected rabbit intralobular arteries and afferent and efferent arterioles, to study the effects of eicosanoids on renal vascular resistance.[101] Whereas $PGF_{2\alpha}$ had no effect on any of the three vessels, arachidonic acid PGE_2 and PGI_2 relaxed norepinephrine-constricted intralobular arteries and afferent arterioles. In the efferent arteriole, only PGI_2 relaxed the vessel and antagonized both catecholamine and angiotensin-induced constriction.[101] Vikse and Kiil have examined the importance of PGE_2 in the autoregulatory vasodilatation of preglomerular vessels in the dog. PGE_2 synthesis, as measured in renal venous plasma, increased during renal autoregulation in response to decreased perfusion pressure. If renal PGE_2 synthesis had already been maximally stimulated by ureteral obstruction, then the canine kidney autoregulated poorly in response to

decreased perfusion pressure.[102] Infusion of arachidonic acid, after autoregulatory vasodilation, potentiated renal PGE_2 release by the canine kidney. It is unknown how renal arterial constriction or ureteral dilatation, maneuvers that vasodilate the afferent arteriole, potentiate the renal prostaglandin synthetic response to arachidonic acid.[82]

Wilcox *et al.* have evaluated the role of thromboxane as a possible mediator of the renal vasoconstriction that ensues after infusion of hypertonic sodium chloride into the canine kidney.[103] Hypertonic sodium chloride infusion stimulated the release of TxB_2 into both urine and hilar lymph, and indomethacin attenuated, but did not abolish, this sodium chloride-induced increase in renal vascular resistance. If arachidonic acid was infused into this canine renal preparation, all urinary prostaglandins increased, whereas lymphatic 6-keto-$PGF_{1\alpha}$ selectively increased, suggesting predominant cortical synthesis of PGI_2 (renal hilar lymph is derived predominantly from the renal cortex). Sodium chloride induced less renal vasoconstriction if it was infused concomitantly with arachidonic acid, leading the investigators to conclude that renal vascular resistance was controlled by a balance between PGI_2 and PGE_2 compared to thromboxane A_2.[103]

Thromboxane A_2, although synthesized by normal renal cortical and medullary tissue, is present in only small amounts in the normal kidney or in urine. It is generally believed that renal thromboxane release, or renal vasoconstriction due to thromboxane release from nonrenal cells, is a manifestation of a pathophysiologic condition. Zipser evaluated a thromboxane synthetase inhibitor, dazmegral, in 20 healthy volunteers over a 14-day treatment.[104] Although dazmegral reduced urinary thromboxane B_2 and serum thromboxane B_2 (a measure of platelet thromboxane release), there was no change in renal blood flow or glomerular filtration rate, nor was there evidence of augmented PGI_2 synthesis due to endoperoxide shunting from thromboxane to PGI_2.[104] Several studies in humans have documented the vasodilatory efficacy of prostaglandins, including PGE_1 and a prostacyclin analog, iloprost.[105,106] PGE_1 was infused into eight normal subjects, and as anticipated, blood pressure decreased and blood flow increased to all organs including the kidneys. Glomerular filtration rate and electrolyte excretion were not quantitated.[105] Iloprost, a stable prostacyclin analog, was infused in nine patients with severe arteriosclerotic vascular disease in order to induce peripheral vasodilatation. After 72 hr of infusion, there were substantial changes in renal function, including increments of glomerular filtration rate (45%) and enhanced excretion of sodium and water. Renal blood flow was not measured. Urinary kallikrein increased dramatically, and surprisingly, plasma renin did not increase.[106] This is one of the few demonstrations of glomerular hyperfiltration induced by vasodilatory prostaglandins. Unfortunately, in the absence of measurements of renal

blood flow, it is not possible to know whether glomerular filtration rate increased because of hemodynamic or direct intraglomerular actions of the infused prostaglandin.

5.2. Interactions of Vasoconstrictor Hormones with Vasodilatory Eicosanoids

α-Adrenergic catecholamines and renal nerve stimulation cause renal vasoconstriction and renal prostaglandin synthesis. Corradi and Arendshorst increased renal nerve activity by renal venous compression, and the subsequent increase in renal vascular resistance was tripled after prostaglandin inhibition, pointing to an important dynamic role of vasodilatory renal prostaglandins to attenuate neurally induced renal vasoconstriction. After renal denervation, increases in renal venous pressure (RVR) did not increase renal vascular resistance before indomethacin, but elicited a 24% increase of RVR after renal venous compression.[107] Cooper and Malik have examined the α-adrenergic receptors that mediate adrenergic-dependent renal prostaglandin synthesis. They concluded that predominantly α-I-adrenergic receptor stimulation resulted in prostaglandin release, whereas α-II adrenoreceptors had a negligible effect, as did β-adrenergic-receptor stimulation.[108]

Vasopressin, like catecholamines, is a vasoconstrictor, but the renal vasoconstriction after infusion of AVP is always less than systemic, nonrenal vasoconstriction. Yared *et al.* evaluated this phenomenon using micropuncture techniques in anesthetized rats.[109] A pressor dose of AVP actually increased renal blood flow and reduced renal vascular resistance relative to systemic vascular resistance. If prostaglandin synthesis was blocked with indomethacin, infusion of AVP resulted in equivalent vasoconstriction in systemic and renal vascular beds.[109] Seino *et al.* infused AVP into the renal artery in anesthetized rabbits before and after prostaglandin inhibition.[110] AVP caused systemic hypertension and renal vasoconstriction, which was followed by renal vasodilatation immediately after cessation of the AVP infusion. Indomethacin potentiated both systemic and renal vasoconstriction and obliterated the post-AVP renal vasodilatation. AVP-stimulated calcium entry into vascular smooth muscle may be important in these responses, as nifedipine, a calcium channel blocker, reduced the actions of AVP to cause vasoconstriction and to increase PGE_2 synthesis.[110] These studies are consistent with the belief that AVP stimulates prostaglandin synthesis in renal blood vessels as well as in other renal cellular sites, such as glomerular mesangial and epithelial cells and renal medullary interstitial cells. This response, mediated by the V1 receptor, moderates the extent of renal vasoconstriction and the fall in GFR.

The contractile actions of ANGII on vascular smooth muscle and

mesangium are also regulated or antagonized by PGE_2 and PGI_2. Satoh *et al.* incubated renal arteries from the dog with ANGII and verified increased synthesis of PGI_2 with smaller amounts of PGE_2.[111,112] This is a calcium-dependent process, as could be surmised from prior publications, which depends on angiotensin II-stimulated calcium entry. Satoh *et al.* blocked ANGII-stimulated prostaglandin synthesis in canine renal arteries with calcium channel blockers, as well as with antagonists of calmodulin.[111] Cooper *et al.* published contrasting results obtained with the isolated perfused rat kidney.[113] In this preparation, ANGII caused vasoconstriction dependent on calcium entry (blocked by calcium channel blockers), whereas ANGII-stimulated PGE_2 and PGI_2 synthesis were mediated by intracellular calcium mobilization and calcium calmodulin (blocked by inhibitors of intracellular calcium release and calmodulin).[113] The latter results also conflict with prior renal cell culture experiments in which several laboratories had shown that ANGII augmented PGE_2 synthesis and this process was dependent on the integrity of calcium influx through calcium channels. Scharschmidt and her co-workers have

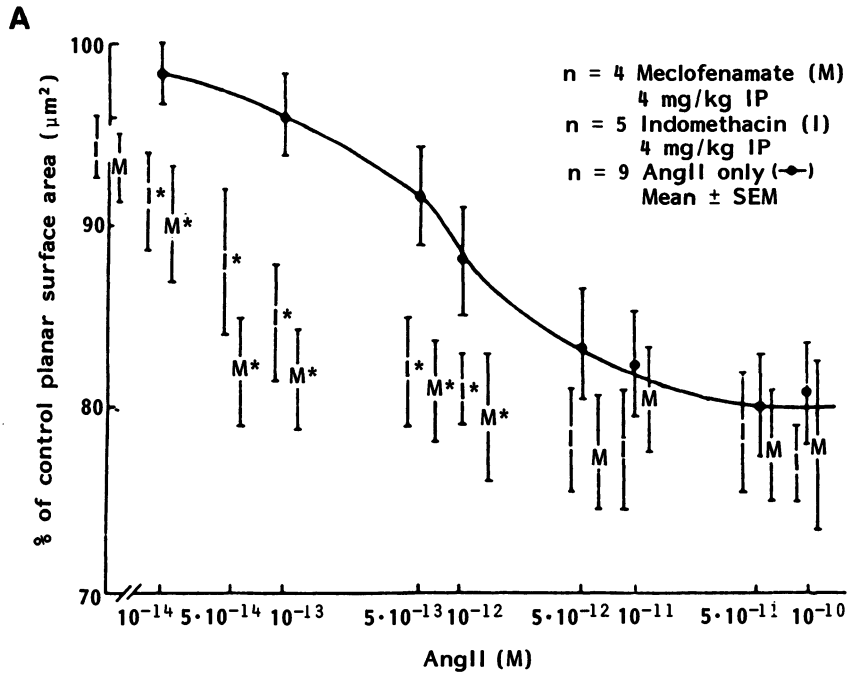

Fig. 6. Summary of experiments with isolated rat glomeruli, with measurements of planar surface area using image analysis microscopy. Increasing concentrations of ANGII caused a 20% reduction of planar surface area, and arachidonic acid antagonized this response (A), whereas indomethacin or meclofenamate potentiated the contraction (B).[114]

studied rat and human glomeruli and demonstrated glomerular contraction when ANGII is added to the media. This ANGII-mediated glomerular contraction undoubtedly was a result of mesangial contraction and was regulated by glomerular prostaglandin synthesis. Augmentation of prostaglandin synthesis with arachidonic acid, or exogenous provision of PGE_2, antagonized ANGII-stimulated glomerular contraction, whereas cyclooxygenase inhibitors potentiated the contraction.[114] Figure 6 depicts these results.

Sodium depletion and diuretics would be expected to increase plasma renin activity and ANGII, thereby attenuating renal blood flow, unless prostaglandins increase in a compensatory fashion. Izumi *et al.* depleted rats with a low-sodium diet and furosemide administration and observed that indomethacin, naproxen, and sulindac reduced glomerular filtration rate and free-water clearance. All the nonsteroidal agents inhibited

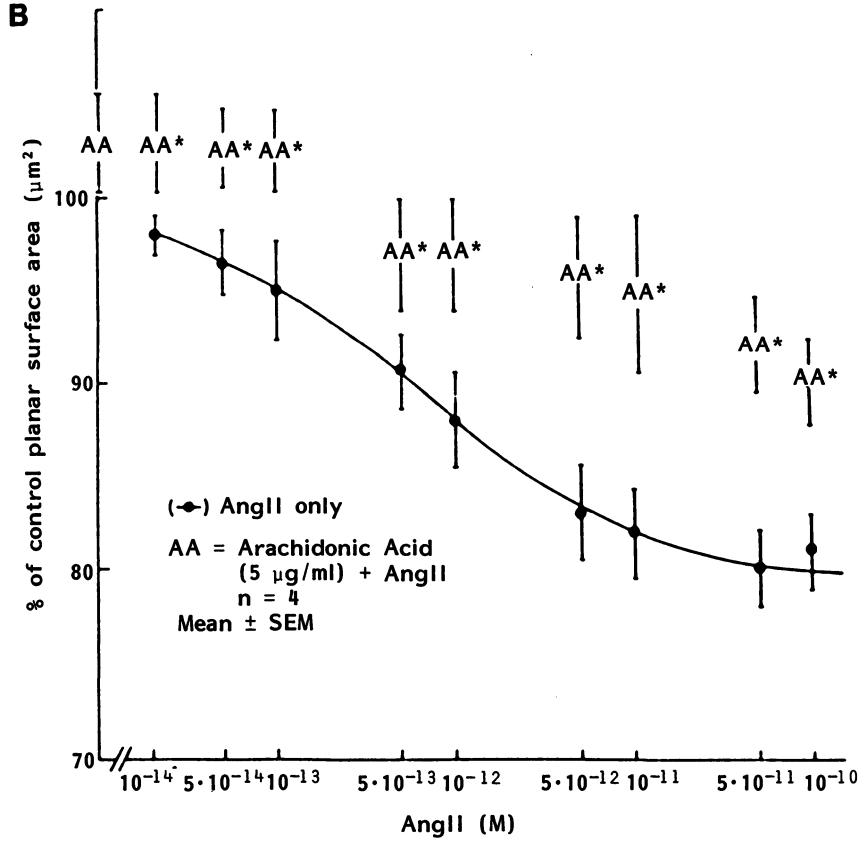

Fig. 6. *(Continued)*

urinary excretion of PGE_2.[115] It is not surprising that Usberti and coworkers, studying normal male volunteers, found that ANGII infusions reduced GFR and RPF to a greater extent after aspirin inhibition of prostaglandin synthesis. Filtration fraction rose after ANGII infusion and aspirin did not alter this response. Urinary PGE_2 increased after ANGII infusion, whereas 6-keto-$PGF_{1\alpha}$ showed equivocal changes.[55] These studies reinforce the prevalent belief that ANGII-stimulated renal PGE_2 synthesis serves an important vasoregulatory feedback role and that prostaglandin inhibition, under circumstances of increased ANGII concentrations, will reduce GFR and RPF.

5.3. The Clinical Nephrotoxicity of Nonsteroidal Antiinflammatory Drugs

The clinical dangers of nonsteroidal antiinflammatory drugs administered to selected patients are well documented. A large number of excellent reviews of this topic have appeared over the last several years. These reviews have emphasized that nonsteroidal antiinflammatory drug-induced renal dysfunction occurs only in patients with predisposing conditions, such as congestive heart failure, severe hepatic disease, chronic renal disease, arteriosclerotic cardiovascular disease in the elderly, and diuretic therapy often accompanied by sodium restriction and sodium depletion. The renal dysfunction or acute renal failure can be nonoliguric or oliguric and is almost always reversible, rarely requiring dialysis.[116–122] A peculiar interaction of triamterene, a potassium-sparing diuretic, has been suspected. Weinberg *et al.* described a patient who developed anuric acute renal failure after treatment with both indomethacin and triamterene. This patient, who had congestive heart failure, would have been at risk for treatment with indomethacin alone, but an 11-day period of anuria suggests that triamterene had an additional synergistic affect to worsen the renal compromise.[123] There is no good explanation for this deleterious interaction of triamterene with indomethacin, and it has not been reported with the other potassium-sparing agents such as spironolactone or amiloride. Favre and Vallotton found that triamterene is the only diuretic or potassium-sparing agent that stimulates renal PGE_2 excretion after oral administration.[124] Perhaps this increment of renal prostaglandin synthesis, after triamterene administration, has an important compensatory role?

Significant interest has been stimulated by claims that sulindac does not inhibit renal prostaglandin synthesis and, hence, is a renal-sparing nonsteroidal antiinflammatory drug. These claims have been based on publications showing that the active form of sulindac, sulindac sulfide, was not excreted in the urine and that urinary prostaglandins were

unaffected in patients treated with oral sulindac in conventional doses. Publications over the last 2 years have heightened, but not substantially clarified, a debate between proponents and exponents of the theory that sulindac is renal-sparing. Sedor and co-workers studied normal female volunteers and compared sulindac to indomethacin.[125] Indomethacin, but not sulindac, reduced urinary excretion of PGE_2, $PGF_{2\alpha}$, and 6-keto-$PGF_{1\alpha}$, as well as urinary sodium and chloride. There were no changes in GFR or RBF with either drug in these normal volunteers.[125] Patients with cirrhosis and ascites are notoriously sensitive to renal nephrotoxicity with nonsteroidal antiinflammatory drugs, and in a series of 15 patients, indomethacin, but not sulindac, reduced creatinine clearance and prostaglandin excretion. However, after intravenous furosemide administration, both indomethacin and sulindac reduced natriuresis and PGE_2 excretion.[126] Laffi *et al.* obtained similar results, indicating a renal-sparing action of sulindac, in patients with cirrhosis and ascites in whom they compared sulindac and ibuprofen and found that ibuprofen, but not sulindac, reduced GFR and RPF.[127] Sulindac should be administered with caution, if at all, to patients with cirrhosis and ascites since hepatobiliary excretion of the drug is impaired and plasma levels can rise to fivefold normal levels. The dangers of sulindac in liver disease were highlighted by studies by Zambraski *et al.* in dogs with biliary common bile duct obstruction. Intravenous administration of either sulindac sulfoxide, the prodrug, or sulindac sulfide, the active form of the drug, resulted in inhibition of renal prostaglandin synthesis and acute reductions of RBF and GFR.[128] Berg and Talseth administered indomethacin or sulindac to patients with chronic renal failure and a control creatinine clearance of approximately 40 ml/min. Indomethacin, but not sulindac, decreased creatinine clearance by 10 ml/min. Sulindac, as well as indomethacin, reduced PGE_2 excretion, but indomethacin had a greater inhibitory effect.[129] Ciabattoni and co-workers reached similar conclusions about the safety of sulindac in patients with chronic glomerular disease in a prospective comparison of ibuprofen versus sulindac over a 7-day treatment period. Ibuprofen reduced renal prostaglandin synthesis, RPF, and GFR, whereas sulindac had no inhibitory effects on RPF, GFR, or urinary prostaglandins.[130]

Swainson and Griffiths studied six patients with chronic renal disease and basal glomerular filtration rates of 18–48 ml/min. After 28 days of treatment with sulindac, 600 mg/day (above the recommended dose of 400 mg/day), there were negligible changes of RPF and GFR, except in one patient who did develop reductions of renal function.[131] Roberts *et al.* arrived at contrasting conclusions based on their studies of 15 normal women who received sulindac, indomethacin, or placebo. Although sulindac appeared less inhibitory than indomethacin, both agents reduced

urinary PGE_2 excretion and both agents reduced furosemide-induced natriuresis.[132] Svendsen *et al.* compared sulindac and naproxen in patients with rheumatoid arthritis and mild heart failure. Both drugs, administered for 14 days, similarly decreased renal prostaglandin excretion without adverse effects on renal function.[133] Although sulindac may be relatively renal-sparing, it should be emphasized that acute renal failure has been reported in elderly patients receiving sulindac in large doses, particularly when the patient's age is taken into consideration.[117,119] Taking into consideration the prior literature not reviewed in this chapter, I believe that sulindac has less effect on renal prostaglandin synthesis than other clinically used nonsteroidal antiinflammatory drugs, and hence, it is relatively renal-sparing and the safest nonsteroidal antiinflammatory drug in situations of enhanced risk due to ineffective circulating plasma volume, old age, or concurrent therapy with diuretics.

5.4. Summary

The vasodilatory eicosanoids, PGE_2 and PGI_2, increase renal blood flow and glomerular filtration rate by reducing renal vascular resistance and possibly through direct intraglomerular actions. Leukotrienes, especially LTC_4 and LTD_4, and TxA_2 are renal vasoconstrictors and reduce RPF and GFR. The vasodilatory actions of PGE_2 and PGI_2, as well as the vasoconstrictor consequences of LTC_4 and TxA_2, are restricted to circumstances of disease and physiologic perturbation. Cyclooxygenase inhibition, with reduction of PGE_2 and PGI_2, has no significant consequences in healthy subjects, and inhibition of TxA_2 synthesis likewise does not alter renal function in normal subjects. Vasoconstrictor compounds, including catecholamines, ANGII, and AVP, stimulate renal PGE_2 and PGI_2 synthesis, which act as vasomodulators. Clearly, nonsteroidal antiinflammatory drugs potentiate the vasoconstrictor action of these agonists on the kidney. Patients at risk for nonsteroidal antiinflammatory drug-induced acute renal failure generally have high plasma levels of catecholamines, AVP, and ANGII. Risk groups include patients with cardiac, hepatic, and renal disease and elderly patients, especially those with diffuse arteriosclerotic cardiovascular disease who are receiving diuretic therapy. The renal compromise is rapidly reversible and is usually accompanied by hyperkalemia disproportionate to the elevation of serum creatinine. Sulindac, when compared with other nonsteroidal antiinflammatory drugs, often has been shown to have less or minimal prostaglandin inhibitory capacity in the kidney, and sulindac preserves RPF and GFR in most circumstances in which other drugs reduce these parameters.

6. Eicosanoids and Renal Disease

It is understandable that research interest has been focused on the beneficial as well as detrimental effects of eicosanoids in renal disease, as PGE_2 and PGI_2 increase RPF and GFR, whereas TxA_2 and leukotrienes can decrease renal function. Therefore, endogenous glomerular production of PGE_2 has been of interest in diabetes mellitus, and exogenous administration of PGE_2 or PGI_2 has been evaluated as therapy for ischemic acute renal failure. Contrariwise, a possible pathophysiologic role of TxA_2 has been carefully examined in diverse conditions ranging from renal transplant rejection to ureteral obstruction. These studies have been enhanced by the growing availability of drugs that are rather selective inhibitors of TxA_2 synthesis.

6.1. Immunologic Glomerular Disease

The major focus of attention within the area of immunopathologic glomerular injury and eicosanoids has been on the deleterious role of increased TxA_2 and also on the possible beneficial effects of enhanced glomerular PGE_2.[134] Stork and Dunn have evaluated glomerular PGE_2 and TxA_2 synthetic rates and the effects of either TxA_2 synthesis inhibition or cyclooxygenase inhibition on RPF and GFR in nephrotoxic serum nephritis in rats.[135] Contrary to our prior work, which showed beneficial effects of TxA_2 inhibition within 3 hr of induction of nephrotoxic serum nephritis, we found that 24 hr and 14 days after initiation of nephrotoxic serum injury, TxA_2 inhibition with two different inhibitors did not enhance RPF or GFR. These negative results were reinforced by superimposing pharmacologic blockade of the thromboxane receptor, which did not unmask any pathophysiologic role of TxA_2. Despite significant increases of glomerular TxA_2 synthesis, 14 days after administration of rabbit antirat glomerular basic membrane antibodies, GFR was normal and RPF increased in association with 10-fold enhancement of glomerular PGE_2 production. Acute inhibition of cyclooxygenase with either meclofenamate or indomethacin caused a 50% decrease in both RPF and GFR in these animals, as shown in Fig. 7. We concluded that despite 10- to 15-fold increments of glomerular PGE_2 and TxA_2 in rats with nephrotoxic serum nephritis, the major hemodynamic effects were mediated by PGE_2 and not by TxA_2.[135]

Lianos *et al.* have further studied glomerular eicosanoid metabolism in rat nephrotoxic serum nephritis and have found substantial increases of arachidonate lipoxygenation to 12-hydroxyeicosatetraenoic acid, a fatty acid that has chemotactic and proinflammatory properties which

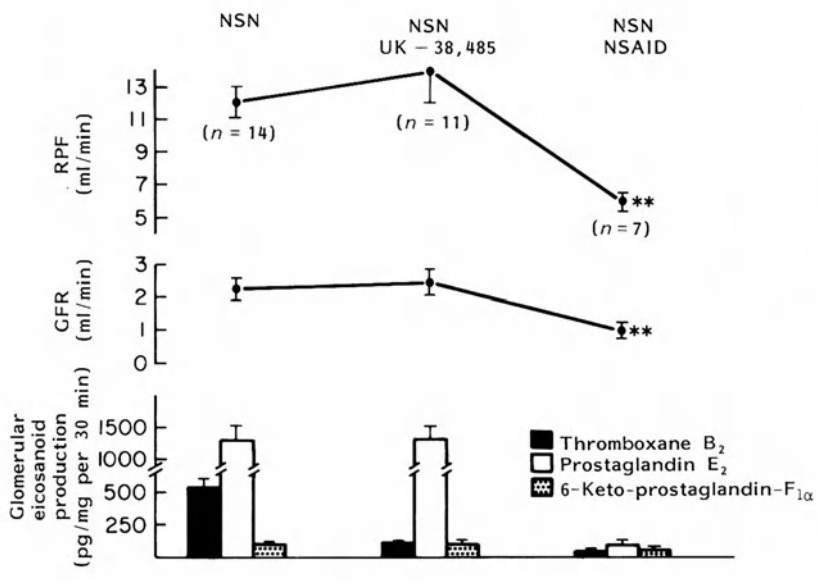

Fig. 7. The effects of thromboxane synthetase inhibition with UK38485 and cyclooxygenase inhibition with indomethacin or meclofenamate (NSAID) in a rat model of nephrotoxic serum nephritis (NSN). RPF and GFR fell significantly after prostaglandin inhibition, but not after thromboxane A_2 inhibition on day 14 of nephrotoxic serum nephritis.[135]

could be important in the mediation of glomerular immune injury.[136] Kelley and co-workers have continued to pursue the role of eicosanoids in murine lupus erythematosus. Using the MRL-lpr murine model of lupus erythematosus, they have confirmed the beneficial effects on the immunologic presentation of the disease, both renal and extrarenal, of increased dietary fish oil, which is composed of eicosapentaenoic acid and docosahexaenoic acid. Fish oil treatment reduced renal dienoic prostaglandins, especially PGE_2 and TxB_2, and may have enhanced trienoic prostaglandin production.[137] It is unknown whether the beneficial effects of fish oil in murine models of lupus erythematosus are secondary to reduction of dienoic thromboxane, and possibly leukotrienes, or whether these changes are coincidental to some other biochemical alteration. Steinhauer *et al.* used another murine lupus model, the NZB-NZW F1 hybrid mouse, to demonstrate that dietary supplementation with histidine or zinc substantially reduced the development of glomerulonephritis and was accompanied by reductions of renal PGE_2 and TxB_2.[138] Kher and co-workers have used an alternative model of immune complex glomerulonephritis induced by the intraperitoneal injection of apoferritin. Apoferritin-injected animals develop chronic glomerular changes due to immune complex deposition within the glomeruli and glomerular

cellular proliferation. If dietary linoleic acid was supplemented, the high-linoleic acid diet reduced proteinuria and renal histologic damage and retarded the development of glomerulonephritis. Whether these changes were due to conversion of linoleic acid to arachidonic acid and subsequent alterations of dienoic prostaglandin synthesis was equivocal.[139]

Saito *et al.* have used a model of immune complex glomerulonephritis induced by the injection of bovine serum albumin in rabbits. Benzylimidazole, an inhibitor of thromboxane A_2 synthesis, was administered to rabbits throughout the course of immunization with bovine serum albumin. The imidazole derivative reduced proteinuria, glomerular infiltration with polymorphonuclear leukocytes and monocytes, and glomerular fibrin deposition.[140] Clearly, it is impossible to determine whether TxA_2 inhibition in platelets and circulating white blood cells had a dominant effect in this model or whether the benzylimidazole was acting within the glomerulus to reduce TxA_2. These renal and extrarenal effects of TxA_2 synthesis are not mutually exclusive, and both may be important. Figure 8 provides a theoretical explanation of the actions of eicosanoids in renal immune injury.

Patrono and colleagues have conducted a clinical investigation of the role of renal eicosanoids in 23 female patients with systemic lupus

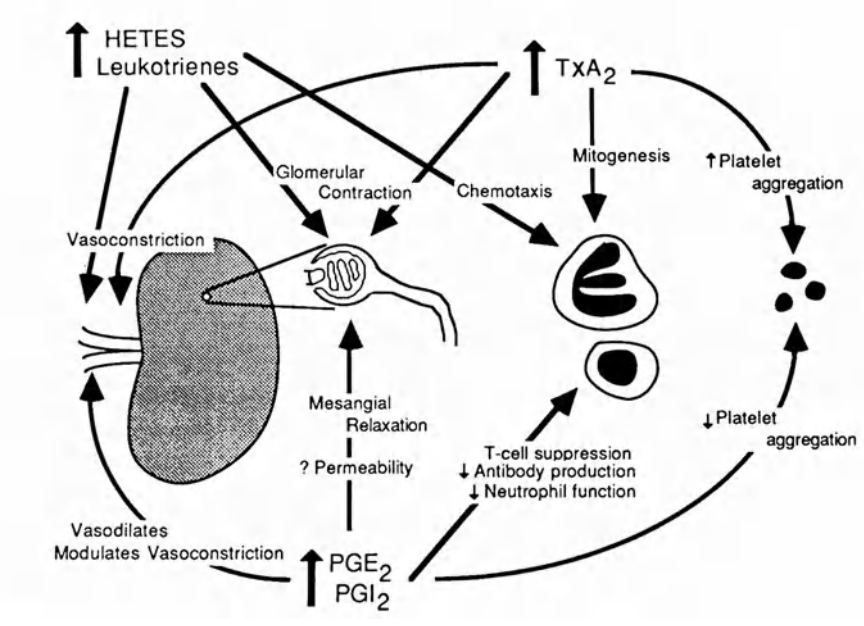

Fig. 8. Summary of the major actions of eicosanoids, derived from both cyclooxygenase and lipoxygenase enzymes, in the mediation of, or modulation of, renal immune injury.[134]

erythematosus, 16 patients with chronic glomerular disease, and 20 healthy control women.[141] Patients with systemic lupus erythematosus had increased urinary TxB_2 and PGE_2 excretion and decreased 6-keto-$PGF_{1\alpha}$, the metabolite of PGI_2. Patients with more active glomerulonephritis on renal biopsy showed a higher TxA_2-to-PGI_2 urinary ratio. Chronic glomerulonephritis was accompanied by reduced 6-keto-$PGF_{1\alpha}$ excretion with no change of TxB_2. These changes of urinary eicosanoids were thought to be a reflection of altered renal synthesis since platelet thromboxane generation was equivalent in all groups, and the excretion of the systemic thromboxane metabolite, 2,3-dinor TxB_2 was unaltered. In the lupus patients, the GFR was inversely correlated with TxB_2 excretion, whereas 6-keto-$PGF_{1\alpha}$ was positively correlated with both GFR and RPF. Inhibition of cyclooxygenase with ibuprofen acutely reduced GFR and RPF, as well as urinary TxB_2 and 6-keto-$PGF_{1\alpha}$, in patients with lupus glomerulonephritis and nonlupus glomerulonephritis, pointing to important hemodynamic effects of these glomerular eicosanoids.[141] These deleterious effects of nonsteroidal antiinflammatory drugs contrast with the beneficial action of cyclooxygenase inhibitors to reduce proteinuria in the nephrotic syndrome. Vriesendorp *et al.* compared indomethacin and naproxen in 10 nephrotic patients. Indomethacin, more than naproxen, reduced proteinuria and prostaglandin excretion. GFR and RPF also fell, suggesting to these authors that the reduction in proteinuria, which is acute and reversible, was mediated by a reduction of the glomerular transcapillary hydraulic pressure and not by any intrinsic alterations of the underlying renal disease.[142] These same investigators have compared sulindac to indomethacin, diclofenac, and flurbiprofen for their therapeutic efficacy to decrease proteinuria in nephrotic patients. Reductions of proteinuria and GFR correlated closely with inhibition of PGE_2 excretion; sulindac reduced neither PGE excretion nor proteinuria; and indomethacin was the most potent agent of the other three drugs.[143]

6.2. Diabetes and Renal Prostaglandins

Schambelan and co-workers have measured glomerular eicosanoid turnover in rats with streptozotocin-induced diabetes mellitus. Glomerular synthesis of PGE_2, $PGF_{2\alpha}$, and TxB_2 was increased in the diabetic rats, and insulin therapy prevented these alterations. Hyperglycemia did not appear to have a direct effect, since glomerular eicosanoid synthesis did not correlate with the extent of hyperglycemia and *in vitro* alterations of glucose did not alter glomerular prostaglandin synthesis.[144] This study left unanswered whether glomerular eicosanoids had any hemodynamic actions in diabetic glomerulosclerosis. Quilley and McGiff measured uri-

nary eicosanoid excretion in diabetic rats and found progressive reductions of PGE_2 and increases of TxB_2 and 6-keto-$PGF_{1\alpha}$ over a 6-month study.[145] These results are not inconsistent with those of Schambelan *et al.*, since urinary excretion of these eicosanoids is derived mostly from the renal medulla and increased glomerular prostaglandin and thromboxane synthesis may not be detectable in 24-hr urine samples.

The interrelations of hyperglycemia, diabetes mellitus, and renal prostaglandin synthesis have been evaluated by Kasiske *et al.* using isolated perfused rat kidneys obtained from normal and diabetic rats. When perfusate glucose was increased, there were parallel changes of GFR, which were partially prevented by prostaglandin inhibition.[146] Glucose-induced changes of renal prostaglandin synthesis were not documented, and it should be noted that cyclooxygenase inhibitors reduce GFR and RPF in all isolated, perfused rat kidney preparations and may well diminish the responsiveness of these kidneys to any type of stimulus. There are few studies in humans of the importance of renal eicosanoids in diabetes mellitus. Esmatjes *et al.* studied 21 patients with insulin-dependent diabetes mellitus and 15 control subjects. As expected, patients with diabetes mellitus had increased RPF and GFR, decreased plasma norepinephrine and plasma renin activity, and no significant differences in the urinary excretion of PGE_2, 6-keto-$PGF_{1\alpha}$, or kallikrein. Acute inhibition of renal prostaglandin synthesis with intravenous lysine acetylsalicylate, in seven diabetic patients, reduced RPF from 640 to 535 ml/min and GFR from 170 to 150 ml/min. The authors attribute the renal hyperperfusion and hyperfiltration to an imbalance between vasodilatory PGE_2 and PGI_2 and vasoconstrictor catecholamines and angiotensin.[147]

6.3. Renal Transplant Rejection

Since the initial reports that rejection of human renal allografts was associated with increased urinary TxB_2 excretion, investigators have focused on experimental models of renal transplant rejection in order to assess the relative importance of TxA_2 in this process. Coffman *et al.* transplanted kidneys across rat species and, by the third day after transplantation, had histologic and functional changes of severe rejection. *Ex vivo* perfusion of the renal allografts showed enhanced TxB_2 release without changes of PGE_2 and 6-keto-$PGF_{1\alpha}$. Acute inhibition of TxA_2 synthesis increased GFR and RPF to values approximately one-third and one-half of native control values. These results indicate that TxA_2, generated within the kidney by invading leukocytes and platelets or synthesized by renal cells, is one of the mediators of renal compromise in transplant rejection.[148] A beneficial effect of PGI_2 infusions for transplant rejection has been suggested by prior publications. Campbell *et al.*

infused PGE_1 into the canine renal artery in order to prevent renal transplant rejection. Continuous infusion of PGE_1 into the renal artery did not prevent allograft failure, but did alter the nature of the cellular infiltrate from predominantly lymphocytic in the untreated controls to polymorphonuclear leukocytes in the PGE_1-treated kidneys.[149] The canine renal transplant, similar to rat transplants, shows increased TxB_2 and PGE_2 synthesis by renal cortical slices incubated *in vitro* during transplant rejection 3 days after transplantation.[150] In clinical studies, Steinhauer *et al.* confirmed the original work of Foegh by showing that urinary TxB_2 was increased in approximately 90% of episodes of renal transplant rejection (24 rejection crises in 34 patients).[151] The increase in urine TxB_2 excretion generally preceded clinical manifestations of transplant rejection by 2 days, suggesting that it is at least a marker of rejection and may be an important mediator of reduced renal function. The pathophysiologic role of thromboxane will be better understood when pharmacologic agents are used clinically which inhibit TxA_2 synthesis from prostaglandin endoperoxides and block TxA_2 receptors.

6.4. Acute Renal Failure

Possible beneficial effects of vasodilatory prostaglandins have been evaluated in ischemic acute renal failure. These therapeutic evaluations seem logical since the initial phase of ischemic acute renal failure is associated with substantial reductions of RPF. Tobimatsu *et al.* administered PGE_1 to dogs after 1–2 hr of complete renal arterial occlusion. PGE_1 administration improved renal cortical blood flow and GFR and prevented tubular necrosis.[152] Similar findings have been reported by Neumayer *et al.*, who treated ischemic acute renal failure with PGE_2 infusions in conscious chronically instrumented dogs. Renal function was measured at 1, 3, and 7 days after complete occlusion of the renal artery, and animals who had a continuous intraaortic infusion of PGE_2 had significantly higher RBF and GFR as well as decreased plasma concentrations of creatinine and urea.[153] Lifschitz and Barnes administered PGI_2 to rats before and after 40 min of renal arterial clamping. If PGI_2 was combined with volume expansion by Ringer's solution, renal injury was remarkably reduced, with preservation of GFR to 50% of normal and minimal tubular necrosis with cast formation.[154] These results, taken together, show that vasodilatory prostanoids, whether PGE_1, PGE_2, or PGI_2, in some way exert a beneficial or protective action in ischemic acute renal failure. These salutary effects may be a result not only of renal vasodilatation and increased RPF, but also of a cytoprotective effect for renal tubular epithelial cells analogous to the cytoprotective effect of prostaglandins for gastric mucosa.

A possible deleterious role of renal TxA_2 in ischemic acute renal failure was studied by Lelcuk *et al.*, who documented acute increments of renal venous TxB_2 concentration after 45 min of renal arterial occlusion in the rat. If animals were treated with a TxA_2 synthesis inhibitor, the increments of renal venous plasma TxB_2 were prevented and serum creatinine concentrations remained normal, as did renal histology. Pretreatment with ibuprofen, which would decrease both PGE_2–PGI_2 and TxA_2, had a deleterious effect, suggesting to these authors that a high PGI_2/TxA_2 ratio is protective against renal ischemia.[155] No clinical trials have been reported in which PGE_1 or PGI_2 was infused or TxA_2 synthesis inhibitors were utilized. The experimental results in animals are sufficiently encouraging that human studies should be initiated.

6.5. Ureteral Obstruction

Ureteral obstruction, especially in the rabbit, augments renal PGE_2 and TxA_2 synthesis secondary to interstitial infiltration of the obstructed kidney by mononuclear cells. TxA_2 inhibition has been shown to improve function after relief of the ureteral obstruction. Lefkowith *et al.* have examined the importance of monocyte–macrophage infiltration in the rabbit kidney after unilateral ureteral obstruction. By employing endotoxin, which is a macrophage agonist, they measured dramatic increases in eicosanoid synthesis by the perfused kidney. Nitrogen mustard blocked this effect, presumably by macrophage depletion. These studies reinforce the conclusion that renal injury induced by ureteral obstruction is followed by interstitial infiltration with monocytes–macrophages, which can produce substantial amounts of TxA_2 and also may stimulate renal synthesis of eicosanoids.[156] These interstitial macrophages may also account for the potent stimulatory effect of platelet-activating factor on PGE_2 and TxB_2 release from the hydronephrotic kidney. Although normal kidneys respond to platelet-activating factor with increased eicosanoid release, hydronephrotic kidneys have an accentuation of this response.[157]

The pathophysiologic role of thromboxane in ureteral obstruction was examined by Ichikawa *et al.* in rats with bilateral ureteral obstruction in which one ureter was released. Although ANGII played an important role to reduce RPF and GFR, high-protein-fed animals showed substantially greater increments of renal vascular resistance and decrements of single-nephron GFR and plasma flow rate than low-protein-fed animals. Thromboxane apparently mediated these changes, as acute inhibition of TxA_2 synthetase increased both single-nephron plasma flow and GFR as well as the glomerular capillary ultrafiltration coefficient in high-protein-fed rats. It is unknown why high but not low protein intake

would unmask or induce TxA_2-mediated vasoconstriction.[158] Thromboxane-mediated decrements of the glomerular ultrafiltration coefficient are consistent with our understanding that the glomerular mesangial cell has receptors for TxA_2 which can induce mesangial contraction and thereby a possible decrement in the ultrafiltration coefficient because of reductions in filtration surface area.

6.6. Miscellaneous Renal Disease

Experimental proteinuria in the nephrotic syndrome, induced by Adriamycin in rats, was associated with increased glomerular TxB_2 synthesis 14 and 30 days after induction of the disease.[159] When Remuzzi and his co-workers treated these animals with a TxA_2 synthetase inhibitor, the proteinuria was reduced by approximately 50%, albeit to levels that were still significantly elevated. Urinary TxB_2 excretion was increased in the nephrotic rats, and the TxA_2 synthesis inhibitor reduced the urinary excretion of TxB_2 to normal.[159] Since this is a noninfiltrative and nonproliferative model of the nephrotic syndrome, induced by glomerular epithelial cell injury, the putative role of thromboxane must be directly related to glomerular synthesis of TxA_2 and an intraglomerular action of TxA_2 within the glomerulus, on protein filtration. Purkerson and co-workers have asked a similar question in rats with subtotal nephrectomy; namely, does TxA_2 alter renal function and would inhibition of TxA_2 synthesis be beneficial? Rats with subtotal nephrectomy (greater than 70% renal ablation) have hypertension, proteinuria, and glomerular sclerosis. Chronic oral treatment with OKY1581, a TxA_2 synthesis inhibitor, improved RPF and GFR, decreased blood pressure, protein excretion, and thromboxane excretion, and preserved renal histology. OKY1581 also reduced platelet aggregation and TxA_2 production. These investigators attributed the beneficial effects of thromboxane inhibition to the antiplatelet action rather than to an intrarenal inhibition of thromboxane.[160] Schwartz *et al.* have reported that partial renal vein constriction in the rabbit induces changes of arachidonic acid metabolism similar to unilateral ureteral obstruction. Isolated perfused rabbit kidneys, with prior partial renal venous constriction, released increased amounts of PGE_2 and TxB_2 which was stimulable with either bradykinin or endotoxin. The tentative sources of these eicosanoids are interstitial fibroblasts and/or macrophages. Whether the released eicosanoids have hemodynamic actions *in vivo* is unproved.[161]

Many studies have appeared of the hepatorenal syndrome and alterations of renal eicosanoid synthesis and excretion in patients with cirrhosis and ascites. Prior to the development of the hepatorenal syndrome, PGE_2 excretion remains normal or increases, whereas during

the incipient stages of renal failure and the hepatorenal syndrome, urinary PGE_2 decreases and TxB_2 increases. Parelon *et al.* have expanded on these findings by showing that the decrement of urinary PGE_2 and increment of urinary TxB_2 in cirrhotics with the hepatorenal syndrome is accompanied by a 50% decline in plasma arachidonic acid. Whether plasma arachidonate serves directly as substrate for renal eicosanoids or whether it must be initially incorporated into membrane phospholipids is a controversial matter. Nonetheless, patients with severe hepatic failure and renal compromise who have renal vasoconstriction and decreased GFR generally have high urinary thromboxane excretion.[162] Because of this, Zipser *et al.* have assessed the therapeutic value of dazoxiben, a TxA_2 synthesis inhibitor, in patients with alcoholic liver disease and renal failure. Although dazoxiben reduced urinary thromboxane to normal, without increasing PGE_2 or 6-keto-$PGF_{1\alpha}$ excretion, there was no improvement in creatinine clearance in these patients.[163] Definitive negative evidence about the role of TxA_2 in the hepatorenal syndrome requires combined treatment with a TxA_2 receptor antagonist as well as a TxA_2 synthetase inhibitor, since the TxA_2 precursor, prostaglandin endoperoxides, can activate the thromboxane receptor.

6.7. Summary

Glomerular immune injuries of diverse types increase glomerular PGE_2 and TxA_2 synthesis; the importance of these eicosanoids varies with the stage of the experimental nephritis. Acutely, TxA_2 may mediate renal vasoconstriction after glomerular immune injury, whereas, after several hours, PGE_2 exerts a dominant effect clearly unmasked when cyclooxygenase inhibitors are administered and acute reductions of RPF and GFR ensue. Dietary alteration of renal eicosanoids by substituting fish oil for a conventional diet substantially reduces the renal injury in experimental lupus erythematosus and also reduces biologically active thromboxane concentrations. The hypothesis has evolved that the balance between TxA_2 on the one hand and PGI_2 and PGE_2 on the other is important in lupus glomerulonephritis and perhaps in other models of glomerular immune injury. Clinical trials of thromboxane synthesis and/or receptor inhibitors in patients with SLE have potential merit. Many forms of renal injury are improved or prevented by inhibition of TxA_2 synthesis. These conditions include renal transplant rejection, Adriamycin nephrosis, progressive injury to remnant nephrons, acute renal failure, and possibly ureteral obstruction. Whether similar results could be obtained by dietary supplementation with fish oil rich in eicosapentaenoic acid and docosahexaenoic acid is untested. The cumulative evidence is excellent, showing a beneficial effect of prostaglandin infusions

in acute renal failure especially secondary to ischemia. Treatment with PGE_1, PGE_2, or PGI_2 reduces postischemic renal injury and improves RPF and GFR in these models. Again, the evidence seems sufficient to warrant clinical trials of PGE_1 or PGI_2 in acute renal failure.

7. Hypertension, Prostaglandins, and Thromboxane

The focus of experiments on the interactions of prostaglandins and thromboxane with the control of blood pressure has emphasized renal arterial hypertension in animals, the role of TxA_2 in spontaneously hypertensive rats (SHR), and clinical studies of prostaglandin excretion in different types of human essential hypertension. Clinical studies have included an evaluation of the interaction of nonsteroidal antiinflammatory drugs with antihypertensive therapy. The experimental models in animals have not revealed any dominant or important role for prostaglandins or thromboxane in the genesis of or maintenance of hypertension. The role of prostaglandins in human hypertension is not entirely clear, but they also appear to be of minor importance. The deleterious effects of nonsteroidal antiinflammatory drugs on the control of hypertension seem closely linked to interference with renal sodium excretion. Grone and Dunn have recently reviewed this topic.[164]

7.1. Experimental Studies in Animal Models of Renal Artery Stenosis

Vandongen and O'Dwyer have evaluated renal prostaglandin excretion in both two-kidney, one-clip and one-kidney, one-clip hypertension in the rat. In two-kidney, one-clip hypertension, the urinary excretion of 6-keto-$PGF_{1\alpha}$ and PGE_2 was not different from that in two-kidney, one-clip rats that remained normotensive. Three weeks' administration of indomethacin to the normotensive group did not increase blood pressure.[165] The one-kidney, one-clip rat model of hypertension was also evaluated by Vandongen *et al.*, and urinary 6-keto-$PGF_{1\alpha}$ and PGE_2 were not increased during the hypertensive phase. After unclipping, blood pressure rapidly returned to normal, and the urinary excretion of 6-keto-$PGF_{1\alpha}$ increased threefold and PGE_2 increased twofold.[166] Other studies by this group have cast doubt on the physiologic importance of renal PGI_2 and PGE_2 synthesis.[167] If rats are maintained on diets rich in fish oil, they reduce the excretion of the dienoic prostaglandins PGE_2 and 6-keto-$PGF_{1\alpha}$; however, after 4 weeks of such dietary treatment, one-kidney, one-clip hypertensive rats had no changes in the level of

their hypertension, and after unclipping, their blood pressure returned to normal in a fashion similar to hypertensive animals maintained on a conventional intake of linoleic acid.[167]

In another study, Codde *et al.* showed that dietary supplementation with linoleic acid (sunflower seed oil) or linolenic acid (linseed oil) had similar small vasodepressor effects on the hypertension in one-kidney, one-clip rats despite stimulation of renal and vascular prostaglandin synthesis by the linoleic acid diet and suppression of prostaglandin synthesis by the linolenic diet.[168] Taken together, these studies do not point to an important role for vascular or renal prostaglandins in the control of blood pressure during renal artery stenosis or after unclipping and correction of the hypertension. In a canine model of two-kidney, one-clip hypertension, urinary PGE_2 excretion was increased in the basal state and increased further after ANGII infusion.[169] In these studies, Watson and co-workers found different renal responses to ANGII with reductions of GFR, RPF, and sodium excretion in normotensive control animals and no changes or slight increments in these measurements in the hypertensive dogs. The results were attributed to either changes in ANGII receptors or the increased renal PGE synthesis in the hypertensive dogs.[169] Changes of renal prostaglandin excretion in experimental hypertension are probably a result of alterations of medullary prostaglandin synthesis. Taverner *et al.* have selectively destroyed the rat renal papilla using bromethylamine, a compound that chemically destroys the renal papilla, resulting in polyuria, no change in GFR, and 75% reductions in the urinary excretion of prostaglandins.[38] Although renal papillary destruction of at least 50% of tissue caused hypertension, this procedure did not accentuate the extent of hypertension after renal artery constriction. Chemical papillectomy did reduce the decrement in blood pressure after unclipping. The authors concluded that the renal medulla synthesizes and releases a vasodepressor substance, perhaps prostaglandins and/or vasodepressor renal medullary lipid, which regulates blood pressure in the rat.[38] Stahl *et al.* have measured glomerular eicosanoid synthesis by glomeruli obtained from two-kidney, one-clip hypertensive rats. Glomeruli from the clipped kidneys synthesized greater amounts of 6-keto-$PGF_{1\alpha}$, PGE_2, and TxB_2 than glomeruli from the contralateral kidney. Acute administration of indomethacin either reduced GFR by 50% or caused anuria in the ischemic kidney, but blood pressure actually decreased by 20 mm Hg. These data suggest that glomerular PGE_2 and PGI_2 served an important vasoregulatory role within the ischemic kidney, but did not influence blood pressure regulation.[170] Dusing *et al.* altered both sodium intake and linoleic acid intake in normal rats. As expected, low sodium intake and linoleic acid supplementation augmented renal prostaglandin synthesis in innermedullary homoge-

nates, whereas blood pressure increased in sodium-loaded animals with restricted linoleic acid intake and, hence, with decreased renal prostaglandin excretion. The authors attributed the increments in blood pressure to reductions in renal sodium excretion that were secondary to decreases in renal prostaglandin synthesis.[171]

7.2. The Role of TxA_2 in Experimental Hypertension

Prior studies have reported increased glomerular TxA_2 in SHR. In addition, positive results have been published showing a vasodepressor effect of inhibitors of TxA_2 synthesis. Shibouta *et al.* have continued to investigate this issue and found that a TxA_2 synthetase inhibitor, CV-4151, administered orally to young SHR would delay the onset of hypertension but did not affect the eventual level of blood pressure, nor did the TxA_2 inhibitor reduce blood pressure in 18-week SHR. Treatment with CV-4151 significantly reduced renal synthesis of TxA_2 and increased PGI_2. Despite these changes in vasoconstrictor–vasodilator autacoid balance, one must conclude that TxA_2 plays only a small role during the onset phase of hypertension in 4- to 6-week-old SHR.[172] Grone *et al.* treated SHR with UK-38485 to inhibit TxA_2 synthesis and also administered a TxA_2 receptor antagonist EP-092.[173] Despite greater than 75% inhibition of glomerular TxB_2 synthesis in both acute and chronic studies, these agents did not reduce blood pressure or increase RPF or GFR in these hypertensive rats. Renal vascular resistance and sodium excretion were unaltered by TxA_2 synthetase inhibition. No endoperoxide shunting, as measured by changes of glomerular PGI_2 or PGE_2 synthesis, could be documented. As a result of these studies, we concluded that enhanced renal or extrarenal TxA_2 synthesis does not contribute to the disordered blood pressure regulation in young SHR.[173] Uderman *et al.* have reported contrasting results using the same thromboxane synthetase inhibitor, UK-38485. Adult SHR treated with UK-38485 for 4 days had a maximum decrease in blood pressure of 25 mm Hg with no evidence of enhanced PGI_2 synthesis.[174] Martineau *et al.* have searched for alterations of renal and extrarenal prostaglandin production in SHR. Using urinary PGE_2 as a measure of renal PGE_2 synthesis and urinary 2,3-dinor 6-keto-$PGF_{1\alpha}$ as a measure of extrarenal PGI_2 synthesis, these investigators found reduced urinary excretion of PGE_2 in SHR and defective increments of the PGI_2 metabolite during salt loading in SHR compared to normotensive controls. These results may point to an important role of renal PGE_2 synthesis and extrarenal role of PGI_2 synthesis in the capacity to handle a sodium load and regulate blood pressure responses to increases of sodium intake.[175]

7.3. Role of Prostaglandins in Essential Hypertension in Humans

Human essential hypertension has been associated with either normal or reduced renal prostaglandin synthesis measured as the urinary excretion of PGE_2, PGF_2, and 6-keto-$PGF_{1\alpha}$. Many investigators have substantiated these findings either in the basal state or after furosemide stimulation of renal prostaglandin synthesis. Scherer *et al.* have compared PGE_2 excretion in 25 normotensive controls and 81 essential hypertensive patients and documented reduced PGE_2 excretion in hypertension 15 min after i.v. administration of furosemide. In addition, they observed that low renin essential hypertensive patients had greater reductions of urinary PGE_2 than the normal renin essential hypertension group. The reduced renal PGE_2 synthesis may cause the defective renin secretion in these older patients with low renin essential hypertension.[176] Mackenzie and co-workers also noted that older hypertensive men had lower PGE_2 excretion than younger hypertensives and normotensive controls. Older normotensive patients did not have reduced urinary PGE_2.[177] Kovatz *et al.* measured urinary PGE_2 excretion in normotensive and hypertensive pregnancy as well as in toxemia. During hypertensive pregnancy, women increased urine PGE_2 above the already increased values in normotensive pregnancy, but with the development of toxemia, renal PGE_2 excretion fell to values one-third of those observed in hypertensive pregnancy and one-half of the normotensive pregnancy excretory rate. Urinary TxB_2 was not measured. Whether these results demonstrate cause or effect is unknown, and no pharmacologic manipulation of cyclooxygenase or thromboxane synthetase was attempted.[178]

7.4. The Interactions of Nonsteroidal Antiinflammatory Drugs with Antihypertensive Therapy

It has been well documented that coadministration of indomethacin with diverse antihypertensive agents consistently increases both systolic and diastolic pressure by 5–15 mm Hg. Drugs whose antihypertensive effects appear to be attenuated by concomitant administration of indomethacin included β-adrenergic blocking agents, thiazide diuretics, converting enzyme inhibitors, hydralazine, and various combinations of the above, thereby making it unlikely that these interactions reflect a specific pharmacokinetic effect rather than a result of prostaglandin inhibition in blood vessels and the kidney. Recent studies have addressed the issue as to whether sulindac had comparable deleterious effects on antihypertensive controls when compared with other nonsteroidal antiinflammatory agents. These studies can be summarized by stating that sulindac did not attenuate the hypotensive effects of thiazides of β-

adrenergic blocking agents, captopril, or various combination therapeutic programs. Studies by Salvetti *et al.* showed no negative interaction between sulindac and beta blockers or captopril, and sulindac had no effect on urinary excretion of PGE_2 and 6-keto-$PGF_{1\alpha}$.[179] Wong *et al.* compared placebo, sulindac, naproxen, and piroxicam in 20 treated hypertensive patients and found significantly higher diastolic blood pressures and lower urinary 6-keto-$PGF_{1\alpha}$ excretory rates after naproxen or piroxicam therapy for 4 weeks when compared to similar 1-month therapy with sulindac.[180] Since sulindac inhibits extrarenal vascular prostacyclin production, the lack of a hypertensive effect when compared with other cyclooxygenase inhibitors argues in favor of the importance of renal prostaglandin synthesis and renal excretion of sodium as an explanation for the results. This conclusion is reinforced by the observations of Trimarco *et al.* that indomethacin as well as ibuprofen increased blood pressure in untreated hypertensive patients in response to a salt load, whereas sulindac had no effects.[72]

7.5. Summary

Studies of essential hypertension in rats (SHR) or experimental models of renal artery stenosis have not shown a significant role of either renal PGE_2 or TxA_2 in the control of blood pressure or renal function. Manipulation of dietary fatty acids may reduce blood pressure in hypertensive animals regardless of the effects on prostaglandin synthesis, since supplementation with different fatty acids, which either decrease or increase vascular and renal prostaglandin production, will reduce blood pressure. Inhibition of TxA_2 synthesis as well as blockade of TxA_2 receptors does not reduce blood pressure or improve renal hemodynamics in SHR, and it is unlikely that TxA_2 contributes to either the renal vasoconstriction or the increased peripheral vascular resistance in SHR.

Human essential hypertension is characterized by reductions of renal prostaglandin excretion, particularly in older patients with low renin essential hypertension. It is possible that the defective renin release in the basal as well as furosemide-stimulated state is a result of decreased renal synthesis of PGE_2 and PGI_2. Nonsteroidal antiinflammatory drugs significantly interfere with the antihypertensive efficacy of adrenergic agents, vasodilators, and diuretics. These actions may be secondary to inhibition of both vascular and renal PGI_2/PGE_2 synthesis. Several studies have confirmed that sulindac does not have negative interaction with antihypertensive therapy, which is presumably related to the failure of sulindac to inhibit renal prostaglandin synthesis. Whether these results can be explained entirely on the basis of preserved renal prostaglandin synthesis and maintenance of sodium balance is unproved.

8. Miscellaneous Actions of Renal Eicosanoids

8.1. Prostaglandins, Hypoxia, and Erythropoietin

Recent studies have linked cellular production of erythropoietin, a hormone that stimulates erythroid precursors in the bone marrow, with renal prostaglandin synthesis. Hagiwara and co-workers cultured human renal carcinoma cells, after serial transplantation into athymic nude mice, in order to study the interrelations of PGE_2 synthesis and erythropoietin production.[181,182] This renal carcinoma cell line was developed from a patient who had erythrocytosis, indicative of excess erythropoietin production by the cancer. The cultured cells showed parallel changes of erythropoietin production and PGE_2 synthesis. Inhibition of PGE_2 synthesis with meclofenamate produced significant decrements of erythropoietin and PGE_2 synthesis. The authors postulate that hypoxia stimulates renal PGE_2 and PGI_2 synthesis, cAMP production, and erythropoietin synthesis and release.[181,182] Kurtz, Jelkmann, and their co-workers have contributed similar evidence of an essential role of prostaglandin synthesis in the renal synthesis of erythropoietin.[183,184] Cultured rat glomerular mesangial cells, stimulated by hypoxia, released increased amounts of PGE_2 and erythropoietin and cyclooxygenase inhibition blocked these increments. Exogenous addition of PGE_2, arachidonic acid, or PGI_2 enhanced erythropoietin production in the mesangial cells under normoxic conditions; these changes were attributed to stimulation of cAMP formation, as forskolin also increased erythropoietin production. These findings with cultured rat glomerular mesangial cells are quite consistent with the data of Hagiwara and Fisher studying cultured human renal carcinoma cells. Figure 9 schematizes these findings. It is unknown whether cyclooxygenase inhibitors would reduce the erythrocytosis in patients with erythropoietin-producing renal carcinomas, nor has it been tested whether prostaglandin-inhibiting drugs interfere with the normal renal responses to hypoxemia in humans.

8.2. Prostaglandins and the Renal Excretion of Calcium, Phosphate, and Ammonia

Urinary calcium excretion is enhanced by PGE_2 infusion and has been reported to decrease after administration of indomethacin both to experimental animals and to patients with idiopathic hypercalciuria. Friedlander and Amiel have further examined the interrelations of renal prostaglandin synthesis and divalent cation excretion in the rat. Cyclooxygenase inhibition with meclofenamate, indomethacin, or piroxicam acutely reduced absolute and fractional excretion of calcium and magnesium in

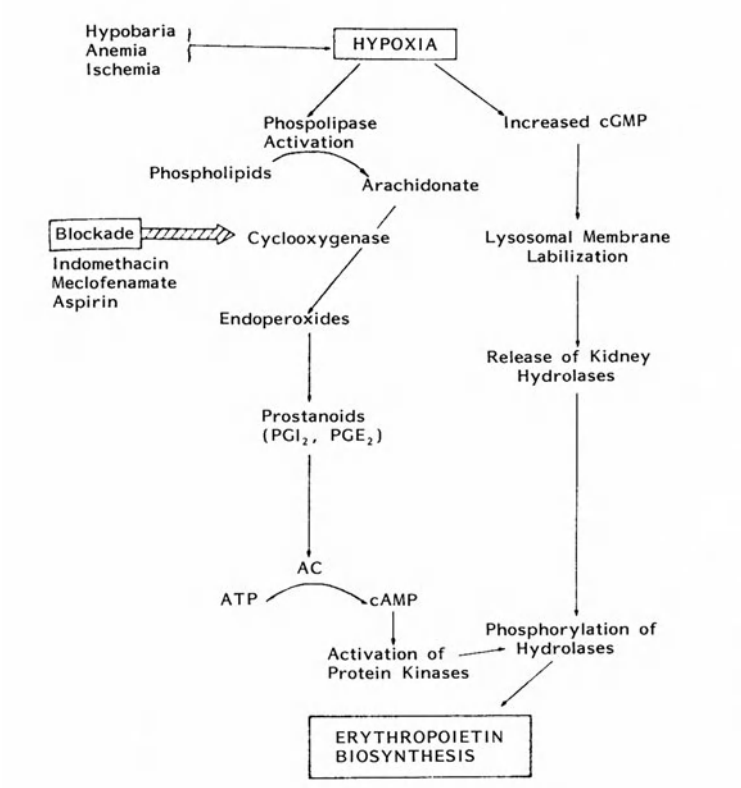

Fig. 9. A model for hypoxic stimulation of erythropoietin biosynthesis by the kidney. Hypoxia stimulates renal cellular (mesangial or juxtaglomerular?) synthesis of prostaglandins which stimulate adenylate cyclase (AC) and thereby stimulate erythropoietin through the actions of cAMP.[182]

intact and thyroparathyroidectomized rats.[185] Roman *et al.* have published corroborating evidence that renal prostaglandins regulate divalent cation excretion. In antidiuretic and diuretic rats, acute administration of meclofenamate reduced by 50% the excretion of calcium, magnesium, and sodium, pointing to an inhibitory action of PGE_2 on the tubular reabsorption of these cations.[90] A patient with hypercalciuria and many features of Bartter's syndrome had reductions of calcium excretion after treatment with either aspirin or indomethacin.[88]

The interrelations of PGE_2 and phosphate reabsorption are complex. *In vivo* studies have suggested that PGE_2 may enhance proximal tubular phosphate reabsorption. Dominguez *et al.* have used the isolated microperfused rabbit proximal tubule to study PGE_2–parathyroid hormone interactions. Both PGE_2 and parathyroid hormone inhibited phos-

phate reabsorption, particularly in the late proximal straight tubule. Simultaneous addition of parathyroid hormone and PGE_2 returned phosphate reabsorption to control values.[186] The effects of PGE_2 on tubular phosphate reabsorption are not limited to antagonism of parathyroid hormone since the phosphaturia induced by bicarbonate loading, volume expansion, or acetazolamide was eliminated or significantly attenuated by concomitant infusion of PGE_2.[187] Yamada *et al.* have published results that both corroborate and contradict the aforementioned papers. In vitamin D-deficient, thyroparathyroidectomized rats, administration of PGE_2 antagonized the phosphaturic effects of calcitonin and blocked calcitonin-stimulated synthesis of 1,25-dihydroxy vitamin D_3. Surprisingly, PGE_2 infusions did not alter the phosphaturic effects of parathyroid hormone or the parathyroid hormone-dependent stimulation of 1,25-dihydroxy vitamin D_3.[188] Renal prostaglandins may also regulate ammoniagenesis and, hence, may have a regulatory action in acid excretion by the kidney. Jones *et al.* stimulated renal ammoniagenesis *in vivo* by cyclooxygenase inhibition with meclofenamate in normal rats as well as those with metabolic acidosis, suggesting an *in vivo* inhibitory effect of a renal prostaglandin. Using rat renal cortical slices, these workers corroborated the *in vivo* results, since inhibition of prostaglandin synthesis stimulated ammoniagenesis and stimulation of prostaglandin synthesis had the opposite effects. Metabolic acidosis stimulated $PGF_{2\alpha}$ synthesis by the rat cortical slices, and the authors speculated that $PGF_{2\alpha}$ may suppress ammoniagenesis and, hence, reduce the ability to excrete an acid load.[189]

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5

Acid–Base Physiology and Pathophysiology

Melvin E. Laski and Neil A. Kurtzman

1. Proximal Tubule

Recent studies of proximal acidification have improved our knowledge of the control and mechanism of bicarbonate reabsorption. The exit step for bicarbonate in the proximal tubule was examined by Sasaki and Berry.[1] Using the *in vitro* perfused rabbit proximal convoluted tubule, they suggested that bicarbonate leaves the cell through a mechanism other than Cl/HCO_3 exchange. They found that removal of chloride from the bath had no effect on bicarbonate flux. The presence of barium, which depolarizes the basolateral membrane potential difference, caused a marked reduction in bicarbonate transport. Since chloride removal would profoundly decrease bicarbonate reabsorption were it mediated by a chloride/bicarbonate exchange, these workers concluded that bicarbonate exited the basolateral membrane not via an exchanger, but through a rheogenic mechanism.

Alpern further examined this issue using the *in vivo* perfused proximal tubule of the rat.[2] He used fluorescent pH-sensitive dye to examine

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the effects of sodium and chloride concentration on cell pH. Gradients were imposed across either the apical or basolateral membrane; the effect of these changes on pH relaxation was then observed. Analysis of the data was consistent with apical Na/H exchange and a conductive basolateral bicarbonate exit step. The ratio of sodium to bicarbonate exit proposed was greater than one. Thus, these data agree with those of Sasaki and Berry.[1]

While electrically neutral Na/H exchange appears to dominate proximal acidification, the coexistence of electrogenic proton secretion in this nephron segment continues to be at issue. Bank *et al.* perfused proximal convoluted tubule *in vivo* with solutions containing the proton ATPase inhibitor DCCD.[3] This agent significantly decreased bicarbonate reabsorption without an effect on glucose transport. This effect was additive to that of amiloride or acetazolamide. These authors felt their data indicated the presence of a luminal proton pump. The lack of an effect of DCCD on glucose transport argues against mitochondrial inhibition, though such a mechanism cannot be excluded.

Amiloride also was found to inhibit proximal bicarbonate reabsorption by Howlin *et al.*[4] Sasaki *et al.*, using intracellular pH electrodes, reported that amiloride or removal of sodium from the tubular perfusate changed cell pH.[5] These data are again consistent with apical Na/H exchange. In the latter study, however, the pH was too high to be the result of simple equilibrium and raised the possibility of active luminal proton secretion or active serosal bicarbonate transport.

The question of active mucosal proton secretion was addressed by Sabolic *et al.* using brush border vesicles from rat renal cortex.[6] They found evidence for a chloride-dependent, ATP-driven, DCCD and FCCP (another proton ATPase inhibitor)-inhibitable proton translocating system. The ultramicroscopic characteristics of these vesicles were similar to the endocytic vesicles involved in protein reabsorption. The significance of this finding is unclear, as is the overall role, if any, of active proton secretion in proximal acidification.

A variety of studies have been performed to further characterize the Na/H exchanger at the brush border of the proximal tubule. Using vesicles prepared from this membrane, the V_{max} was shown to be increased in material obtained from rabbits subjected to 7/8 nephrectomy prior to study.[7] This observation is in accord with increased bicarbonate reabsorptive capacity characteristic of both animals and humans with chronic renal failure. Similar data were obtained when vesicles from NH_4Cl-loaded rabbits were examined.[8] Glucocorticoid administration also increased the V_{max} of this exchanger.[9] Furosemide-induced metabolic alkalosis also stimulated the rate of Na/H exchange.[10] In contrast, PTH and cAMP both inhibited amiloride-sensitive sodium entry, sug-

gesting that these agents interfere with proximal acidification *in vitro* by inhibiting sodium entry.[11]

A note of caution was raised by the studies of Mircheff *et al.*[12] These workers purified their brush border membranes by adding an additional countercurrent separation technique. They found four subpopulations of material, only one of which had characteristics strongly suggestive of a true brush border membrane vesicle. Thus, the issue of the purity of the preparation studied must be settled before the true significance of these types of experiments can be understood.

It is conceivable that concentration gradients developed across the proximal tubule might affect bicarbonate transport by a passive diffusion. Such a process requires a high permeability. Alpern *et al.* previously reported a proximal bicarbonate permeability high enough to allow for passive bicarbonate movement.[13] Hamm *et al.*,[14] using intact proximal tubules, and Ives,[15] using brush border membranes, both derived permeability values for hydroxyl/proton ion. These values were too low to account for any significant fraction of bicarbonate transfer to be mediated by passive hydroxyl/proton transport. Thus, any passive movement of bicarbonate must be by bicarbonate diffusion *per se*.

Alpern examined the relationship between bicarbonate and volume reabsorption in the intact proximal tubule.[16] Volume and bicarbonate flux were linearly related when carbonic anhydrase activity was intact. The relationship was lost when the enzyme was inhibited. These data were interpreted to denote a barrier to bicarbonate diffusion at the basolateral membrane which is reduced by volume flow across this membrane. Inhibition of carbonic anhydrase activity prevents the uptake of bicarbonate across the apical membrane and thus obviates the relationship between the two parameters.

Alpern and Rector[17] proposed a model of proximal bicarbonate transport that postulated axial and radial heterogeneity similar to the earlier model of Wang and Deen,[18] but which also included the effect of flow rate on an apical unstirred layer and load stimulation of proton secretion. The model also included a role for passive bicarbonate backleak.

Recent work has suggested that volume expansion increases bicarbonate excretion mainly, or solely, by increasing filtered load.[19] Bichara *et al.*, using micropuncture, demonstrated that volume expansion depressed proximal bicarbonate reabsorption independent of filtered load. This indicates that volume expansion directly inhibits proton secretion and/or increases bicarbonate backleak. Maddox and Gennari found a direct relationship between load and reabsorption in the proximal tubule of the Munich–Wistar rat,[20] a finding similar to that reported by Alpern *et al.*[21] We think it reasonable to conclude that both load and direct

proximal transport mediate the delivery of this ion out of the proximal tubule.

2. Metabolic Alkalosis

Despite intense and prolonged effort, the critical factors responsible for the maintenance phase of metabolic alkalosis remain controversial. The work of Cogan and Liu argues that the failure of subjects with metabolic alkalosis to excrete sufficient bicarbonate in the urine to correct the disorder is mainly the consequence of a decreased filtered load and that volume expansion corrects the alkalosis by increasing GFR and hence filtered load.[22]

Galla *et al.* studied the role of chloride depletion on the maintenance of metabolic alkalosis induced by peritoneal dialysis of rats.[23] As far as they could tell, there was no change in effective arterial blood volume. While much clearance and micropuncture data concerning chloride reabsorption were obtained, we think the most interesting finding was a marked decrease in both whole-kidney and single-nephron GFR. The authors' conclusion that chloride depletion alkalosis "can be corrected by the provision of chloride without volume expansion or alterations in the intranephronal distribution of fluid reabsorption" depends on previous work from their laboratory. The current study suggests that the effect they observe with chloride administration may be mediated by changes in GFR and filtered load.

This suggestion is supported by another study by these investigators that measured proximal and distal sngfr in rats with chloride-depletion alkalosis.[24] Sngfr was decreased when measured distally, but unchanged from control when proximal samples were collected. This indicates that chloride depletion activates glomerular–tubular feedback to decrease GFR. The decreased GFR would perpetuate metabolic alkalosis by decreasing the filtered load of bicarbonate.

Further emphasizing the importance of reduced GFR in maintaining metabolic alkalosis is the effect of infusing atrial natriuretic factor to volume-contracted rats with metabolic alkalosis. Cogan found that this markedly increased bicarbonate excretion.[25] In a sense, the argument over the relative importance of filtered load and enhanced tubular absorption of bicarbonate in the maintenance of metabolic alkalosis seems unresolvable. Both must play a role. This certainty was emphasized by the work of Berger, Cogan, and Sebastian, who showed that both reduced GFR and enhanced bicarbonate reabsorption maintained metabolic alkalosis in humans.[26]

3. Renal Cortical P_{CO_2}

The P_{CO_2} surrounding the proximal tubule is about 20 mm Hg greater than that of the blood. The source of this CO_2 continues to occupy the efforts of two groups. DuBose and colleagues measured cortical CO_2 with varying rates of renal blood flow and after administration of metabolic poisons.[27,28] Renal blood flow could be dissociated from CO_2, and no gradient for CO_2 was found between tubule and peritubular blood, despite large changes in arterial P_{CO_2} and proximal bicarbonate reabsorption. Infusion of carbonic anhydrase lowered cortical CO_2. The latter observation suggests that carbonic anhydrase is not present in peritubular vessels. The possibility that a disequilibrium pH exists in these structures must be considered. Since metabolic poisons markedly lowered cortical CO_2 production, the same group published a mathematical model of proximal CO_2 production which required that CO_2 be produced by both acidification and metabolism.[29]

Atherton *et al.* found a gradient between proximal tubular and peritubular CO_2.[30] Accordingly, they produced a model of cortical CO_2 generation based on a complex analysis of blood buffering.[31] Naturally, this model predicts a difference in CO_2 across the proximal tubule about equal to what they find in their experiments. It also predicts an inverse relationship between renal blood flow and cortical CO_2 and that 50% of CO_2 results from metabolism.

While major differences exist between the way these two groups view this phenomenon, both postulate a role for metabolism and diffusion (secondary to CO_2 generation from proton secretion) in cortical CO_2 production. Interestingly, Hogg *et al.* found a CO_2 level in liver much higher than in blood.[32] High CO_2 levels were not found in muscle or brain. The liver, of course, is an organ that generates large amounts of bicarbonate (from lactate); so this finding is not too surprising.

4. Loop of Henle

The loop of Henle has long been held to play no role in renal acidification. This view has been challenged by two studies. Good, Knepper, and Burg studied ammonia and bicarbonate transport in the thick ascending limb.[33] The study clearly indicated bicarbonate transport in this segment. To further examine the issue, Good perfused rat cortical thick ascending limbs.[34] Bicarbonate reabsorption, measured as total CO_2 flux with microcalorimetry, was 10 pmoles/mm per min. It was inhibited by acetazolamide without a change in transepithelial voltage.

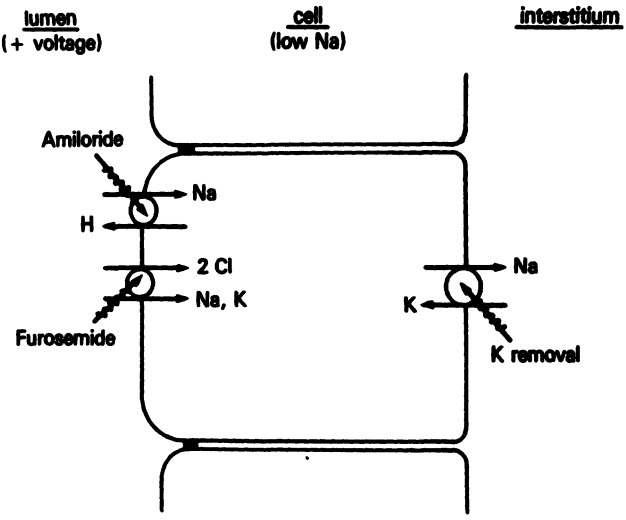

Fig. 1. The proposed mechanism of proton secretion responsible for bicarbonate absorption in the cortical thick ascending limb of the loop of Henle. In addition to the previously suggested apical Na,K,2 Cl entry site and basolateral Na,K-ATPase, an apical Na–H exchange mechanism is postulated. Acetazolamide inhibits bicarbonate reabsorption; the source of protons is probably carbonic anhydrase dependent. Ouabain inhibits bicarbonate absorption because it causes loss of the driving force for apical sodium entry to the cell which drives the exchanger. Perfusate amiloride and zero sodium perfusate inhibit the exchanger directly. The model also explains the effect of furosemide to increase acidification in this segment. Inhibition of the Na,K, 2 Cl entry mechanism with continued function of the basolateral ATPase makes the Na–H exchanger the principal entry site for sodium to the low-sodium environment of the cell, increasing the rate of exchange. (Reprinted from Good, *Am. J. Physiol.* **247**:F35,1984 with permission.)

Choline replacement of sodium inhibited transport and transcellular voltage. Amiloride and a zero potassium bath also inhibited bicarbonate transport, while furosemide increased bicarbonate flux. From these data, Good proposed a model of bicarbonate transport in the ascending limb mediated by sodium for hydrogen exchange (Fig. 1).

If this view of acidification in the ascending limb holds, considerable readjustment in our thinking of the control of bicarbonate reabsorption is required. Certainly, it will not be possible to assess the role of deep versus superficial nephrons by comparing delivery of bicarbonate to the bend of Henle's loop with that to the early superficial distal tubule.

5. Distal Acidification

Although distal acidification was held to be mediated by a proton pump for years, no direct evidence for the existence of this transporter

in mammalian kidney had been offered. In 1984, however, Gluck and Al-Awqati studied renal medullary vesicles obtained from bovine renal medulla.[35] Using fluorescence of acridine orange, they showed that acidification by these vesicles was ATP dependent and electrogenic. Acidification was inhibited by the proton ATPase inhibitors DCCD, NBD-Cl, PCMBS, and NEM, but not by mitochondrial inhibitors or vanadate. The conclusion of this work is that there is a plasma membrane proton pump in renal medulla, presumably in the medullary collecting tubule.

Additional work is needed to characterize and localize this pump along the nephron. It will be of considerable interest to know whether the microstructures noted in the collecting tubule by other investigators are, in fact, proton pumps. These membrane structures, pits and rods, are found in the apical membranes of acidifying cells. Their number changes due to membrane amplification by fusion of intracellular vesicles to apical membranes when cell pH falls. Gluck *et al.* used fluorescent techniques to show that respiratory acidosis caused low pH vesicles to fuse with the surface membrane of the turtle bladder.[36] More recently, Schwartz andAl-Awqati, using a similar method, showed that these vesicles moved to the apical membrane of the collecting tubule when cell pH fell.[37] This work suggests that these vesicles are the proton pump that is inserted into the luminal membrane of the distal nephron when increased acidification is required. It also indicates that the collecting tubules adapt to acidosis as would be expected, i.e., by increasing the number of active proton pumps.

In this regard, Jacobson showed that the medullary collecting tubule of the rabbit increased acidification in response to an *in vitro* increase in carbon dioxide tension.[38] He also showed a similar response to a lowering of bath bicarbonate concentration. These observations reinforce the adaptive capacity of the collecting tubule to acidosis. Levine also showed, using *in vivo* microperfusion, adaptation by the distal nephron to metabolic acidosis. His study additionally showed no effect of varying distal deliveries of sodium or potassium on distal acidification[39] The conclusion that distal sodium flux is not an important modulator of distal acidification, however, is not supported by the data. The range of sodium loads perfused was not large, and studies were not performed with inhibition of sodium transport.

The mechanism of aldosterone-dependent and -independent acidification continues to be of considerable interest. Kornandakieti and Tannen studied distal acidification during aldosterone deficiency in the isolated perfused kidney.[40] They found that the ability to generate urinary pH gradients was intact in aldosterone-deficient kidneys, but that their capacity to develop pH gradients was diminished, compared to controls, when distal buffer administration was increased with creatinine

infusion. Amiloride inhibited acidification in both aldosterone-deplete and -replete kidneys. These data indicate that aldosterone deficiency inhibits the rate of the distal proton pump but does not reduce its force. They also demonstrate an important role for sodium-linked distal acidification. A more recent study by Mujais *et al.* also emphasizes this point.[41] These workers showed that distal acidification, as assessed by the ability to generate CO_2 gradients or lower urinary pH during sodium sulfate infusion, was the same in rats with aldosterone deficiency as compared to control animals. Amiloride administration inhibited distal acidification in aldosterone-deficient animals, as it does in controls. These data emphasize the importance of sodium-dependent aldosterone-independent distal acidification.

Using papillary micropuncture, Higashihara *et al.* studied the role of aldosterone on papillary collecting tubule acidification.[42] They showed that during acidosis both control and adrenalectomized animals lowered urinary pH along the collecting tubule. They also showed that DOCA administration increased the capacity of adrenalectomized rats to lower pH. These results suggest that the terminal collecting duct participates in generating urinary pH gradients and that this process is influenced by aldosterone.

Two studies investigated the mechanism of carbonic anhydrase-independent (CAI) distal acidification. Frommer *et al.*, using micropuncture in the rat, showed continued distal acidification after acetazolamide infusion.[43] A portion of this process was amiloride inhibitable. They also detected large biocarbonate gradients between collecting tubule urine and vasa recta. As much as 70% of the filtered bicarbonate can be reabsorbed without carbonic anhydrase activity. These data suggest that much, perhaps all, of the bicarbonate reabsorbed in the presence of CAI may be mediated by two processes: (1) passive transport of bicarbonate down concentration gradients and (2) an acceleration of proton secretion in the cortical collecting tubule driven by a lumen negative transepithelial voltage itself the consequence of amiloride-inhibitable sodium transport.

To further examine the role of transepithelial voltage on CAI acidification, Sabatini and Kurtzman studied the effect of voltage clamping on proton secretion in the turtle bladder.[44] They found that favorable voltage exerted the same stimulatory effect on acidification with CAI as without, though the curve defining the relationship was set at a lower level and had a lesser slope (Fig. 2). These results provide more support for the regulatory role of voltage in the distal nephron on CAI acidification.

The turtle bladder not only secretes protons, it secretes bicarbonate as well. The bladder is made of two types of cells, granular and mitochondrial rich. The former mediate sodium transport and the latter

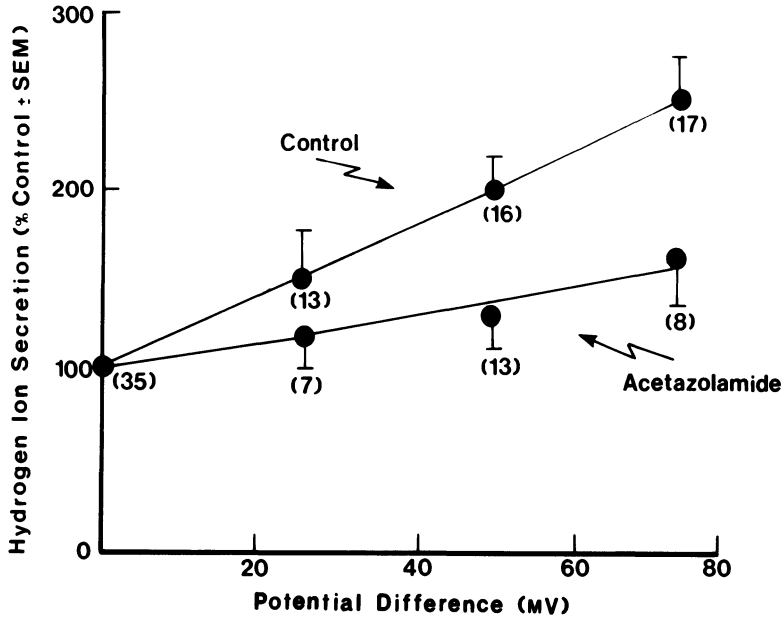

Fig. 2. The change in proton flux as transepithelial potential is increased by manipulation of the voltage clamp across carbonic anhydrase-inhibited bladders. The data in the graph are standardized to 100% of rates at zero transepithelial potential; these baseline rates differ in control and acetazolamide groups. Whether or not carbonic anhydrase is inhibited, increasing the negative voltage across the tissue increases the rate of acidification, although the slopes of the lines and absolute rates differ. These data provide evidence that acidification may be driven by favorable voltage even if carbonic anhydrase is inhibited and may explain at least one mechanism by which carbonic anhydrase-independent acidification occurs. (Reprinted from Sabatini and Kurtzman, *Miner. Electrolyte Metab.* 11:277, 1985, with permission.)

modulate acidification. Since the only difference between proton and bicarbonate secretion is the polarity of the transporting cell, bicarbonate transport should be mediated by the mitochondrial-rich cell. Fritsche and Schwartz studied the issue using separated mitochondrial-rich and granular cells from turtle bladder.[45] They found that acetazolamide and the bicarbonate transport inhibitor SITS had no effect on metabolism in the granular cell and had an inhibitory and additive effect on metabolism in the mitochondrial-rich cell. They concluded that bicarbonate transport in this membrane is effected by the mitochondrial-rich cell.

A similar issue was studied by Stetson and Steinmetz.[46] Using ultramicroscopy, they found two types of carbonic anhydrase-containing (mitochondrial-rich) cells in turtle bladder. Alpha cells had rod-shaped particles and increased their surface area in response to acidosis. Beta cells had sparse rod-shaped particles and did not increase surface area during acidosis. These authors felt that the beta cell mediated bicarbon-

ate secretion. In other words, they believe that one cell subtype controls bicarbonate transport while another cell subtype controls proton secretion. This view is contrary to the proposition that the same cell controls both processes by changing its polarity. It is in accord with the work of Sabatini, who found that cyclic AMP had no effect on proton secretion in the turtle bladder, but markedly stimulated bicarbonate secretion.[47] If both processes were mediated by the same cell type, cAMP should affect both.

Stetson *et al.* showed that cAMP-stimulated bicarbonate secretion was associated with enhanced chloride transport in the opposite direction, as well as by changes in short-circuit current and transepithelial resistance.[48] Inhibition of chloride conductance decreased the change in short-circuit current induced by cAMP. Despite this relationship between bicarbonate and chloride transport, phosphodiesterase inhibition increased bicarbonate secretion in the absence of chloride. These data were interpreted to indicate the presence of both an apical chloride/bicarbonate exchanger and an apical bicarbonate pathway. This would explain both the chloride dependence and independence of bicarbonate secretion. The energy for this process, according to this view, comes from a proton pump at the basolateral membrane. Cyclic AMP would stimulate apical bicarbonate conductance, but only in this cell subtype. The model of bicarbonate secretion envisaged by Stetson is shown in Figs. 3 and 4.

Bicarbonate secretion has also been studied in the mammalian collecting tubule. McKinney and Burg found bicarbonate secretion not to be chloride dependent.[49] Laski *et al.* did show cloride dependence, but could not tell whether this dependence was due to anion exchange or voltage effects.[50] Garcia-Austt *et al.*, also using rabbit collecting tubules, noted high rates of bicarbonate secretion in bicarbonate- but not acid-loaded animals.[51] Bicarbonate secretion was reduced when glutamate or sulfate was substituted for luminal chloride. Star *et al.* noted that chloride transport was dependent on bicarbonate transport.[52] Thus, it is reasonably certain that collecting tubule bicarbonate transport is, at least in part, the result of chloride/bicarbonate exchange.

Not surprisingly, Schuster showed that dibutyryl cAMP stimulated bicarbonate secretion in the cortical collecting duct of the rabbit, a metaphor of the turtle bladder.[53] Isoproterenol had a similar effect. This investigator also noted a constant decay in bicarbonate secretion, an effect that must be considered when evaluating other studies of bicarbonate transport by this epithelium.

Almost all the work reported with the technique of *in vitro* nephron perfusion has used the rabbit kidney. Most clearance studies have used the rat or the dog, while most micropuncture experiments have used

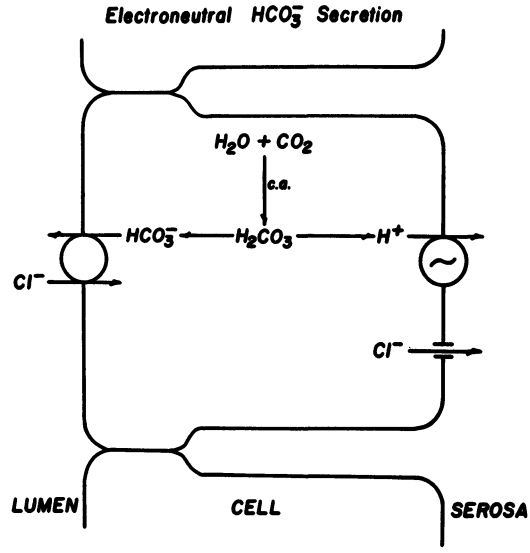

Fig. 3. The mechanism proposed for the process of electroneutral HCO_3 secretion by the turtle bladder. Apical Cl–HCO_3 exchange is present, as suggested in the past; the addition to the model over those previously proposed is the presence of a basolateral proton pump mechanism. (Reprinted from Stetson *et al., Am. J. Physiol.* **249**:F112, 1985, with permission.)

the rat. Because of the alkaline ash diet of the rabbit and its different requirements for renal acid–base homeostasis, considerable questions have been raised about the relevance of data, particularly bicarbonate secretion, obtained from isolated rabbit collecting tubules. Atkins and Burg were able to overcome the technical difficulties inherent in perfusion of the rat tubule and study bicarbonate transport in this tissue.[54]

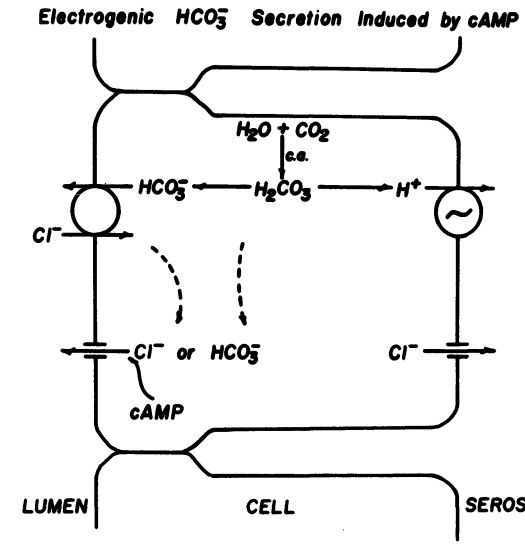

Fig. 4. Working model of a second mechanism in the bicarbonate-secreting cell in the turtle bladder which is present in addition to the process shown in Fig. 3. This cell is proposed to explain the effects of cAMP on bicarbonate secretion, which is to in crease secretion and also to alter transepithelial potential. The increase in bicarbonate secretion in response to cAMP is not inhibitable by DIDS. The increase in secretion after cAMP is prevented by addition of 9AA, an inhibitor of Cl conductance pathways. These data lead to the placement of an inducible anion conductance path in the apical membrane which may serve as an exit for bicarbonate formed behind the basolateral proton pump. (Reprinted from Stetson *et al., Am. J. Physiol.* **249**:F112, 1985, with permission.)

They found that cortical collecting tubule secreted bicarbonate after alkali loading and that amiloride loading reversed the lumen negative potential difference normally observed. Bicarbonate secretion was limited to the cortical tubule; i.e., the medullary tubule always absorbed bicarbonate. These data are in agreement with those obtained from the rabbit. Thus, it is likely that the isolated tubule data from the rabbit can be extrapolated to other mammalian species.

6. Clinical Studies of Distal Acidification

One of the biggest problems encountered in studying patients with distal acidification defects has been the difficulty inherent in performing acid-loading tests and phosphate and/or sulfate infusions. Although these tests provide useful diagnostic information, they are cumbersome, time consuming, and require intravenous infusion of solutions that must be specially prepared by the pharmacy. In terms of convenience without any sacrifice in diagnostic reliability, the development of the furosemide test to characterize distal defects has been a real advance.[55] The oral administration of 40–80 mg of furosemide produces an effect on both acid and potassium excretion virtually identical to that of sodium sulfate. Thus, patients with voltage-dependent defects have reduced potassium and acid excretion after furosemide. The latter defect can easily be detected by the failure of urine pH to fall below 5.5.

In another study, Rastogi and colleagues showed that adrenalectomized rats lowered urine pH and increased acid excretion after furosemide administration.[56] They suggested, as did Kurtzman earlier,[57] that furosemide stimulates distal acidification by increasing distal delivery of sodium while making chloride an impermeant anion. In other words, furosemide converts sodium chloride to, in a functional sense, sodium sulfate.

There are data, however, that suggest that furosemide may exert an effect on acidification proximal to the collecting tubule. Hropot *et al.* noted an effect of the diuretic on ammonia delivery, titratable acid, and pH proximal to the early distal tubule which they attributed to an action in the ascending limb.[58] Good showed bicarbonate reabsorption in the ascending limb which was stimulated by furosemide.[34] Thus, some of the effect of this drug on acid excretion may be due to a loop effect. That it drives urine pH to 5.0 or less requires some effect in the collecting tubule.

Maher and co-workers gave furosemide chronically to patients with renal insufficiency and renal tubular acidosis.[60] As expected, they showed an increase in both acid and potassium excretion. Though other expla-

nations are possible, this observation is best explained by an action of this drug in the collecting tubule, as described earlier in this section. Maher and associates also studied hyperkalemic distal renal tubular acidosis. They showed that dietary potassium restriction caused an increase in serum bicarbonate. They attributed this finding to two effects of potassium restriction: first, a rise in bicarbonate concentration attributable to an extrarenal effect; second, renal retention of this extra bicarbonate due to decreased potassium stores. Thus, the cornerstone of treatment of the hyperkalemic acidoses is increased distal delivery of sodium, using furosemide if necessary, and reduction of serum potassium concentration, which in itself may result from increased distal delivery of sodium. Mineralocorticoid hormone may be added to the regimen of those patients with this syndrome who have aldosterone deficiency and who do not respond to the treatment just described. Such patients are likely to be few, however.

7. Ammonia

Several studies examined the issue of ammonia transport *per se,* i.e., whether ammonia or ammonium is the species moved. Although we have long believed that collecting tubule ammonium was formed in the proximal tubule and found its way to the distal nephron by nonionic diffusion, doubt was expressed that this was the only mechanism for ammonia transport. Ammonium pumps exist in lower organisms, and the low intracellular pH that prevails in ammonia-transporting cells requires that ammonium be the predominant species.

Arruda and co-workers examined ammonia transport in the turtle bladder.[61] They varied the NH_3/NH_4^+ ratio to determine which moiety crossed the membrane. They found that raising either increased transport. They also observed an increase in short circuit which was proportional to ammonia flux. They concluded that both NH_3 and NH_4 are specifically transported by turtle bladder, the former by diffusion, the latter perhaps by a pump. The same group studied the relationship between proton secretion and ammonia transport.[62] Eliminating acid secretion by establishing a rate-limiting transepithelial pH gradient also stopped ammonia transport. Similarly, acetazolamide, SITS, and DCCD—agents that inhibit acidification through different mechanisms—also reduced ammonia transport. CO_2 addition increased both processes. When similar experiments were performed at a serosal pH of 8.4, the transport of ammonia increased even though proton secretion was reduced, indicating that the two parameters are not inextricably linked. These workers felt that the connection between proton and ammonia transport was

mediated by pH changes at the apical and basolateral unstirred layers which altered the NH_3/NH_4 ratios in the immediate periepithelial environment, which would alter the availability of NH_3 for diffusion.

The development of sensitive and accurate assays for ammonia content of nanoliter-scale samples allowed similar investigations to take place in the mammalian nephron. Hamm *et al.* studied ammonia transport in the cortical collecting tubule of the rabbit and showed that entry from bath to lumen did occur, but at a limited rate.[63] NH_3 permeability was calculated to be about 0.007 cm/sec, but this apparent permeability decreased when CO_2/HCO_3 was also present in the system. The permeability of the proximal convoluted tubule was greater than the CCT. The general feeling was that the permeability was low in the CCT, that entry was flow dependent, and that the process seemed to be due to NH_3 diffusion. In a second paper Hamm *et al.* investigated the effect of the presence of ammonia on the transport of other ions.[64] These authors noted that adding ammonia to the bath resulted in decreased transepithelial potential and, on further investigation, decreased transport of sodium and potassium. The effect resembled the addition of amiloride or tetramethyl ammonium and was reversible by lowering bath P_{CO_2}. The results suggest that ammonia may interfere with the apical sodium pore.

Knepper and colleagues also investigated ammonia secretion in the cortical collecting tubule of the rabbit.[65] These investigators used DOCA-treated rabbits and simultaneously measured ammonia and total CO_2 transport in the cortical collecting tubule. They found that NH_3 and bicarbonate secretion paralleled each other under normal conditions and that NH_3 movement seemed to follow the negative transepithelial potential. Unlike the results in the bladder, however, no effect of ouabain or zero potassium was noted. Because of the relationship between total CO_2 flux and ammonia flux, the authors attempted to dissociate these two by infusing perfusates with added carbonic anhydrase to remove any disequilibrium pH which might lead to ammonia trapping. When this was performed, ammonia entry decreased. They concluded that entry of ammonia was due to diffusion and that acid disequilibrium pH led to increased ammonia trapping.

The same authors also studied ammonia and total CO_2 transport in cortical collecting tubules obtained from DOCA-loaded rats.[66] Their findings were similar to the rabbit study in that ammonia appeared to be entering by nonionic diffusion and that the disequilibrium pH led to increased trapping, but they also noted that increases in ammonia were associated with decreased bicarbonate secretion, which did not occur in the rabbit. Carbonic anhydrase infusion lowered ammonia entry but not

bicarbonate secretion. This study agrees with both the rabbit data and the turtle bladder study of Arruda *et al.*[60,62,65] These results in the rat are important because of the problem of applying data derived from an animal that does not normally depend on ammonia secretion for acid–base balance to other species that do, i.e., humans.

Good and Burg examined 10 different nephron segments and found that ammonia was produced to some degree by all, including the glomerulus, but that the S1 and S3 segments of the proximal tubule had the highest levels of production.[67] Prior loading with NH_4Cl increased production in S1 and S3 if glutamine was provided, while HCO_3 loading decreased production by the S1, results in concordance with the effects of alkalosis and acidosis from whole-animal studies. Nagami and Kurokawa used isolated mouse proximal tubules and a unique assay system to demonstrate that perfused tubules had higher rates of ammonia production than nonperfused tubules.[68] If the perfusate contained glutamine, the production rate rose still higher. The effect of perfusion to alter production casts doubt on studies performed in slices or suspensions.

To determine whether respiratory acidosis stimulated ammonia production, Tannen and Hamid either exposed rats to high P_{CO_2} in an environmental chamber or provided NH_4Cl in drinking water.[69] When isolated kidneys from these animals were examined, glucose use and ammonia production were increased in the animals with metabolic acidosis, but not in the animals with respiratory acidosis. The same results were seen in tubule suspensions. If the pH of the media of tubule suspensions was lowered, glucose production increased in only the metabolic acidosis group. Tannen and Hamid concluded that only metabolic acidosis was stimulatory. In contrast to this result is a study that showed an increase in basolateral membrane vesicle glutamine uptake in respiratory acidosis in tissues from the dog.[81] This finding is consistent with adaptation to respiratory acidosis.

Simon *et al.* used surface micropuncture, in the rat, to study ammonia transport along the accessible nephron and found data substantially in agreement with the microperfusion studies just reviewed; i.e., ammonia added along the proximal tubule disappeared in the loop and was again added in the distal tubules.[70] Proximal permeability appeared to be higher than distal, and no diffusion equilibrium was present. In acidosis, greater rates of NH_4 addition along the proximal tubule were found, but this increase dissipated along the loop, possibly owing to a countercurrent effect.

Excretion of ammonia and titratable acidity in metabolic acidosis was examined by Wilcox and co-workers.[71] In this micropuncture study in the rat, chronic administration of DOCA was found to increase am-

monia excretion in adrenalectomized animals, whereas acute DOCA had no effect. Acute saline loading in chronically expanded animals did not alter excretion of titratable acidity unless the animals were acidotic, but ammonia excretion was increased by saline infusion generally. The proximal and distal convoluted tubules were found to be the major sites of ammonia addition. Halperin and colleagues also studied ammonia excretion in acidosis.[72] Using metabolic inhibitors (ouabain, mercaptopicolinate), they found ammonia production to be related to the ability to generate ATP and also found that ammonia production was linearly related to GFR and proximal reabsorption. These findings thus agree with the effects of volume expansion just discussed. No correlation of ammonia production with gluconeogenesis was noted.

The final study of ammonia metabolism we shall consider evaluated the effects of prostaglandin inhibitors and also the relationship between prostaglandin levels and ammonia production.[73] Clearance and cortical slice studies were performed. Meclofenamate was seen to increase ammonia excretion in normal rats, whereas it decreased GFR and renal blood flow. The effect was also noted in animals with alkalosis and mild acidosis, but not if the acidosis was severe. Ammonia excretion and prostaglandin levels were compared. The results seem to clearly indicate that prostaglandin has a major role in control of ammoniagenesis, specifically as a feedback inhibitor. How this factor relates to all the above studies seems likely to be a fertile area of investigation in the future.

8. Renal Adaptation to Respiratory Change

Although conventional wisdom has long held that the kidney responds to respiratory acidosis by increasing acid excretion, this assertion was cast in doubt by the observation that the difference between urine and blood P_{CO_2} actually decreased during acute respiratory acidosis.[74] Two questions were raised by this study; first, was urine P_{CO_2} during bicarbonaturia truly a reasonable measure of distal proton secretion, as suggested long ago by Pitts, and second, did the kidney in fact respond to elevation of P_{CO_2} by increasing acid secretion? Studies performed in the past 2 years provide ample justification for the historical assertions.

In a beautiful series of experiments, DuBose and Caflisch have shown that Pitts was correct in proposing that rising urine P_{CO_2} during bicarbonaturia represented the effect of proton secretion.[75] Rats were prepared for papillary micropuncture, and pH *in situ*, disequilibrium pH, and P_{CO_2} *in situ* were measured in acid and HCO_3 loading. In addition to baseline for these conditions, the effects of amiloride, lithium, post-

obstructive uropathy, and amphotericin were examined. Administration of HCO_3 resulted in finding a disequilibrium pH in controls, but amiloride, lithium, and postobstructed animal models lost this and the elevation of P_{CO_2}. The amphotericin model continued to show a disequilibrium pH and retained elevation of P_{CO_2}. These results are precisely those predicted by urine P_{CO_2} measurement studies.

If urine P_{CO_2} remains a good measure of distal acidification, then why did the urine–blood P_{CO2} gradient fall during respiratory acidosis? One possibility, the one reached by Androgue *et al.*, is that acute respiratory acidosis decreases distal acidification.[74] Intuitively, this is hard to accept. Another possibility is that the urine–blood P_{CO_2} gradient does not reflect distal acidification during respiratory acidosis. Batlle and colleagues addressed this issue in two studies.[76,77] They found that both acute and chronic respiratory acidosis were associated with urine P_{CO_2}'s greater than seen during normocapnia. They noted that the rise in urine P_{CO_2} from prebicarbonate infusion to postinfusion was greater during hypercapnia than normocapnia. They also found that the infusion of carbonic anhydrase to hypercapnic animals decreased urine P_{CO_2} to levels lower than that of blood, an observation not found during normocapnia. This means that vasa recta P_{CO_2} during hypercapnia must be lower than systemic blood. If such is the case, then the urine–blood P_{CO_2} is an artifact which happens to work during normocapnia (because vasa recta and systemic blood are the same), but which gives aberrant results during hypercapnia. Under these conditions, the best way to assess distal acidification using the urine P_{CO_2} would be to measure the difference between urine P_{CO_2} during bicarbonate loading before and after the infusion of carbonic anhydrase. The greater the difference, the greater the distal acidification. Using this criterion, Batlle *et al.* concluded that acute respiratory acidosis was associated with enhanced distal acidification.

9. Lactic Acidosis

While most clinicians will readily admit that treatment of severe lactic acidosis with bicarbonate is usually futile, such therapy is more or less universally given because attempts to reverse the underlying cause of the lactate production, such as shock or hepatic failure, are not frequently successful. Graf, Leach, and Arieff have examined the effect of bicarbonate therapy on cardiac function and blood pressure in dogs with lactic acidosis reduced by ventilation with hypoxic gas mixtures.[78,79] Animals were given either maintenance fluids, volume expansion with sa-

line, or bicarbonate infusions. Bicarbonate-infused animals had higher blood lactate levels, lower blood pressure and cardiac index, and greater gut lactate production rates. The authors conclude from these data that bicarbonate infusion is not helpful and is in fact detrimental to the overall status of the animal. In contrast to the results with bicarbonate, the same authors noted in another paper that dogs with hypoxic lactic acidosis responded positively to the infusion of dichloroacetate.[80] In this study, dichloroacetate infusion was compared to saline infusion in hypoxic animals. Dichloroacetate infusion resulted in higher blood pH and bicarbonate and lowered blood lactate levels. Hemodynamic parameters were not altered. Muscle and liver intracellular pH was higher in the dichloroacetate-treated group and the lactate levels were lower. Gut and carcass lactate production decreased with dichloroacetate infusion. In addition, hepatic lactate extraction was increased in the treatment group.

The general impression from the data is that dichloroacetate may be more beneficial than bicarbonate in this disorder. Several problems remain, however. The model used may not mimic the usual clinical situation to a useful degree. In addition, dichloroacetate has not been proven innocuous. Finally, while lactate production is a sign of poor perfusion and tissue injury, decreasing lactate by such metabolic manipulation as dichloroacetate administration may not save the dying cells. The best recommendation for care of an individual with lactic acidosis is still that the underlying cause of the acidosis must be identified and corrected. Given life-threatening acidemia, i.e., pH 7.0, the temptation to infuse bicarbonate may prove irresistible.

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6

Mineral Metabolism

Roger A. L. Sutton and E. C. Cameron

1. Vitamin D Endocrine System

1.1. Vitamin D Metabolism

The vitamin D endocrine system utilizes metabolites of vitamin D in the regulation of a wide variety of metabolic processes.[1] Calcitriol (1,25-dihydroxy vitamin D) is the most biologically active metabolite, and this compound functions as an important steroidal hormone.[2]

Vitamin D_3, the major precursor of vitamin D metabolites, is synthesized in the skin by photochemical and thermal conversion processes.[3] Vitamin D_3 is also present in certain foods, such as fish oils, and may be absorbed by the lymphatics of the small intestine as a fat-soluble vitamin. In human beings, the natural diet is a trivial source of vitamin D, but foods may be fortified with vitamin D_2, which is derived from irradiated plant sterols.[4] Dietary sources of vitamin D may become important in individuals with reduced sunlight exposure.[5] The ability of the skin to photosynthesize vitamin D_3 has been shown to be markedly diminished in the elderly compared with younger individuals.[6] Blacks appear to have diminished synthesis of vitamin D in the skin because of increased pigment.[7]

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Vitamin D and its metabolites are transported in the circulation bound to proteins, particularly albumin and α_2 globulin vitamin D-binding protein (DBP). This glycoprotein is synthesized in the liver and is responsible for most of the transport of vitamin D-related compounds. It has been shown to be a member of the gene family that encodes other serum proteins, including albumin and α-fetoprotein.[8] In the circulation, DBP is the principal carrier protein of calcitriol and albumin is the major secondary carrier, especially in patients with low DBP levels.[9]

Vitamin D is enzymatically hydroxylated in the liver to calcidiol (25-hydroxy vitamin D) to become the major circulating and storage metabolite. Serum calcidiol levels are not closely regulated and largely reflect sun exposure and cutaneous synthesis of vitamin D.[4] A circulating level of >20 nmoles/liter of calcidiol appears to provide adequate substrate for calcitriol production in most individuals.[5] Bell *et al.*[10] have demonstrated effective feedback regulation of calcidiol production by calcitriol, and they have shown that supraphysiological doses of calcitriol completely inhibit the increase in serum calcidiol produced by vitamin D challenge in normal subjects. The mechanism of this regulation is unclear, since calcitriol receptors have not been identified on hepatocytes. Further, the physiologic significance of this feedback mechanism is not certain, since patients with elevated calcitriol levels do not have low calcidiol levels.[11] The liver also excretes vitamin D metabolites in the bile, probably as part of the degradation process, rather than as a conserving enterohepatic circulation, as was previously postulated.[4]

Calcidiol is the substrate for calcitriol hormone production. The enzyme that possesses 1α-hydroxylase activity (25-OHD-1α-hydroxylase) is a cytochrome P-450-dependent monooxygenase localized in the mitochondria of the proximal convoluted tubule and the proximal straight tubule.[12] Calcidiol may also be metabolized to 24,25-dihydroxy vitamin D [24,25$(OH)_2$D] by the enzyme 25-OHD-24-hydroxylase. There appears to be a reciprocal change in the activities of these two enzymes such that when 1α-hydroxylase activity is stimulated, 24-hydroxylase activity is suppressed, and vice versa.[12]

The normal concentration of calcitriol and 24,25$(OH)_2$D is 80 pmoles versus 4 nmoles/liter, and the daily production of these metabolites is 1.4 nmoles versus 48 nmoles.[13] In contrast to calcitriol, the production of 24,25$(OH)_2$D is not closely regulated, and plasma levels are directly related to the precursor calcidiol concentration.[14]

A number of studies appear to have demonstrated bioactivity of 24,25$(OH)_2$D, particularly in bone.[15] However, whether or not 24,25$(OH)_2$D has a specific regulating function has been a matter of controversy.[16] Aside from calcitriol, the role of the many other vitamin D metabolites in the expression or catabolism of vitamin D bioactivity

remains to be clarified. Data concerning the actions of $24,25(OH)_2D$ will be discussed later.

1.2. Regulation of Renal Calcitriol Production

Calcitriol has been shown to have a number of specific biologic actions, and its synthesis by the kidney, the major site of physiologic production, is closely regulated under normal conditions. Halloran *et al.*[17] have shown that the serum concentration of calcitriol does not undergo large fluctuations and is maintained within approximately 20% of its overall 24-hr mean. However, the levels of circulating calcitriol are not as tightly regulated in children as in adults.[18]

Calcitriol has been shown to modulate its own production, and basal levels of renal 25-OHD-1α-hydroxylase vary inversely with serum calcitriol levels.[19] Further, calcitriol induces the 24-hydroxylase, and may itself be further hydroxylated to $1,24,25(OH)_3D$, which initiates catabolism.[20]

A number of studies have shown that parathyroid hormone (PTH) is the principal regulator of calcitriol synthesis.[1] The fact that the serum calcitriol level varies with the concentration of calcidiol in patients with hypoparathyroidism indicates that substrate concentration largely determines the production of calcitriol in the absence of PTH.[21] *In vitro* studies have demonstrated the direct stimulation of 1α-hydroxylase activity by PTH and indicate that this stimulatory effect is mediated through cAMP.[22] Kawashima and Kurokawa have shown that exogenous cAMP restores the reduced 25-OHD-1α-hydroxylase in the proximal convoluted tubule of thyroparathyroidectomized vitamin D-deficient rats.[12] Lo Cascio *et al.*[23] observed that 1α-hydroxylase is loosely regulated in patients with primary hyperparathyroidism and that in these patients, circulating levels of calcitriol were more dependent on the prevailing concentration of calcidiol than in normal adults.

Increased immunoreactive PTH in obese compared with nonobese subjects was confirmed by Bell *et al.*[24] These obese subjects also had increased calcitriol levels and a decreased urinary calcium excretion. Such changes in calcium and vitamin D metabolism in obesity may be a factor in increasing skeletal mass.

Kawashima *et al.*[25] measured the 1α-hydroxylase activity of various nephron segments in vitamin D-deficient rats given calcitonin. This study showed that calcitonin stimulated 1α-hydroxylase activity in the proximal straight tubule, but did not affect the enzyme activity in the proximal convoluted tubule. The study also showed that PTH stimulates the 1α-hydroxylase in the proximal convoluted tubule via cAMP, while calcitonin stimulates the 1α-hydroxylase in the proximal straight tubule in-

dependently of cAMP. It has been suggested that the calcitonin-sensitive 1α-hydroxylase system may be of importance during fetal development by stimulating calcitriol production in the presence of an elevated fetal serum ionized calcium concentration.[12]

There is evidence that calcium, phosphate, and magnesium levels can directly modulate calcitriol production independently of PTH. Bushinsky *et al.*[26] have shown in rats that the blood ionized calcium concentration can regulate serum levels of calcitriol independently of serum phosphorus or PTH. These studies demonstrated that lowering of the blood calcium concentration within and below the normal range progressively raised serum calcitriol levels despite a constant high PTH infusion and stable serum phosphorus levels. Studies by Hulter *et al.*[27] in humans and dogs showed that in these species also, hypercalcemia prevents the elevation of calcitriol levels associated with increased PTH secretion.

Plasma phosphate is a potent regulator of plasma calcitriol levels, in that phosphate deprivation increases and phosphate supplementation decreases calcitriol synthesis by the renal tubule.[28] A study in healthy men has shown that reductions and increases in dietary phosphorus can induce rapid (1–2 days) large and persisting changes in calcitriol levels by altering the production rate of calcitriol without significant changes in serum calcium or PTH levels.[29] A linkage between renal tubular reabsorption of phosphate and calcitriol synthesis is indicated in two human genetic disorders. In patients with X-linked hypophosphatemic rickets, it has been shown that calcitriol levels tend to be low despite hypophosphatemia. These patients appear to have a deficient 1α-hydroxylase system and high 24-hydroxylase activity.[30] In contrast, patients with hyperphosphatemic tumoral calcinosis and decreased tubular phosphate excretion had increased levels of calcitriol.[31]

Gray and Garthwaite[28] showed, in studies in rats, that the presence of growth hormone is necessary for the increased calcitriol synthesis that is observed with phosphate deprivation. These authors suggest that since the kidney is a principal site of somatomedin production, this action of growth hormone may be mediated by somatomedins.

Studies in hypocalcemic magnesium-deficient patients by Rude *et al.*[32] showed that serum calcitriol concentrations are frequently low in patients with magnesium deficiency and may remain low for up to 2 weeks of parenteral magnesium administration despite a high serum concentration of PTH. These studies suggested that calcitriol production may be impaired by magnesium deficiency and that vitamin D metabolism may be more sensitive to this deficiency than it is to either PTH secretion or the effect of PTH on skeletal or renal cAMP generation.

1.3. Extrarenal Production of Calcitriol

Although the renal conversion of calcidiol to calcitriol is of prime importance for normal calcium and phosphorus homeostasis, extrarenal synthesis of calcitriol has been described in a number of circumstances.

Evidence of extrarenal production of calcitriol was described in a patient with sarcoidosis by Barbour *et al.*[33] The extrarenal synthesis of calcitriol has subsequently been confirmed in patients with sarcoidosis and appears to be operative in other gramulomatous disorders as well as lymphomas with hypercalcemia.[34–35]

Low, but detectable, levels of calcitriol have been measured in nephrectomized dialysis patients, indicating extrarenal production of this compound.[36] It has been suggested that inflammatory granulomas in the liver resulting from silicon particles from dialysate tubing may be responsible for this synthesis of calcitriol.[1]

The human placental unit can synthesize calcitriol, and increased circulating levels of this hormone are found throughout pregnancy.[37] Whether this hormone production plays a role in the mineral homeostasis of the developing fetus remains speculative. Zerwekh and Breslau[38] showed that in women with PTH-resistant hypoparathyroidism, in whom the renal capacity to synthesize calcitriol is impaired, placental production of this compound takes place in the mitochondria of the trophoblasts and is not impaired.

1.4. Actions of Calcitriol

Receptors for calcitriol have been identified in a wide variety of hormonal and neoplastic tissues, with the interesting exceptions of the osteoclasts and liver.[39] The mammalian calcitriol receptor is generated in the nucleus of the cell, and based on its molecular size, DNA-binding property, subcellular location, and capacity to induce new protein synthesis, the calcitriol receptor resembles estrogen and thyroid hormone receptors.[40]

Sher *et al.*[41] have shown, in studies using intact human breast cancer cells, that receptors for calcitriol are processed by a nuclear mechanism analogous to that of other steriod hormones. Receptor loss, resembling the downregulation of peptide hormone receptors, was demonstrated and may represent a sensitive mechanism for control of cellular responsiveness to this hormone.

Defective calcitriol receptors have been shown to be responsible for pathologic conditions in both animals and humans. Studies of Adams *et al.*,[42] using cultured dermal fibroblasts, indicated that the occurrence of

vitamin D-resistant osteomalacia in New World primates is a result of decreased high-affinity, receptor-mediated uptake of calcitriol by the target cells. In humans with end-organ resistance to vitamin D (vitamin D-dependent rickets type II) defects in the intracellular receptor mechanism of calcitriol have been demonstrated in fibroblasts cultured from skin biopsies and in peripheral mononuclear cells.[43] Hirst *et al.*[44] have described a kindred with end-organ resistance to vitamin D in whom cultured skin fibroblasts exhibited normal calcitriol binding, but appeared to have a defect in subsequent binding of the receptor to DNA.

1.4.1. Actions of Calcitriol on Intestinal Cells

Extensive evidence supports the existence of a steroid hormone-like mechanism for calcitriol-mediated calcium absorption in the intestine.[45] It has been shown that calcitriol induces *de novo* synthesis of one or more calcium transport components, including a vitamin D-dependent calcium-binding protein, which are essential for the integrated response of intestinal calcium transport.[2]

It appears that multiple mechanisms are involved in calcitriol-stimulated calcium transport by the intestinal cell. Nemere *et al.*[45] have shown that calcium flux across the duodenal brush border membrane of vitamin D-replete chicks increased in response to calcitriol within 15 min. Such transport does not appear to require new protein synthesis of transport components. Although changes in membrane fluidity affect calcium transport in the brush border membrane, Bikle *et al.*[46] did not find evidence in chicks to support the proposal that calcitriol-stimulated changes in calcium transport are mediated through changes in membrane fluidity. However, Bikle and Munson[47] have shown that calcitriol increases calmodulin binding to specific proteins in the chick duodenal brush border membrane. Since this mechanism could cause protein activation without new protein synthesis, these authors suggest that this process may be of importance in intestinal calcium transport.

Shedl *et al.*[48] demonstrated decreased absorption of calcium in the proximal small intestine of spontaneously hypertensive rats and have postulated that altered membrane transport of calcium and sodium may be causal to the hypertension in these animals.

Glucocorticoid administration decreases intestinal calcium absorption in humans and animals. Korkor *et al.*,[49] in studies in dogs, showed that chronic prednisone administration did not decrease intestinal calcitriol binding and therefore suggested that glucocorticoids may in some way dissociate the sequence of transcriptional and translational events

that intervene between calcitriol receptor binding and the calcium transport process.

Calcitriol exerts a direct effect on intestinal epithelial cell membranes to increase the transport of inorganic phosphate independent of calcium transport. Karsenty *et al.*[50] used isolated enterocytes from normal vitamin D-replete rats to demonstrate a rapid direct effect of calcitriol in stimulating phosphate entry across the cell membrane, which may be additional to the important long-term effects on intestinal phosphate absorption.

1.4.2. Actions of Calcitriol on Bone Cells

Calcitriol is involved in bone formation and resorption by both indirect and direct mechanisms.[51] The maintenance of plasma calcium and phosphorus levels, through the actions of this hormone on the gastrointestinal tract and kidney, supports normal mineralization in bone tissue. A number of direct actions of calcitriol have been reported, including increased numbers and activity of osteoclasts[52] and both increased and decreased collagen synthesis by osteoblastic cell lines.[51]

Studies by Key *et al.*[53] on the treatment of congenital osteopetrosis with high-dose calcitriol clearly demonstrated stimulation of osteoclastic bone resorption by calcitriol. A pretreatment bone biopsy specimen from children with this condition contained no osteoclasts with ruffled borders, a feature of active osteoclasts. After 11 days of calcitriol, ruffled borders were noted, and after 3 months, numerous osteoclasts with ruffled borders as well as associated bony disruptions were evident. These authors suggested that calcitriol stimulated cellular differentiation, resulting in cells capable of bone resorption, and stimulated these cells to resorb bone at a higher level than normal. Since calcitriol receptors have not been convincingly demonstrated on osteoclasts, it is probable that this hormone is involved in the differentiation of osteoclasts from stem cells in the bone marrow.

Chen *et al.*[54] examined the functional bioresponses of bone to calcitriol and dexamethasone in rat osteoblast cells. Their studies showed that both calcitriol and dexamethasone reduced collagen synthesis and that calcitriol induced the production of osteocalcin (Gla protein) and stimulated 24-hydroxylase activity in these cells. The physiologic significance of such studies remains to be clarified.

Silve *et al.*[55] developed techniques that permit the evaluation of the metabolic properties of bone-derived cells *in vitro*. Their work showed that osteoblastic cells from children with vitamin D-resistant rickets were resistant to calcitriol, but that children with acroosteolysis with osteo-

porosis and hyperphosphatasia with osteoectesia responded normally to this hormone.

1.4.3. Actions of Calcitriol on Renal Tubular Cells

The renal action of calcitriol has recently been reviewed by Kawashima and Kurokawa.[12] These authors detailed the conflicting results obtained in studies of the role of calcitriol on the renal transport of both calcium and phosphate by the renal tubule and pointed out that these discrepant findings may result from the differences in the vitamin D and dietary status of the experimental animals and the different dosages of calcitriol that were used.

Yamamoto *et al.*[56] demonstrated that repletion of vitamin D in D-deficient rats facilitated renal calcium reabsorption and enhanced the responsiveness of the tubule to PTH (see Section 4.2). Vitamin D-dependent calcium-binding protein is found almost exclusively in the distal tubule.[57]

Studies in isolated renal tubular cells showed that calcitriol increases phosphate uptake by renal tubular cells, a process that was blocked by inhibitors of protein synthesis.[58] Kurnik and Hruska[59] studied the effects of calcitriol on phosphate transport in the kidney using a model of partial vitamin D depletion in weanling rats. They were able to demonstrate that vitamin D depletion was associated with decreased inorganic phosphate transport which was rapidly corrected with physiologic amounts of calcitriol. This effect was manifest in the brush border membrane of the renal proximal tubular cell at the level of the Na^+-dependent active transport mechanisms. Similar results were reported by Egel *et al.*,[60] who also showed that the calcitriol-induced increase in phosphate reabsorption at the brush border membrane of the proximal tubule required a permissive dose of PTH and was associated with a reduction in renal cortical gluconeogenesis.

1.4.4. Actions of Calcitriol on Endocrine Glands

The finding of calcitriol receptors in a number of endocrine glands, including pancreas, parathyroid, pituitary, and ovary, has raised the question of the role of calcitriol in the regulation of hormone release from endocrine glands.

Hochberg *et al.*[61] found that children with defective binding or lack of receptors of calcitriol had no significant abnormalities in hormone secretion from pituitary, pancreas, and testis. However, there is evidence from *in vitro* studies that vitamin D metabolites are involved in the regulation of endocrine β-cell function, including insulin secretion.[62] This

possibility has important implications for uremic patients, in whom impaired carbohydrate metabolism is a common finding. Akmal *et al.*[63] have shown that the state of secondary hyperparathyroidism in chronic renal failure plays a major role in the genesis of the associated glucose intolerance by reducing insulin secretion. However, the effect of calcitriol deficiency on the impaired insulin release associated with chronic renal failure remains to be clarified.[64]

The presence of calcitriol receptors in parathyroid cells has raised the possibility of a regulatory effect of the vitamin D hormone on PTH secretion. Cantley *et al.*[65] showed a significant suppression of PTH secretion by cultured bovine parathyroid cells when incubated with, but not acutely exposed to, calcitriol. These authors point out that the results of studies performed to test for acute effects of calcitriol on PTH secretion *in vitro* have been contradictory, suggesting that this reflects the varying methodology used by different investigators. Seshadri *et al.*,[66] in a recent study using a sensitive PTH bioassay, were unable to demonstrate an effect of acute intraperitoneal administration of calcitriol to suppress PTH secretion in vitamin D-deficient hypocalcemic rats. Other recent studies[67] have suggested that calcitriol may suppress PTH secretion *in vivo* (see also Section 2.2.3).

1.4.5. Actions of Calcitriol on Cells of the Hematopoietic and Immune System

In the early 1980s, the discovery of calcitriol receptors on cells of the hematopoetic and immune system stimulated a major new area of research relative to the vitamin D endocrine system.[39]

In vitro evidence suggests that calcitriol may enhance differentiation of myeloid progenitor cells predominantly toward mononuclear phagocytes.[68] Since monocytes are probable precursors of osteoclasts, this may be the mechanism by which calcitriol increases osteoclast numbers and activity.[14]

Studies have provided increasing evidence for the interaction between macrophages and the vitamin D endocrine system. In sarcoidosis, pulmonary alveolar macrophages have been shown to convert calcidiol to calcitriol *in vitro*.[69] Reichel *et al.*[68] examined pulmonary alveolar macrophages obtained by bronchial lavage and showed that these macrophages were able to synthesize calcitriol and that activation of this process could be induced by γ-interferon and lipopolysaccharides.

The evidence that calcitriol is involved in regulation of the immune system has recently been summarized by Manolagas[70]: cells from the monocyte/macrophage series contain receptors for calcitriol regardless of their activation stage, and cells of the lymphoid series express this

receptor only at certain stages of differentiation and require activation; calcitriol promotes differentiation of monocytes toward the macrophage phenotype and may enhance the function of macrophages in phagocytosis and antigen presentation. Calcitriol is a potent inhibitor of interleukin-2 production by activated lymphocytes, has suppressive effects on T and B lymphocytes, and may be involved in the differentiation of T lymphocytes in the thymus.[70]

The relationship between these *in vitro* actions of calcitriol on hematopoietic and immune cells and clinical manifestations in diseases such as uremia promises to be a fascinating and clinically important area of continuing research.

1.5. Actions of $24,25(OH)_2D$

The role of $24,25(OH)_2D$ in the vitamin D endocrine system remains controversial.[71] $24,25(OH)_2D$ is produced in greater quantity and circulates at higher levels than calcitriol.[13] Studies by Horst *et al.*[72] in humans and pigs showed that the kidney is the major site of $24,25(OH)_2D$ production, but that extrarenal production could be demonstrated in pigs given pharmacologic doses of vitamin D.

Data with regard to a possible inhibitory action of pharmacologic doses of $24,25(OH)_2D$ on PTH secretion are conflicting in both animal and human studies.[73] Canterbury *et al.*[74] reported that 2 μg of $24,25(OH)_2D$ daily in uremic dogs for 3 weeks resulted in suppression of immunoreactive PTH. More recent long-term studies in uremic dogs by Olgaard *et al.*[73] using 2.5 μg of $24,25(OH)_2D$ daily for 1 year failed to show suppression of PTH secretion or skeletal action. Large doses of $24,25(OH)_2D$ in hypercalcemic, hyperparathyroid postrenal transplant patients did not suppress PTH secretion.[75] Further, no effect of long-term pharmacologic doses of $24,25(OH)_2D$ was demonstrated in patients with primary hyperparathyroidism.[76]

The data with regard to the effect of $24,25(OH)_2D$ on bone development are also conflicting. Parfitt *et al.*[77] used 24,25-difluoro-25-hydroxy cholecalciferol, a synthetic compound that can undergo 1-hydroxylation but not 24-hydroxylation, in studies in rats. Their results indicated that calcitriol is the only metabolite that is both necessary and sufficient for normal bone growth and development. However, in vitamin D-deficient chicks, Rambeck *et al.*[78] demonstrated a synergistic effect of calcitriol and $24,25(OH)_2D$ on increasing bone ash content. Using autoradiographic techniques, $24,25(OH)_2D$ receptors have been identified in cartilage cells of rats and chicks and suggest a role for this compound in the maturation of bone.[79] Results of studies concerning the use of $24,25(OH)_2D$ in uremic osteodystrophy will be discussed later.

2. Parathyroid Hormone

2.1. Secretion

PTH is synthesized as a large precursor, prepro-PTH, on the rough endoplasmic reticulum. After cleavage of the signal presequence, pro-PTH is transported to the Golgi apparatus, where the prosequence is removed. PTH is then moved to the secretory granules of the parathyroid cells and is secreted by exocytosis.[89] Certain proteins, including rat preproinsulin, have been successfully processed by *Escherichia coli* and yeast cells after introduction of the eukaryotic gene into these cells. Using recombinant DNA techniques,[81,82] human prepro-PTH cDNA was introduced into GH4 cells, a rat pituitary cell line that is capable of regulated secretion of prolactin and growth hormone. These cells were found to transport, cleave, and secrete PTH into the medium. Secretion was stimulated by TSH, a secretagogue for pituitary GH4 cells. By contrast, when placed in *E. coli* or in yeast cells, prepro-PTH was synthesized but not further processed. Preparation of mutant prepro-PTH DNA molecules will permit further analysis of the roles of different portions of the prepro sequence.[80]

Phosphorylation of PTH by human or bovine parathyroid tissue has recently been demonstrated and shown to occur on serine residues in the N-terminal portion of PTH.[83] The extent to which this phosphorylation is controlled *in vivo*, or may influence the transport and activity of PTH, remains to be investigated. Kemper *et al.*[84] first noted that a glycoprotein (secretory protein I) was cosecreted with PTH by parathyroid cells. It is present in secretory granules, and its function is unknown. The protein is actively phosphorylated,[85] and a similar secretory protein is present in catecholamine secretory granules (chromogranin A) and in other polypeptide hormone-producing tissues.[86]

2.2. Factors Affecting PTH Secretion

2.2.1. Calcium

The major factor controlling the release of PTH from parathyroid cells is the extracellular fluid ionized calcium level. The normal parathyroid cell shows an inverse sigmoidal relationship between PTH secretion and the extracellular ionized calcium concentration.[87] Ultracytochemical studies by Dietel *et al.*[88] have shown that parathyroid cell cytosolic calcium levels are reduced by incubation of the cells in a low-calcium environment, that DB-cAMP and lithium lower intracellular calcium in a normocalcemic environment, while high-calcium, aluminum, and calcitriol increase cellular calcium and suppress PTH secretion.

Brown and associates have used the Quin-2 technique to examine the relationship between PTH secretion and cytosolic calcium concentration in dispersed bovine parathyroid cells.[89,90] A close correlation was observed between the effects of extracellular calcium on PTH release and cytosolic calcium. The effects of elevated extracellular calcium on PTH release were mimicked by addition of the divalent cation ionophore A23187 at a fixed calcium concentration. These data suggested that alterations in the sensitivity of parathyroid tissue to extracellular calcium might result from changes in the relationship between the extracellular and cytosolic calcium concentrations. This possibility was further explored by LeBoff *et al.*[91] In these studies the relationship between extracellular calcium, cytosolic calcium, and PTH secretion was studied using Quin-2 in adult, neonatal, and cultured bovine as well as pathologic human parathyroid cells. Cells from the parathyroid glands of neonatal calves, which show reduced sensitivity to the inhibitory effect of calcium on PTH release, showed a greater set-point for secretion than adult cells (extracellular calcium concentration causing half-maximal inhibition of PTH release 1.27 ± 0.11 versus 1.06 ± 0.11 mmole), and a slightly higher calcium was necessary to raise cytosolic calcium to a given level in the neonatal than the adult bovine parathyroid cells. There was a close correlation between the set-point for PTH secretion and the set-point for cytosolic calcium in neonatal and adult cell preparations. In cells from parathyroid adenomas, the extracellular calcium concentration necessary to raise cytosolic calcium concentration to a given level was higher than in neonatal cells. In four of five preparations of human parathyroid cells, there was a correlation between the set-points for secretion and cytosolic calcium. In parathyroid cells from a fifth parathyroid adenoma, there was only 29% suppression of PTH release at 1 mmole of extracellular calcium, despite an increase in cytosolic calcium to levels comparable with that in adult bovine parathyroid cells. The poor suppressibility of this cell preparation suggests a defect distal to the mechanism regulating the cytosolic calcium concentration.

Since both neonatal bovine and pathologic human parathyroids show cellular hyperplasia, the relationship was studied between cellular proliferation and the regulation of PTH release by cytosolic calcium concentration in cultured bovine parathyroid cells. On successive days, during which cellular proliferation occurred, high calcium inhibited maximal PTH release progressively less from 59% on day 1 to 17% on day 4. Cytosolic calcium at 3 mmole extracellular calcium was 701 nmole on day 1 and 314 nmole on day 4. These studies suggest that during active proliferation, there is altered regulation of cytosolic calcium on PTH release. It is possible that the low cytosolic calcium concentration promoted enhanced cellular proliferation, and that parathyroid cells divide

to maintain an increased hormonal output at a lower calcium concentrations, as postulated by Parfitt.[92]

Although PTH secretion varies inversely with higher parathyroid intracellular free calcium levels, at lower levels PTH release may be independent of free calcium concentrations.[90] Extracellular calcium has also been shown to influence parathyroid gland adenylate cyclase, with elevated calcium concentrations inhibiting the enzyme.[93] Cyclic AMP may therefore be involved in the mediation of changes in PTH secretion by calcium.

2.2.2. Magnesium and Aluminum

Although calcium is the principal regulator of PTH secretion, other factors are known to influence PTH release,[94,95] including α- and β-adrenergic antagonists, histamine, prostaglandins, secretin, magnesium, vitamin D, aluminum, and the hypocalcemic agent WR 2721. The effects of magnesium are complex: profound hypomagnesemia inhibits the secretion and peripheral actions of PTH.[96,97] Lesser degrees of hypomagnesemia stimulate PTH secretion, while hypermagnesemia suppresses it, but in this respect magnesium is only one-half to one-third as potent as calcium.[98] Cholst *et al.*[99] have observed that intravenous administration of magnesium sulfate to pregnant women (to suppress premature labor), resulting in marked hypermagnesemia (mean 6.1 mg/dl), was associated with hypocalcemia (mean 7.6 mg/dl at 3 hr) and PTH suppression. Thus, marked hypermagnesemia in human subjects suppresses PTH and the parathyroid response to the concomitant hypocalcemia.

The effects of aluminum on PTH secretion are of clinical interest since aluminum intoxication in hemodialysis patients is associated with relatively low PTH levels.[100] *In vitro* studies[101–104] have shown that low aluminum concentrations stimulate PTH release, while higher levels decrease PTH release and cause parathyroid cell damage.

2.2.3. Calcitriol

The role of calcitriol in regulating PTH secretion has been controversial.[105–112] However, parathyroid glands have specific receptors for calcitriol, and recently, convincing evidence has emerged that calcitriol may exert an important modulating influence on PTH secretion in both pathologic and physiologic circumstances. Slatopolsky *et al.*[67] showed that intravenous administration of calcitriol in uremic patients resulted in marked suppression of PTH levels. A 20% decrease in PTH occurred without a significant change in serum calcium. In a subgroup of patients, an increase in serum calcium produced by oral calcium carbonate pro-

duced only a 25% decrease in PTH, while a similar increase in serum calcium produced by calcitriol resulted in a 73% decrease in PTH. These studies indicate that calcitriol, administered intravenously, in contrast to orally (which did not alter PTH levels), resulted in a substantial suppression of parathyroid activity. The authors suggested that intravenous calcitriol was effective because this route of administration avoided possible degradation of the hormone by the intestine. These studies also suggest the possibility that a component of secondary hyperparathyroidism in renal failure may result from a decreased calcitriol effect on the parathyroid glands.

Silver *et al.*[113] showed that calcitriol decreased prepro-PTH messenger RNA levels in a dose-dependent manner in primary cultures of isolated bovine parathyroid cells. Subsequently, the regulation of prepro-PTH mRNA by calcitriol has been examined *in vivo* in the rat.[114] After 50 or 100 pmoles of calcitriol given intraperitoneally, prepro-PTH mRNA levels decreased to 50% of basal at 6 hr and 25% at 24 hr. A second dose of 50 or 100 pmoles at 24 hr decreased prepro-PTH messenger RNA levels at 48 hr to 4% of basal. There was no increase in serum calcium. These results indicate that calcitriol plays an important role in regulating the synthesis of PTH *in vivo*.

2.2.4. WR 2721

Glover *et al.*[115] first reported hypocalcemia and inhibition of parathyroid secretion after the administration of WR 2721 [S-2 (3 aminopropylamino) ethylphosphorothioic acid] a radioprotective and chemoprotective agent. Attie *et al.*[116] showed that intravenous injection of 15 mg/kg WR 2721 in dogs caused a 25% fall in serum calcium and a fall in PTH. However, WR 2721 also induced a fall in serum calcium in parathyroidectomized dogs and in PTH-infused dogs. In experiments in rats, WR 2721 was shown to inhibit the entry of calcium into the circulation, presumably from bone, and in primary chick osteoclast cultures, WR 2721 inhibited bone resorption. Thus, the drug appears to have a direct effect on bone resorption in addition to its effect on the parathyroid glands. *In vitro* experiments showed that WR 2721 reduced the release of PTH from bovine parathyroid cell suspensions.[115] WR 2721 protects normal tissues against radio- or chemotherapy after being dephosphorylated. Hirschel-Scholz *et al.*[117] have shown in rat experiments that the phosphorylated and dephosphorylated forms of WR 2721 have an equal hypocalcemic potency in parathyroid-intact animals. In the rat, the drug did not impair the actions of PTH on renal calcium and phosphorus handling or urinary cAMP excretion. In TPTX rats, however, WR 2721 had a PTH-independent action to inhibit renal tu-

bular calcium resorption, which contributes to its hypocalcemic effect. The drug also causes a fall in serum magnesium,[115,117] but prevention of hypomagnesemia by magnesium infusion did not reduce the hypocalcemic effect.[117] Thus, this unique hypocalcemic agent appears to cause hypocalcemia by direct bone and renal tubular effects as well as by suppressing PTH release. This agent could become useful for the medical treatment of hyperparathyroidism: its use has already been reported in a patient with refractory hypercalcemia due to recurrent parathyroid carcinoma.[118]

2.3. Parathyroid Hormone Structure–Activity Relationships and Development of Inhibitors

For PTH to exhibit bioactivity, it must first interact with receptors in target cells. Full activity of 1-34 PTH has been shown in multiple PTH assay systems.[80] The 3-34 sequence is necessary for binding, while amino acids in the 1 and 2 position are required for hormone action.[119] An analog of the 3-34 sequence Nle8, Nlel8, Tyr34, bPTH 3-34 NH2 proved to be a true competitive inhibitor *in vitro*.[120] However, *in vivo* it failed to display inhibitory properties because of weak agonist effects,[121–123] including elevation of serum calcium, phosphaturia, and increased calcitriol production. Progressive truncation from the N-terminus showed that the 7-34 sequence was still bound, though much less avidly than the 3-34 sequence. Substitution of tyramide enhanced activity *in vitro*.[80] This led to the production of Tyr34, bPTH7-34 NH_2, which was purified.[124] Simultaneous infusion in a 30- to 200-fold molar excess with bovine 1-34 PTH completely inhibited phosphaturia and the increase in urinary cAMP in the rat. Similarly, the antagonist inhibited PTH's calcemic effect in the TPTX rat.[119] Further development of these antagonists may result in the production of agents of value in treating hypercalcemia associated with PTH excess and perhaps hypercalcemia of malignancy, if it is, as recently suggested, mediated by non-PTH humoral factors interacting with the PTH receptor.[125]

2.4. PTH Receptor

As with other hormones, photoaffinity labeling has been used in an attempt to identify PTH receptors.[126,127] A membrane component corresponding to the receptor or a binding subunit of Mr about 70,000 has been identified in renal, bone, and skin cells. The phenomenon of desensitization has been studied using photoaffinity labeling in cells cultured from a giant cell tumor of bone and has been shown to result from decreased receptor number and availability.

2.5. PTH Assays

Since Berson *et al.* first described radioimmunoassay of PTH in 1963,[128] numerous assays have been described. For a number of years, C-terminal assays have mainly been used for clinical purposes and have proved quite effective in differentiating patients with primary hyperparathyroidism from normal subjects. Recently, a number of new assays have been described. So called midregion (PTH 44-68) assays have proved to be clinically useful.[129] However, these assays suffer from the same problem as C-terminal assays with respect to accumulation of immunoassayable fragments in the serum in patients with renal impairment. Segre[130] has described the development of an amino-terminal assay using an antiserum to synthetic human 1-34 PTH. Results with this assay were reported to correlate well with those obtained using the cytochemical bioassay.[131] This assay has shown that PTH secretion is pulsatile in normal subjects, but rarely do these pulsatile changes interfere with the usefulness of static measurements in the differentiation of primary hyperparathyroidism from normal.

A commercially available PTH assay, the intact assay, which involves a two-step procedure with initial extraction of PTH using the antiserum followed by radioimmunoassay, also appears to provide a much sharper separation of normal from hyperparathyroid subjects than previously available assays.[132,133]

It may be unrealistic to expect any PTH assay to provide a complete separation of normal from hyperparathyroid subjects, since a set-point error in the glands may merely result in the secretion of normal amounts of PTH at an abnormal (increased) serum ionized calcium level.

2.6. PTH as a Uremic Toxin

The possibility that high levels of PTH may exert a toxic effect in chronic renal failure has been systematically explored by Massry and colleagues over the past few years. Evidence for a toxic effect in the central nervous system, the peripheral nervous system, skeletal muscle, the heart, and the hematopoietic system, has been presented.[134] Recently, PTH has been incriminated as a contributing cause of glucose intolerance in chronic renal failure. In detailed studies PTH was shown to interfere with the ability of β cells to increase insulin output in the face of peripheral insulin resistance.[63]

With respect to erythrocyte survival in chronic renal failure, Akmal *et al.*[135] have shown that the shortened red-cell survival in chronic renal failure in the dog is a consequence of excess blood levels of PTH, and when these are prevented by parathyroidectomy, red-cell survival is nor-

mal. However, McGonigle *et al.*[136] have provided evidence that PTH does not act as an inhibitor of erythropoiesis in renal failure.

3. Calcitonin

Studies in the rat have suggested that the calcitonin gene encodes five distinct peptides. It is thought that tissue-specific gene processing produces distinct precursors; in the first, produced in the thyroid, calcitonin is flanked by amino-terminal and c-terminal peptides; in the second, produced in the nervous system, calcitonin is replaced by calcitonin gene-related peptide (CGRP), flanked by the same amino-terminal peptide as procalcitonin, but by a different c-terminal peptide.[137–139] Rat CGRP has many actions, including vascular effects, but its physiologic role is not yet clear. Human CGRP has been shown to be a potent vasodilator in humans.[140] The human calcitonin precursor contains the C-terminal flanking peptide katacalcin, which has a different sequence from that of the rat, and is secreted and circulates in normal subjects,[141] paralleling calcitonin levels. Levels are greatly elevated in medullary thyroid carcinoma. The physiologic role of katacalcin is uncertain.

4. Calcium: Physiology and Pathophysiology

4.1. Renal Handling of Calcium

This subject has recently been extensively reviewed.[142] More than 50% of filtered calcium is reabsorbed along the proximal tubule. The nature of proximal tubular calcium reabsorption has been examined in detail by Bomsztyk *et al.*[143] In *in vivo* microperfusion studies of the proximal tubule of the rat, using a variety of different solutions containing different anions together with mannitol sufficient to reduce net fluid flux to near zero, electrochemical driving forces across the tubule wall were calculated from measurements of transepithelial voltage and of calcium ion activity in perfused and collected fluids. Ion-selective electrodes were used to determine luminal calcium ion activity. Proximal calcium reabsorption was shown to depend on luminal calcium ion activity and transepithelial voltage. At zero transepithelial electrochemical driving force, calcium absorption was significantly greater than zero, implying the presence of active calcium transport, as had previously been detected by Ullrich *et al.*[144] but not by Ng *et al.*[145] With certain anion replacements, calcium and sodium transport were clearly dissociated.

For example, with EGTA, calcium transport decreased, whereas sodium transport was not significantly changed. With sulfate and citrate solutions, the reduction in calcium absorption was substantially larger than the reduction in sodium absorption. In a preliminary report,[146] Bourdeau examined calcium transport across the pars recta of rabbit cortical proximal tubules and observed net calcium reabsorption that could be accounted for by diffusion (probably paracellular), driven by the favorable electrochemical gradient, when tubules were perfused with simulated "late proximal tubule fluid."

Although the net result of PTH on renal calcium reabsorption is an enhancement, many studies have shown that the effect within the proximal tubule is a diminution of bulk reabsorption and of calcium reabsorption. Scoble *et al.*[147] have recently studied calcium transport in canine renal basolateral membrane vesicles and have examined the effect of PTH. Evidence was obtained for electrogenic Na^+–Ca^{2+} exchange activity. There was a sodium gradient-independent calcium flux. The sodium-dependent efflux was very rapid. Both calcium fluxes were decreased in vesicles from parathyroidectomized dogs, and both were stimulated by PTH infusion. The authors suggest that *in vivo* the sodium-dependent calcium flux could result in an elevation of cytosolic calcium which, it is postulated,[148] would decrease apical entry of calcium and sodium and hence contribute to the observed decrease in solute transport in the proximal tubule.

With respect to calcium reabsorption in the subsequent nephron segments, calcium reabsorption in the thick ascending limb of Henle's loop is in large part driven by the lumen-positive transepithelial PD in this segment.[149] There is also evidence suggesting the presence of a separate active calcium reabsorptive process in the cortical thick ascending limb.[150] With respect to the component of passive calcium transport in this segment, current models involve the Na,K-ATPase pump at the basolateral cell membrane, which lowers the intracellular sodium concentration, and an Na/K/2 Cl cotransporter at the luminal surface, which transports K and Cl into the cell against their electrochemical gradients.[151] Intracellular chloride then exits preferentially at the basolateral surface, resulting in the lumen-positive electrochemical PD. This PD may then be the driving force for reabsorption of cations including calcium[149] and magnesium.[152] Recent studies of frog skin,[153] a membrane that exhibits cAMP-stimulated chloride-dependent calcium secretion, are of interest in relation to this model. It is suggested that in this membrane, as in other chloride-secreting membranes,[154] the Na/K/2 Cl cotransporter and the Na,K-ATPase pump are both located on the basolateral (serosal) cell surface. Furosemide pretreatment abolished both chloride and calcium secretion; substitution of chloride with nitrate

blunted calcium secretion. Further studies of this membrane may shed light on the relationship of calcium to chloride transport in the thick ascending limb. Agus has suggested that there could be coupled calcium–chloride cotransport at the mucosal surface in frog skin (perhaps induced by cAMP) and at the basolateral surface in the thick ascending limb.[155]

In the distal convoluted tubule and collecting duct, calcium transport is active and proceeds against an electrochemical potential difference. Bourdeau and Hellstrom-Stein have shown that a small passive net secretory flux does occur in the collecting duct at negative voltages, but it is small because calcium permeability is very low.[156]

4.2. Factors Affecting Calcium Reabsorption

Net calcium reabsorption is enhanced by PTH, metabolic alkalosis, thiazide diuretics, and amiloride, while net reabsorption is decreased by metabolic acidosis, furosemide, and phosphate depletion.[142] The influence of vitamin D on tubular calcium reabsorption has been controversial, vitamin D having been shown to have a hypocalciuric effect[157,158] or a hypercalciuric effect.[159,160] Recently, in clearance studies in rats, Yamamoto *et al*[56] have shown that in parathyroidectomized rats, the "threshold of calcium excretion" (i.e., the theoretical serum level at which urinary calcium is zero) was lower in D-deficient than in D-replete animals. Administration of PTH at a dose of 0.75 units/hr increased the threshold of calcium excretion in D-replete rats but had no effect in D-deficient animals. A higher PTH dose (2.5 units/hr) raised the threshold in the D-deficient animals to an extent similar to the lower dose in D-replete animals. These results strongly suggest that D deficiency in the rat is associated with an impairment of renal tubular calcium reabsorption and a resistance to the hypocalciuric effect of PTH. Burnatowska *et al.*,[161] however, in a study of vitamin D-replete hamsters, were able to show that in parathyroidectomized animals infused with a low dose of PTH, insufficient to fully correct hypocalcemia, the superimposition of intravenous calcitriol resulted in a significant increase in the fractional excretion of both calcium and magnesium. The latter results suggest that in vitamin D-replete animals, additional calcitriol may antagonize the effect of PTH and thus have a net hypercalciuric effect. Thus, the influence of vitamin D on tubular calcium reabsorption may depend on the vitamin D status of the animal and the vitamin D metabolite being administered.

With respect to amiloride, Costanzo,[162] using *in vivo* microperfusion techniques in the rat, has recently shown that this agent inhibits sodium reabsorption but promotes calcium reabsorption in the second half of the

distal convoluted tubule, while thiazide has a similar effect in the first half of the distal convoluted tubule. Thiazide and amiloride therefore have additive hypocalciuric actions, making this drug combination an attractive choice for the management of hypercalciuric disorders.[163,164]

A tracer microinjection study of the hypercalciuria of phosphate depletion has recently suggested the presence of a defect in calcium reabsorption at a site(s) between the late proximal and early distal tubules.[165] The defect was not correctable with PTH infusion. In addition to the factors mentioned earlier, chronic prolactin excess has been shown to cause hypercalciuria by a PTH-independent action, probably a direct inhibitory action on tubular calcium reabsorption.[166]

4.3. Hypercalcemia

4.3.1. Primary Hyperparathyroidism

4.3.1.1. Pathophysiology. The underlying defect in the parathyroid glands in primary hyperparathyroidism may involve both an alteration of the set-point for PTH release and proliferation of parathyroid cells. Many studies have shown that PTH secretion in primary hyperparathyroidism is not autonomous, but does respond to exogenous calcium.[167–171] Insogna *et al.*[172] have shown that even modest changes in the dietary calcium intake from 400 to 1000 mg/day may be associated with significant changes in immunoreactive PTH and in urinary nephrogenous cAMP, as well as in plasma levels of calcitriol.

Although calcitriol levels would be expected to be elevated in primary hyperparathyroidism and have frequently been reported to be so, Hulter *et al.*[27] have shown that normal human subjects, undergoing 12 days of continuous intravenous PTH infusion to induce sustained moderate hypercalcemia and hypophosphatemia, actually show a significant decrease in plasma calcitriol. In experiments in dogs, these authors showed that similar reduction in plasma calcitriol concentration was not seen if the hypercalcemia was prevented during PTH infusion by a simultaneous EGTA infusion. In these animals, the PTH resulted in a sustained increase in the plasma calcitriol concentration. These data suggest that hypercalcemia can prevent and even reverse the elevated calcitriol level associated with chronic hypersecretion of PTH.

Gardin and Paillard[173] have examined the mechanism accounting for normocalcemia in some cases of primary hyperparathyroidism. In a group of patients with primary hyperparathyroidism and stable plasma calcium levels ranging from 9.5 to 13.7 mg/dl, these authors found no significant correlation between immunoassayable PTH or nephrogenous cAMP and the degree of hypercalcemia. Similarly, all patients displayed

similar degrees of net bone resorption and intestinal calcium absorption. However, the relationship between urinary calcium excretion and serum calcium level was examined and was compared with the same relationship in subjects with normal parathyroid function. Tubular reabsorption of calcium was observed to be increased in the hypercalcemic patients, but was normal or subnormal in the patients with normal serum calcium levels. These data suggest that the main determinant of the plasma calcium value in primary hyperparathyroidism is the tubular reabsorption of calcium. Since tubular calcium reabsorption was not related to PTH levels, these authors suggest that undetermined factors must interfere with the tubular action of PTH in the normocalcemic patients. After surgical removal of the parathyroid adenomas, fasting hypercalciuria and intestinal absorption of calcium returned to normal, indicating that these patients were not suffering from a preexisting renal tubular calcium reabsorptive defect ("renal hypercalciuria") such as has been previously proposed.[174] The latter authors suggested that so-called renal hypercalciuria might cause initially secondary and ultimately autonomous (tertiary, or apparent primary) hyperparathyroidism, but such a sequence of events would not account for the observations of Gardin and Paillard.

With respect to vitamin D metabolism in primary hyperparathyroidism, LoCascio *et al.*[23] have observed that the administration of 50 μg of calcidiol for a month in normal volunteers did not result in any change in serum calcitriol levels, whereas in six patients with primary hyperparathyroidism a sharp increase in calcitriol occurred, and there was a significant positive correlation between calcidiol and calcitriol levels. These observations indicate that, unlike normal subjects in whom calcitriol levels are not a function of calcidiol levels, in primary hyperparathyroidism calcitriol levels are dependent on calcidiol levels, as they are in sarcoidosis. The data suggest that prevailing levels of calcidiol should be accounted for in studies of the role of calcitriol in primary hyperparathyroidism.

4.3.1.2. Preoperative Evaluation of Primary Hyperparathyroidism. The diagnosis is usually suggested by the presence of hypercalcemia, though "normocalcemic" hyperparathyroidism is being increasingly recognized.[173] Confirmation of the diagnosis may be obtained by means of improved PTH assays and/or by observing an increased urinary (or nephrogenous) cAMP excretion. The parathyroid glands, in primary hyperparathyroidism, exhibit a variable sensitivity to exogenous calcium.[172] Prior to surgical treatment (parathyroidectomy), localization of the enlarged parathyroids may be attempted (in primary, secondary, or so-called tertiary hyperparathyroidism) by means of high-resolution, real-time ultrasonography[175–177] or by subtraction nuclear scanning.[178,179]

The latter technique, when combined with a color computer comparison, appears highly sensitive.[180] However, the precise role and value of these techniques in the patient about to undergo a first parathyroid exploration are not yet clear. In the patient with a previous failed neck exploration, additional methods for localization of the abnormal parathyroid(s) are available, including computed tomography,[181] nonselective digital arteriography[182] (which appears relatively insensitive[183]), selective arteriography, venography, and PTH assay,[184–186] and parathyroid aspiration guided by ultrasound,[187] CT,[188] or angiography[189] and combined with cytology and histology of the aspirated cells[187] or PTH assay.[188,189] A comparison of some of these methods, to determine their respective values, has been reported,[179] but further evaluation is required.

4.3.1.3. Treatment of Primary Hyperparathyroidism. In the past, surgery was recommended for all patients with primary hyperparathyroidism, since even patients with mild hypercalcemia were considered to be at risk for a variety of complications, including declining renal function, acute severe hypercalcemia, and progressive osteopenia. Recently, with the recognition that the condition is frequently asymptomatic, and is most common in older women,[190] conservative (nonoperative) treatment has become commoner, but most physicians continue to advise surgery when the serum calcium is above 11.0 mg/dl. Paterson *et al.*[191] have recently reported on a group of 14 patients with serum calcium levels above 11 mg/dl who were followed for 5–23 years. Their serum calcium levels did not tend to rise, and they came to little obvious harm from not having surgery, leading to the suggestion that conservative management may be appropriate in the asymptomatic patient, even in the presence of moderate hypercalcemia. However, some of these patients did suffer from fractures and renal calculi, and systematic studies of bone density were not performed.[192] There is general agreement that patients with renal calculi, peptic ulcers, pancreatitis, and osteitis fibrosa should be treated surgically. More information is required with respect to risk factors for the development of complications in patients who admit to few or no symptoms, in order to decide when surgery should be recommended for these patients. A prospective randomized clinical trial has been proposed to evaluate the risks and benefits of surgical versus nonsurgical treatment of primary hyperparathyroidism.[193]

A number of possible medical alternatives to surgical treatment have been examined, including diphosphonates[194] and estrogen,[195] but the future role of these agents (or of WR 2721—see Section 2.2.4) as alternatives to surgery is not clear at present.

With respect to parathyroidectomy, the patient with a solitary adenoma requires removal of the abnormal gland. However, solitary ad-

enomas may be subtle and small.[196] Optimal management of the patient with primary parathyroid hyperplasia remains controversial. Differentiation of adenoma from hyperplasia on the basis of random biopsy specimens is unreliable.[197] Total parathyroidectomy with autotransplantation has been recommended[198] but carries a risk of permanent hypoparathyroidism or probably of persisting hyperparathyroidism.[199] Bondeson *et al.* have recently recommended an individualized approach whereby, when all glands are moderately enlarged, a subtotal (3 1/2 gland) parathyroidectomy is performed, whereas when one or more glands are of normal size, one gland is left intact, to reduce the risk of permanent hypoparathyroidism.[200] Examples of accidental seeding of nonmalignant parathyroid cells at surgery have been described,[201] and glands should be handled carefully at operation.

In patients undergoing arteriography following unsucessful parathyroid surgery, percutaneous infarction of the parathyroid tumor by staining with the contrast agent has been recommended for mediastinal adenomas with an internal mammary supply and has given satisfactory long-term results.[202]

4.3.2. Familial Hypocalciuric Hypercalcemia

This disorder, first described as familial benign hypercalcemia in 1972,[203] has recently been reviewed by Law and Heath.[204] These authors reviewed 125 patients belonging to 21 families. The main features of the syndrome include symptomless, nonprogressive hypercalcemia of autosomal dominant inheritance with normal serum immunoreactive PTH levels and parathyroid glands with normal gross and histologic features, relatively low urinary calcium excretion, and a failure to achieve normocalcemia after subtotal parathyroidectomy. Law and Heath reported that their large series of patients fared well clinically, with normal longevity and no discernible increase in other medical problems, except gallstones. Unlike the situation in primary hyperparathyroidism, the parathyroid glands were not detected by high-resolution ultrasonography. Plasma calcitonin and calcitriol levels were normal or low.[205] Skeletal mass was normal, as assessed by photon absorptiometry of the radius and lumbar spine, and fractures were not increased in frequency. In addition to the normal immunoassayable PTH levels, normal PTH activity has also recently been reported using the cytochemical bioassay.[206] Law *et al.* found that parathyroid gland weight was usually within the normal range and that histologic features of hyperplasia were usually absent.[207] With respect to the fundamental defect in familial hypocalciuric hypercalcemia, renal cAMP responses to PTH are normal. The

enhanced tubular reabsorption of calcium persists after total parathyroidectomy.[208] Studies of responses to diuretics suggest that the enhanced reabsorption of calcium may be occurring in the thick ascending limb of Henle's loop.[208,209] Since the serum calcium level reciprocally influences calcium reabsorption in the loop of Henle,[210] it is possible that this enhancement of calcium reabsorption represents a blunting of the normal inhibitory effect of hypercalcemia on calcium reabsorption in this segment, which is associated with a blunting of the normal inhibitory effect of hypercalcemia on the parathyroid glands. However, the parathyroid glands do respond appropriately to increases or decreases of the prevailing serum calcium level, as anticipated with a "set-point" abnormality.[204]

In support of the concept that familial hypocalciuric hypercalcemia may reflect a global defect of cell calcium transport, Hoare and Paterson[211] have reported the finding of increased active calcium efflux from the erythrocytes of patients with familial hypocalciuric hypercalcemia but not from the cells of patients with primary hyperparathyroidism. Subsequently, Mole and Paterson[212] have reported that the activity of the calcium-stimulated, magnesium-dependent ATPase of erythrocyte ghost membranes from patients with familial hypocalciuric hypercalcemia is significantly higher than in normal subjects, though not significantly higher than in those from patients with primary hyperparathyroidism.

An association between neonatal severe primary hyperparathyroidism and familial hypocalciuric hypercalcemia was reported by Marx *et al.* in 1982.[213] Subsequently, Marx *et al.*[214] have reported additional data suggesting that severe neonatal hyperparathyroidism may be the homozygous manifestation of a gene that in heterozygotes is expressed as mild intermittent hypercalcemia. As Marx *et al.* indicate,[214] it is interesting to speculate to what extent mild, intermittent familial hypocalciuric hypercalcemia might contribute to the variability of serum calcium values in the normal population.

The diagnosis of familial hypocalciuric hypercalcemia cannot be made confidently in an isolated person.[204] There is an overlap in urinary calcium excretion between primary hyperparathyroidism and familial hypocalciuric hypercalcemia, although a calcium:creatinine clearance ratio of 0.01 or less is suggestive of familial hypocalciuric hypercalcemia. The diagnosis should not be made without family screening. Other conditions reported to cause hypercalcemia in association with hypocalciuria include primary hyperparathyroidism associated with chronic renal failure[215] and hypothyroidism.[216] In the latter report it was postulated that the hypercalcemia was associated with an altered parathyroid set-point, presumably resulting from thyroid deficiency, and the hypocalciuria was secondary to elevated PTH levels.

4.3.3. Malignant Hypercalcemia

It has been suggested[217,218] that it may be advantageous to classify hypercalcemia of malignancy into three clinical categories: (1) hematologic cancers, (2) solid tumors with bone metastases, and (3) solid tumors without bone metastases. With respect to hematologic malignancies, those associated with hypercalcemia include myeloma and lymphoma. A recently described retrovirus-associated adult T-cell lymphoma has a particularly frequent association with hypercalcemia.[219] The hypercalcemia in these hematologic malignancies is associated with the production of osteoclast-activating factors. Malignant lymphoid cells may also produce inhibitors of osteoclastic bone resorption, including leukocyte interferon.[220] An interleukin-1-like factor that stimulates bone resorption *in vitro* has been reported and could account for hypercalcemia associated with some monocytic malignancies.[221] In addition, calcitriol could be produced locally by bone marrow cells, since macrophages have the ability to make calcitriol.[222] Increased serum calcitriol levels have been reported in some patients with adult T-cell lymphoma.[223] Since hypercalcemia is frequently associated with renal impairment in myeloma, impaired renal calcium excretion may be required for hypercalcemia to occur.

In patients with extensive metastases, particularly breast cancer, increased bone resorption is produced by tumor cells and by osteoclasts. Prostaglandins may be involved in the stimulation of osteoclasts. Incubation of cultured human breast cancer cells with estrogens or antiestrogens leads to release of bone-resorbing activity and of prostaglandins of the E series.[224] Indomethacin inhibits the release of bone-resorbing activity and the release of prostaglandins. Bone-resorbing activity is released only by breast cancer cells with estrogen receptors.[224]

The syndrome of hypercalcemia associated with solid tumors without bone metastases has recently been called humoral hypercalcemia of cancer. The humoral factor was initially thought to be PTH, but recent studies have shown that tumor tissue from tumors associated with hypercalcemia usually does not contain PTH messenger RNA.[225] Two other humoral protein factors that might be responsible for hypercalcemia of malignancy are "PTH-like" factors and transforming growth factors. The PTH-like factors[226,227] appear to bind to some, but not all, PTH receptors, causing increased renal cAMP generation and phosphaturia, but not enhanced calcitriol production. The effects of PTH-like factors *in vitro* are inhibited by synthetic PTH antagonists, but these factors do not interact with antiserum to PTH. It is possible that the factor responsible for renal phosphate wasting in oncogenic osteomalacia could be related to these PTH-like factors.

Transforming growth factors, which are polypeptide mitogens secreted by virus-transformed cells or neoplastic cells, confer neoplastic characteristics on target cells.[228] Transforming growth factor (TGFα) binds to the epidermal growth factor receptor and appears to be responsible for bone resorption in several animal models of humoral hypercalcemia of malignancy.[217] The TGFα produced by these tumors appears to be abnormal.[229] Two other related substances, TGFβ and platelet-derived growth factor, may be involved in hypercalcemia of malignancy.[218] There are homologies between one chain of platelet-derived growth factor and the protein encoded by the v-sis oncogene.[230,231] Mundy *et al.*[218] suggest that cellular oncogene expression (possibly c-sis) may lead to activation of the genes for PTH-like factors and transforming growth factors. This would lead to secretion of both classes of proteins, which may together produce hypercalcemia. Transforming growth factors may act predominantly to increase bone resorption, and human TGFα has recently been shown to be a potent bone-resorbing agent,[232] while PTH-like factors may act predominantly on the kidney, producing both phosphaturia and calcium retention. The latter effect may be important, in conjunction with increased calcium release from the bone, in bringing about hypercalcemia of malignancy.[233,234]

With respect to the clinical differentiation of malignant hypercalcemia from primary hyperparathyroidism, a recent study has examined which laboratory tests are most useful.[235] These authors found that, among the tests performed as part of a routine biochemical profile, plus full blood count, blood pH, and serum immunoreactive PTH levels, the serum albumin, chloride, and PTH were the most useful indices, malignant hypercalcemia being characterized by a tendency to a lower serum albumin, a lower serum chloride, and a lower PTH level than primary hyperparathyroidism. Serum phosphate levels were not useful. Urinary calcium, phosphate, and cAMP were not included in this study.

4.3.4. Granulomatous Disorders

The incidence of hypercalcemia in sarcoidosis is reported to vary from 2% to 63%.[236] The hypercalcemia is associated with increased circulating levels of calcitriol, and the case report of Barbour *et al.*[33] indicated that the calcitriol is not of renal origin. Adams *et al.*[237] demonstrated that cultured alveolar macrophages from patients with sarcoidosis produced calcitriol from calcidiol. This *in vitro* synthesis of calcitriol was specific for alveolar macrophages from patients with active sarcoidosis. The vitamin D derivative produced by the macrophages was subsequently isolated and structurally identified as being 1,25-dihydroxy vitamin D_3.[69] Mason *et al.*[222] have since demonstrated *in vitro* synthesis of a calcitriol-like metabolite by a sarcoid lymph node.

With respect to other granulomatous disorders, hypercalcemia, and, perhaps more commonly, hypercalciuria have been reported in tuberculosis,[34] berylliosis, and coccidiodomycosis, as well as in association with granulomatous inflammation induced by cosmetically injected silicone.[238] Monocytic phagocytes, the principal cells of granulomas, have been shown to metabolize 25-hydroxy vitamin D_3 *in vitro*.[239] The ectopic production of calcitriol by granulomas is not regulated in the same way as its production in the kidney.[240] Most patients with granulomatous hypercalcemia also have impaired kidney function.[240] Gkonos *et al.* reported studies on a dialysis-treated patient with end-stage renal disease who had an increase in calcitriol levels and hypercalcemia at a time when his clinical course was complicated by active tuberculosis.[34] Elevated calcitriol levels have been observed in hypercalcemia associated with lymphoma.[35] A patient with Hodgkin's disease and hypercalcemia has been reported in whom elevated serum calcitriol levels were present. After spontaneous correction of hypercalcemia, ultraviolet irradiation caused a recurrence of hypercalcemia and high calcitriol levels. After chemotherapy, ultraviolet irradiation did not produce these effects. In this patient hypercalcemia was apparently caused by abnormal vitamin D metabolism, quite likely in the Hodgkin's tissue.[241]

Hypercalcemia has recently been reported in two patients with the acquired immunodeficiency syndrome and disseminated cytomegalovirus infection.[242] PTH and calcitriol levels were suppressed, and it is suggested that hypercalcemia may have resulted from increased osteoclastic bone resorption induced by infection of osteoclasts with either cytomegalovirus or HTLV-3.[242]

4.4. Hypocalcemia

The presence of hypocalcemia usually reflects either lack of PTH or an end-organ resistance to the hormone. In pseudohypoparathyroidism, a defect in the PTH receptor was initially postulated. However, some patients with this condition also have hypothyroidism, hypogonadism, mental retardation, and short stature. These patients have been shown to have a deficiency in the stimulatory guanine nucleotide-binding protein component (G_S) of the adenylate cyclase complex.[243] The defect is usually inherited in an autosomal dominant pattern. Other patients with pseudohypoparathyroidism and a generally normal appearance have normal G_S activity,[244] and resistance is limited to PTH. In a few patients with an abnormal appearance and pseudohypoparathyroidism, no defect in G_S has been demonstrated. The nature of the lesion in such patients in unclear. In pseudopseudohypoparathyroidism, the red-cell membranes have been found to show the same 50% deficiency in the G protein as in subjects with pseudohypoparathyroidism.[245] Several such patients

have had high immunoassayable PTH levels and increases in TSH, suggesting resistance to both PTH and TSH. Hurley *et al.*[246] have recently demonstrated homologies in the amino acid sequence of the G proteins and the protein products of the ras oncogene.

Despite the high immunoassayable PTH levels in pseudohypoparathyroidism, Fischer *et al.*[247] have found normal or low bioactive PTH levels. They have also demonstrated subnormal recovery of bioactive PTH when added to the plasma of pseudohypoparathyroid patients, indicating the presence of a factor in the circulation that interferes with the activity of PTH, perhaps by blocking PTH receptors. The relationship of this factor to the deficiency of the G protein in the syndrome is at present unclear. Allgrove *et al.*[248] have reported similarly elevated PTH levels using the cytochemical bioassay in pseudohypoparathyroidism and in vitamin D deficiency, and in both conditions bioactive PTH decreased in response to normocalcemia. However, in pseudohypoparathyroidism, immunoassayable N-terminal PTH did not return to normal in response to normocalcemia, suggesting continuing production of biologically inactive fragments which, it is speculated, could inhibit the biologic activity of PTH in the cytochemical bioassay.

The pathophysiology of the hypocalcemia associated with acute pancreatitis is controversial. Deposition of calcium in areas of fatty necrosis, hyperglucagonemia, PTH deficiency, and hypomagnesemia have been proposed as causes of hypocalcemia. In a recent study,[249] induction of acute pancreatitis in the dog was associated with hypocalcemia and with increased calcium content of the liver and especially muscle, as well as pancreas. It was suggested that pancreatic enzymes may have a widespread effect on cell membranes, allowing calcium to accumulate in soft tissues, and that this may make a significant contribution to the hypocalcemia. With respect to the clinical consequences of hypocalcemia, in addition to the familiar manifestations including tetany, seizures, etc.[142] hypocalcemic heart failure has recently been emphasized. A patient has been reported[250] with reversible cardiomyopathy and congestive heart failure associated with untreated hypoparathyroidism. Following normalization of the serum calcium level, there was rapid reversal of the signs and symptoms of heart failure.

5. Calcium Nephrolithiasis

The major advance in the therapy of renal calculi has been the introduction of extracorporeal shock wave lithotripsy, which is now becoming widely available in North America and elsewhere.[251,252] By dramatically reducing the morbidity associated with stone removal, this tech-

nique may have major impact on the extent of metabolic investigation and the aggressiveness of long-term preventive measures in stone patients. Nevertheless, the underlying pathophysiology and the efficacy of prophylaxis of calcium-containing renal stones continue to be intensively investigated.

5.1. Idiopathic Hypercalciuria

5.1.1. Renal Calcium Wasting

The issue whether idiopathic hypercalciuria usually reflects primary intestinal calcium hyperabsorption, primary renal calcium wasting, or neither remains unresolved.[253] Certain data have suggested the presence of renal tubular abnormalities in calcium stone patients,[254–258] as well as in a rat model of idiopathic hypercalciuria.[259,260] However, Coe *et al.*[261] have observed that, after a prolonged period on a very low calcium intake, patients with idiopathic hypercalciuria continue to waste abnormally large amounts of calcium in the urine and develop an appreciable negative calcium balance, but nevertheless maintain higher serum calcium levels and lower PTH levels than normal subjects. These observations strongly suggest augmented skeletal resorption in stone formers. The contribution of elevated calcitriol levels to this apparent increase in bone resorption is unclear, since elevated calcitriol levels have been observed in some,[262,263] but not all,[261,264] groups of patients with idiopathic hypercalciuria. Further evidence suggesting abnormal skeletal resorption in calcium stone patients has recently been obtained from studies of urinary hydroxyproline excretion.[265] In these studies, whereas normal subjects showed a suppression of urinary hydroxyproline in the daytime, presumably related to calcium ingestion,[266] stone patients did not show this normal circadian rhythm. Several authors have interpreted their data as favoring a single pathophysiologic basis for idiopathic hypercalciuria, rather than separate "absorptive" and "renal" types.[255,261,265,267] However, Sakhaee *et al.*[257] using the acute response to hydrochlorothiazide to examine renal tubular function, have reported that natriuretic responses were exaggerated only in so-called "renal" hypercalciuria (defined as hypercalciuria during fast plus elevated C-terminal PTH levels). They interpret this finding as suggesting the presence of a proximal tubular defect only in their "renal hypercalciuric" subgroup of patients. It is difficult to reconcile this finding with that of Sutton and Walker,[255] who found abnormal responses to hydrochlorothiazide in their large subgroup of stone formers with hypercalciuria during fasting, who comprised more than 50% of unselected patients, and who did not have elevated PTH levels. These conflicting findings probably result from

different methods of selection of patient groups for study. Jaeger *et al.*[258] have found evidence of a variety of tubule defects in from 3 to 17% of 214 patients with diverse types of urolithiasis. They found that these defects were not specific to any particular etiologic group of patients, but rather were related to the presence of large pyelocaliceal stones at the time of study, and they conclude that the apparent tubulopathy is the consequence rather than the cause of stones. While this may be true of the defects they describe, the abnormal responses to hydrochlorothiazide, interpreted as suggesting an underlying proximal tubule defect,[255,257] were present in patients who were not harboring large stones, and similar abnormalities of proximal tubule function have been observed in a hypercalciuric rat model, in the absence of stones.[259]

5.1.2. Vitamin D Metabolism

Broadus and colleagues[263] have reported increases in circulating calcitriol levels in patients with so-called absorptive hypercalciuria, defined as hypercalciuria, while ingesting a diet containing 1000 mg of calcium per day, evidence of intestinal hyperabsorption of calcium based on an oral calcium load test, and normal or suppressed results for PTH and nephrogenous cAMP. Serum calcitriol levels initially responded briskly to an increased calcium intake (falling from high to normal levels), but in a small number of patients calcitriol levels "escaped," despite the continued high-calcium intake, suggesting disordered control of calcitriol production in "absorptive hypercalciuria." By contrast, Netelenbos *et al.*[264] found no significant differences in calcitriol levels between large groups of normal subjects, normocalciuric, and hypercalciuric stone formers, ingesting similar calcium intakes.

In the spontaneously hypercalciuric rat model,[260] hypercalciuria persists relative to controls despite the equalization of calcitriol levels by vitamin D deprivation, indicating that hypercalciuria is not mediated by calcitriol in this model. Rather, the elevated calcitriol levels were felt to be secondary to the hypercalciuria, and presumably mediated by increased parathyroid activity. The relevance of these observations to human idiopathic hypercalciuria, in which PTH levels are usually not raised and calcitriol may[263] or may not[264] be elevated, is not clear.

It is possible that renal calcium wasting and altered calcitriol production may both be manifestations of a subtle alteration of renal tubular function in idiopathic hypercalciuria, and that apparent "renal" hypercalciuria may result when the renal calcium wasting predominates, while apparent "absorptive" hypercalciuria may result when increased calcitriol production and therefore intestinal calcium absorption is more than sufficient to offset the renal calcium leak. Although it is tempting to

attribute both renal calcium wasting and disordered calcitriol production to a proximal tubule defect, a selective disturbance of renal calcium handling would seem more likely to result from distal tubule defect.[142] Such a distal tubule defect has generally been assumed to involve impaired calcium reabsorption, but could include increased net calcium secretion in the collecting tubule if the calcium permeability were increased.[156]

In a series of studies in healthy men[268–270] Maierhofer *et al.* have shown that exogenous calcitriol stimulates bone resorption when dietary calcium intake is low, and that an increased dietary calcium intake prevents a negative calcium balance when calcitriol levels are high. Hydrochlorothiazide given with the calcitriol lowered urinary calcium excretion, made calcium balance less negative, and reduced urinary hydroxyproline excretion, despite no changes in PTH or urinary cAMP excretion, implying a reduction in bone resorption.

It is likely that the suppressibility of the parathyroids may be another determinant of urinary calcium excretion. More complete PTH suppression in response to ingested calcium would lead to a reduction in tubular calcium reabsorption. Since calcitriol appears to cause parathyroid suppression,[67,113,114] it is possible that increased calcitriol levels, if present in idiopathic hypercalciuria, could facilitate postprandial parathyroid suppression. Furthermore, there is evidence that, under certain conditions,[161] calcitriol may antagonize the effect of PTH to promote tubular calcium reabsorption, and in this way calcitriol might also promote hypercalciuria.

5.1.3. Dietary Sodium, Acid–Base Factors, and Idiopathic Hypercalciuria

Sodium intake is known to exert an acute influence on urinary calcium excretion. Summarizing available data, Lemann *et al.*[271] concluded that dietary sodium intake, within the usual ranges, causes relatively small changes in calcium excretion. Sutton and Walker[272] found a significant positive correlation between calcium and sodium excretions in idiopathic calcium stone formers, both during fasting and while ingesting free diets. They concluded that dietary sodium may be a determinant of urinary calcium excretion , and that a high sodium intake may produce "fasting" hypercalciuria. Subsequently, Muldowney *et al.*[273] reported similar findings. An increase of 100 meq in daily sodium excretion was associated with an average increase of 97 mg in daily calcium excretion. Recently, Silver *et al.*[274] described a small group of stone patients in whom hypercalciuria appeared to be dependent on sodium intake. These patients were felt to show an amplified calciuretic response to

dietary sodium loading. Likewise, Sutton and Walker observed that following oral furosemide, stone patients had a greater increment in calcium excretion for any given increase in sodium excretion.[272] Both groups[272,274] felt that their data were consistent with impaired distal calcium reabsorption. In another recent study, increased excretions of sodium, potassium, and phosphate, as well as calcium, were found in children with hypercalciuria, and in their parents and siblings, and were thought likely to be a result of increased sodium ingestion.[275] Breslau *et al.*[276] reported that a change from a 10-meq- to a 250-meq-sodium intake during a constant calcium intake of 400 mg/day in normal subjects resulted in an increase in urinary calcium excretion from 110 to 167 mg/day, which was associated with increased calcitriol levels and increased intestinal calcium absorption, suggesting that sodium-induced hypercalciuria may be offset by a vitamin D-mediated increase in intestinal calcium absorption.

With respect to dietary acid–base factors, an increase in renal net acid excretion is associated with increased urinary calcium excretion which is not accompanied by a commensurate rise in intestinal calcium absorption, suggesting that increased skeletal resorption supplies the increment in urinary calcium.[271] Recently, Lemann *et al.*[277] have reported that variations in renal net acid excretion in the range expected with normal dietary variation (0–200 meq/day) can change fasting urinary calcium excretion from a low-normal to an elevated value, and hence fixed acid production from the diet also needs to be taken into account in the evaluation of fasting urinary calcium excretion.

5.2. Citrate Excretion

A possible role for decreased urinary citrate excretion in the pathogenesis of calcium stones has been recognized since the 1930s. There has been a major revival of interest in this subject, as well as in the use of citrate in the treatment of stones. A reduction in urinary citrate has been reported in from 19 to 63% of calcium stone formers.[278–280] In the latter study, low urinary citrate excretions were found in all categories of stone formers except patients with primary hyperparathyroidism and hyperuricosuric calcium oxalate lithiasis. Renal tubular acidosis, enteric hyperoxaluria, and potassium depletion associated with thiazide diuretics are recognized causes of hypocitraturia, but many cases are idiopathic.[280] Urinary citrate excretion is known to be dependent on acid–base status and to be influenced by potassium depletion. Metabolic acidosis increases renal cell citrate metabolism and decreases urinary excretion, while metabolic alkalosis decreases renal citrate metabolism and increases urinary excretion. Jenkins *et al.*[281] recently demonstrated that metabolic

acidosis increases sodium-dependent citrate uptake across renal brush border membranes. This effect is presumably additional to the effect of metabolic acidosis on renal citrate metabolism. Potassium depletion decreases urinary citrate excretion.[281]

In a series of studies relating to the use of citrate for the treatment of renal calculi, Pak *et al.* have shown that slow-release potassium citrate administered two or three times daily to a dose of 60 meq/day results in a sustained increase in urinary citrate excretion.[283] Potassium citrate simultaneously decreases urinary calcium excretion, presumably by an effect on tubular calcium reabsorption, while equivalent doses of sodium citrate increase urinary calcium excretion. Thus, potassium citrate has been recommended for the treatment of calcium stones.[283,284] In patients treated for calcium stones with thiazides, in whom low urinary citrate excretions may result from potassium depletion, the use of potassium citrate corrected the hypocitraturia and the hypokalemia without influencing the hypocalciuric action of the thiazide.[285] Thus, in patients with calcium stones treated with thiazides, potassium citrate appeared to be an appropriate form of potassium replacement. In patients unresponsive to thiazide therapy for hypercalciuric nephrolithiasis, low urinary citrate excretions are frequent, and a combination of thiazide with potassium citrate has been shown to correct the hypocitraturia and to improve the control of recurrent stone formation.[285] In patients with distal renal tubular acidosis, potassium citrate has been shown to prevent recurrent calcium stone formation,[286] while long-term treatment with potassium citrate in patients with idiopathic hypocitraturic calcium oxalate nephrolithiasis prevented further stone formation in approximately 90% of patients and resulted in a marked decline in the rate of stone formation.[287]

Potassium citrate therapy appears to be effective both for calcium nephrolithiasis and for uric acid lithiasis. With respect to uric acid stones, potassium citrate increases urinary pH and therefore uric acid solubility, while by increasing urinary citrate excretion and maintaining urine pH generally in the range of 6–7, the activity of urinary inhibitors (citrate and pyrophosphate) is increased, and urinary ionized calcium concentration is decreased, thus reducing the urinary saturation of calcium oxalate.[288]

5.3. Inhibitors of Calcium Oxalate Stone Formation

Human urine inhibits the growth of calcium oxalate monohydrate crystals. The major inhibitors include glycosaminoglycans[289] and acidic glycoproteins.[290] In a recent study,[291] a glycoprotein has been identified in human urine that inhibits calcium oxalate crystal growth strongly at

concentrations of 10^{-7} M. Isolation of this inhibitor from the urine of normal people and patients with calcium oxalate stones showed several differences. The glycoprotein from patients contained no detectable γ-carboxyglutamic acid, whereas normal glycoprotein inhibitor contains two to three residues of γ-carboxyglutamic acid per molecule. Second, in an *in vitro* system, the inhibitor from patients had much less crystal growth-inhibiting activity than the inhibitor from normal patients. Finally, glycoprotein inhibitor from patients showed an attenuated surfactant quality. These interesting findings suggest that, in addition to the familial metabolic abnormalities that may underlie calcium oxalate nephrolithiasis, there may also be rather specific chemical abnormalities in glycoprotein crystal growth inhibitors which may also contribute to stone formation.

6. Renal Osteodystrophy

In 1943, Liu and Chu[292] introduced the term "renal osteodystrophy" to describe collectively the various skeletal complications associated with chronic renal failure. Since the application of dialysis therapy and the increased survival of patients with chronic renal failure, renal osteodystrophy (ROD) has become one of the most common metabolic bone disorders in developed countries.

In 1957, Stanbury[293] proposed the following pathogenesis of ROD: " . . . a disorder of chronic course, it may consist of several distinct components of probably differing pathogenesis, which are active in varying degrees in different patients; these individual components may occur alone or variously admixed; and in the course of time, one pattern of bone disease may be transformed into another." Subsequent research has substantiated this hypothesis.

6.1. Histopathology of Renal Osteodystrophy

Histomorphometric techniques, including the use of double tetracycline labeling, demonstrate that ROD may be classified according to the rates of bone formation and mineralization in addition to other morphologic features.[294] There are considered to be four major histologic patterns of ROD: osteitis fibrosa ("fibrotic"), pure osteomalacia ("malacic"), combined features of osteitis fibrosa and osteomalacia ("mixed"), and "aplastic."[295–297] Ott *et al.*[295] included a "mild" category for biopsies with early features of osteitis fibrosa.[298]

Biopsies classified as osteitis fibrosa show increased numbers of osteoclasts and osteoblasts, woven bone, and marrow cavity fibrosis. These

biopsies may have increased osteoid surface and area of both woven and lamellar type. The bone formation and mineralization rates are usually increased.[294]

Osteomalacic bone biopsies have a marked increase in lamellar osteoid area and have a low mineralization rate and bone formation rate with an increased mineralization lag time. Characteristically, such biopsies show decreased number of osteoclasts and osteoblasts and do not feature woven bone or marrow fibrosis.[295] Similarly, aplastic biopsies show little evidence of bone cellular activity. These biopsies do not have an increased quantity of lamellar osteoid because there is virtual cessation of bone formation and mineralization.[296]

The mixed biopsies have increased osteoclastic activity and marrow fibrosis, as well as wide osteoid seams.[298] In such biopsies there is increased bone resorption but a decreased mineralization rate.[297,298]

The incidence of the specific biopsy types of ROD that have been reported in studies of chronic renal failure patients varies according to patient selection, histologic criteria for classification, and treatment regimes being used in the patients. For example, in a recent series, including 94 hemodialysis and peritoneal dialysis patients, Chan *et al.*[297] reported 18 with osteitis fibrosa, 26 with pure osteomalacia, and 50 with mixed lesions. In contrast, Llach *et al.*[296] in a study of 142 hemodialysis patients, reported 96 with osteitis fibrosa (including mixed lesions), 36 with osteomalacia, and 10 with aplastic bone lesions.

With the introduction of methods for quantitating the aluminum content of bone biopsies by direct measurement[299] and by aluminum staining,[295] it has become evident that there is a correlation between aluminum deposition in bone and the biopsy histomorphometric pattern. In a retrospective study of 315 bone biopsy samples, Ott *et al.*[295] found bone aluminum to be positively correlated with osteoid area (osteomalacia) and negatively correlated with bone formation and mineralization. These findings have since been confirmed in other large clinical biopsy studies.[296,297,300]

6.2. Pathogenesis of Renal Osteodystrophy

Fibrotic ROD is the result of secondary hyperparathyroidism.[298] It is well recognized that phosphate retention, in patients with end-stage chronic renal failure, is a major indirect stimulus to increasing PTH secretion by decreasing bone responsiveness to PTH with consequent hypocalcemia.[301] This effect of the increased level of extracellular fluid phosphate may be mediated through a direct action on bone cells and/or by decreased calcitriol production.[302] However, increasing PTH secretion is observed early in chronic renal failure before phosphate retention

has occurred.[303] Wilson *et al.*[302] studied vitamin D, PTH, and divalent ion metabolism in 12 patients during the early stages of chronic renal insufficiency. They showed a low serum phosphate, increased urinary phosphate excretion, low urinary calcium excretion, an elevation of PTH and urinary cAMP, and an impaired calcemic response to endogenous PTH. Low levels of calcitriol were present in these patients, and a significant correlation between levels of calcitriol and creatinine clearance was observed. After treatment with calcitriol, the impaired calcemic response to PTH improved and the renal handling of phosphate became normal. These authors suggest that a mild deficiency of calcitriol is present in early renal failure and may, at this stage, mediate the abnormal divalent ion metabolism. As previously discussed (see also Sections 1.4.4 and 2.2.3), calcitriol deficiency may be a component in the production of secondary hyperparathyroidism in early chronic renal failure by raising the level of ionized calcium necessary to suppress PTH secretion in parathyroid cells.

The pathogenesis of osteomalacic, aplastic, and mixed types of ROD is complex, involving aluminum toxicity, altered PTH secretion, disordered vitamin D metabolism, and other possible factors.[298]

First, it is clear from a number of clinical biopsy studies that malacic, fibrotic, and mixed ROD have marked osteoidosis, and therefore dynamic factors, such as bone formation and mineralization rates, must be measured in order to accurately differentiate these lesions and to assess the effects of therapy, such as the use of calcitriol.[297,298]

These is little evidence that calcitriol deficiency *per se* is an important pathogenic factor in the production of the low mineralization rate in osteomalacic, aplastic, and mixed ROD.[303] In contrast to other vitamin D-deficiency states, most patients with end-stage chronic renal failure have adequate levels of extracellular fluid calcium and phosphate to promote mineralization despite low calcitriol levels. Calcitriol therapy does reduce osteoidosis in certain patients, but this is probably a result of reduced PTH secretion and, possibly in some individuals, increased extracellular fluid calcium and phosphate levels.[304]

The role of $24,25(OH)_2D$ in the pathogenesis and treatment of ROD remains to be clarified. $24,25(OH)_2D$ is the major circulating metabolite of 25 OHD.[13] Levels of $24,25(OH)_2D$ are reduced in patients with chronic renal failure; levels appear to be maintained by extrarenal production.[305,306] There is little evidence for a major physiologic role for $24,25(OH)_2D$ (see also Section 1.5), but studies by Hodsman *et al.*[307] indicated that combined therapy with dihydrotachysterol (DHT_2) or calcitriol and $24,25(OH)_2D$ improved mineralization in some patients with osteomalacic ROD. In retrospect, the role of aluminum in the production of the osteomalacic ROD was not fully appreciated in this study; however,

pharmacologic $24,25(OH)_2D$ did appear to have a beneficial effect on the bones of many of those patients.[71] van Demien-Steenvoorde *et al.*[15] used combined $24,25(OH)_2D$ and DHT_2 therapy in 10 children on dialysis, eight of whom had treatment with DHT_2. Addition of $24,25(OH)_2D$ resulted in decreased serum calcium levels and permitted higher doses of DHT_2. Histomorphometric studies showed decreased osteoclasts and increased mineralization as measured by dual photon absorptiometry after $24,25(OH)_2D$ was added. These authors suggest that $24,25(OH)_2D$ interfered with DHT_2 metabolism and increased DHT_2 tolerance, resulting in increased bone mineralization. It is probable that $24,25(OH)_2D$ does not have important biologic activity with regard to the pathogenesis of ROD, but pharmacologic treatment with $24,25(OH)_2D$, particularly in combination with calcitriol or DHT_2, may be beneficial in the treatment of ROD since the drug may affect other aspects of vitamin D action and metabolism.[307]

Aluminum toxicity is now clearly established as a major pathogenetic factor in the production of osteomalacic, aplastic, and mixed ROD lesions.[250] Currently, since most renal units remove aluminum from dialysate water to provide an aluminum dialysate concentration of <10 μg/liter, the source of aluminum is gastrointestinal absorption from oral phosphate-binding agents.[308] There are several possible mechanisms of aluminum toxicity to bone: First, it is possible that aluminum interferes directly with the mineralization process, and second, aluminum has been shown to be toxic to osteoblasts.[309,310] Further, aluminum is toxic to parathyroid cells and reduced PTH secretion, which appears to increase the deposition of aluminum.[311] de Vernejoul *et al.*[312] differentiated ROD biopsies into two groups on the basis of bone formation rate. Measured directly, the aluminum content of bone was not different in the two groups. However, stainable aluminum on the trabecular surface was greater in the group with low formation rate, demonstrating that decreased formation was more related to recent than total aluminum deposition. Also, as observed in a number of studies, PTH levels were lower in patients with low osteoblastic activity, suggesting a possible synergistic effect of these two factors. Ott *et al.*[313] have shown that desferrioxamine removal of aluminum from some patients with "malacic" osteodystrophy resulted in a decreased surface bone aluminum, increased PTH secretion, and increased bone formation rate.

Calcitriol and pharmacologic-dose vitamin D therapy could influence the production of aluminum-related osteodystrophy by increasing phosphate absorption, thus necessitating a greater intake of aluminum-containing oral phosphate-binding agents, and by reducing PTH secretion.

As Stanbury postulated,[293] ROD consists of several distinct com-

ponents of differing pathogenesis. The currently recognized major components include altered PTH secretion, disordered vitamin D metabolism, and aluminum toxicity. The relative importance and interrelationship of these factors will vary from patient to patient, resulting in a wide spectrum of histopathologic changes on bone biopsy.

6.3. Manifestations of Renal Osteodystrophy

The frequent association of malacic and aplastic ROD with fractures and encephalopathy has been reported in many clinical studies.[296,314,315] Compared with patients with fibrotic ROD, osteomalacic and aplastic patients have been shown to have higher serum calcium and lower phosphate levels in some series,[315,316] but not in others.[296,297] PTH, alkaline phosphatase, and Gla protein levels have been found to be lower in patients with malacic versus fibrotic ROD.[317] Plasma levels of Gla protein have been shown to correlate with parathyroid hormone secretion and bone formation rate in predialysis and dialysis patients, and it has been suggested that levels of Gla protein may be a more reliable index of hyperparathyroidism than levels of alkaline phosphatase.[317,318]

Netter *et al.*[319] demonstrated the accumulation of aluminum in synovial fluid in chronic renal failure patients treated with hemodialysis and aluminum-containing compounds. These patients also had arthropathy, and toxicity of aluminum to joints was suggested.

6.4. Desferrioxamine Infusion Test

Desferrioxamine (DFO), a naturally occurring trihydroxamic acid, forms a chelate with aluminum (aluminoxamine) in plasma and can mobilize aluminum from bone and other tissues.[320] Aluminoxamine is removed by dialysis, and this method has been widely used to treat aluminum-induced osteomalacia.[313]

In an attempt to develop a noninvasive method to diagnose aluminum-related osteodystrophy, Nebeker *et al.*[321] studied the relationship of bone histologic findings and bone aluminum content to plasma aluminum concentration and plasma aluminum concentration after a sudden infusion of DFO. These authors demonstrated that a baseline plasma aluminum concentration of greater than 200 μg/liter was associated with aluminum-related osteodystrophy (>93%), but that a lower concentration did not exclude the diagnosis. After DFO infusion (40 mg/kg following dialysis), an increment of plasma aluminum concentration of less than 200 μg/liter excluded most aluminum-related osteodystrophy (sensitivity 94%), and an increment greater than 500 μg/liter included most

aluminum-related osteodystrophy (specificity 91%). These authors suggest that a bone biopsy should be done to make the diagnosis of aluminum-related osteodystrophy if the increment in aluminum was between 200 and 500 μg/liter or the patient has severe symptoms. Malluche *et al.*,[322] using a different protocol involving lower doses of DFO (28.5 mg/kg during dialysis), failed to find the DFO test useful in distinguishing between patients with and without aluminum osteodystrophy. Likewise, Hodsman *et al.*,[323] using different criteria ("high" serum aluminum level >133 μg/liter) and a higher dose of DFO (6 g following dialysis), found a higher serum aluminum level had a diagnostic sensitivity of 60%. However, after DFO infusion, neither the peak serum aluminum level nor its increment improved the distinction between patients with osteomalacia and secondary hyperparathyroidism. On the basis of these conflicting results, it is probable that most centers without extensive experience with this test will use the bone biopsy to confirm a diagnosis of suspected aluminum-related osteodystrophy.

6.5. Prevention and Management of Renal Osteodystrophy

Because phosphate retention is a major factor in the pathogenesis of ROD and since phosphate is not adequately removed by either hemodialysis or peritoneal dialysis, it is necessary to reduce absorption of phosphate from the gastrointestinal tract in patients with chronic renal failure.[324,325] Dietary protein restriction (e.g., 1 g/kg per day) and avoidance of phosphorus-rich foods, such as dairy products and nuts, will reduce availability of phosphate for absorption.[301,326] However, a severely phosphate-restricted diet is unpalatable and limited in other nutrients, and therefore, methods for binding phosphate in the gastrointestinal tract are necessary.

The most effective binding agents in current clinical use are poorly soluble aluminum-containing gels such as aluminum hydroxide and aluminum carbonate.[327] More recently, the basic aluminum salt of sucrose octasulfate has been shown to be an effective phosphate binder.[327] Unfortunately, large doses of these compounds are usually required, and there is substantial evidence to suggest that their long-term use results in significant absorption of aluminum, which may lead to associated toxicity (see Section 6.2). Therefore, other phosphate-binding agents being used or being investigated include calcium carbonate, magnesium hydroxide, cation-loaded heteropolyuronic acid, and anion-exchange resins.[327,328]

Fournier *et al.*[328] recently reported the results of two studies using calcium carbonate. In the first study, calcium carbonate alone, 5–20 g/day

(mean 9.5 g/day), prevented hyperphosphatemia in most patients as effectively as the combination of calcium carbonate and aluminum hydroxide. In the second study, treatment with calcium carbonate versus the combination of calcitriol and aluminum hydroxide showed similar levels of serum calcium were achieved, but the latter treatment resulted in higher serum phosphate and aluminum levels. Complications of calcium carbonate therapy included poor compliance, hypercalcemia, and diarrhea.

The use of magnesium hydroxide as a phosphate-binding agent is limited, since the gastrointestinal absorption of magnesium results in elevation of serum magnesium levels. Also, magnesium hydroxide may cause diarrhea. This compound alone is not adequate to provide good control of serum phosphate levels.[327]

Schneider *et al.*[329] have developed a new group of aluminum-free substances for intestinal binding of phosphate which are homo- and heteropolyuronic acids charged with cations. A calcium-charged polymer was used in a pilot clinical study. Phosphate levels were controlled with a total of 5–10 g of this substance daily. The phosphate-binding capacity of the polymer depended on the calcium that was released. No major undesirable side effects were reported, and constipation, a frequent consequence of aluminum hydroxide, ceased.

Burt *et al.*[327] reported *in vitro* studies comparing the uptake of phosphate with anion-exchange resins and aluminum-containing phosphate binders. Maximum capacities for uptake of phosphate were greater with aluminum-containing phosphate binders on a mg/g basis. Bile acids were bound by the resins but did not interfere with phosphate binding. These authors concluded that certain resins in the taurocholate form would be potential candidates for *in vivo* testing as alternative phosphate-binding agents to the aluminum-containing gels.

As previously discussed (see Section 6.2), the use of DFO for chelation and removal of aluminum from the body is indicated when there is no evidence of aluminum toxicity.[321] Many regimes for DFO administration have been used, but a relatively standard approach is weekly intravenous infusion of 2–6 g of DFO near the end or after hemodialysis for as many weeks as required to relieve symptoms of aluminum toxicity and to reduce serum aluminum to nontoxic levels. In peritoneal dialysis patients, DFO maybe given intramuscularly (e.g., 1–2 g weekly).

The role of vitamin D and its analogs and metabolites in the prevention and management of ROD remains to be fully clarified. Large doses of vitamin D, dihydrotachysterol, and calcidiol and lower doses of 1α-hydroxycholecalciferol and calcitriol have been shown to increase serum calcium and decrease PTH secretion, osteitis fibrosa, and oste-

oidosis in patients with chronic renal failure.[303,324] Since hypercalcemia is the major side effect of such therapy, the shorter half-life of calcitriol and 1α-hydroxycholecalciferol makes these agents safer to use.[324] However, there is evidence that "vitamin D" therapy may reduce renal function when used in patients with moderate renal failure,[303] and in dialysis patients it may result in the increased use of aluminum-containing phosphate-binding agents because of increased absorption of phosphate by the gastrointestinal tract.[328] Since calcium carbonate has been reported to be as effective as calcitriol in preventing secondary hyperparathyroidism, treatment with this compound may be preferred in adults.[328] In children, however, there is evidence that calcitriol improves bone growth.[303,324]

In some patients with long-standing hyperparathyroidism, treatment with calcitriol or calcium carbonate may result in severe hypercalcemia without effective suppression of PTH secretion.[301] In such circumstances, subtotal parathyroidectomy or total parathyroidectomy with autotransplantation may be required, following which it may be necessary to use large doses of calcitriol and calcium in the immediate postoperative period and lower doses in long-term therapy in order to maintain normocalcemia. Felsenfeld *et al.*[330] showed that postoperative decrement in serum calcium after parathyroidectomy correlated with the histologic severity of osteitis fibrosa.

As previously discussed (see Sections 1.4.4 and 2.2.3), intravenous calcitriol appears to suppress PTH secretion more effectively than oral administration, but further trials are required to determine the clinical application of these observations.[67,298]

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7

Recent Advances in the Role of the Renal Nervous System and Renin in Hypertension

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1. Introduction

Considerable evidence indicates that the kidneys play an important role in blood pressure regulation under a variety of physiologic conditions and in several forms of experimental, as well as human, hypertension. The kidneys can influence blood pressure homeostasis through a variety of afferent neurogenic as well as hormonal mechanisms, which include the renin–angiotensin system, prostaglandins, and the kallikrein–kinin system. The renal mechanisms, on the other hand, are under the influence of and, therefore, are regulated by arterial baroreceptors, cardiopulmonary mechanoreceptors, chemoreceptors, and the central nervous system. The purpose of this chapter is to critically analyze the most current views concerning the role of the renal neurogenic and the renin–angiotensin system under physiologic conditions and in the pathogenesis of hypertension.

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2. The Renal Sympathetic Nervous System

2.1. Renal Neuroanatomy and Its Integrative Connections

The kidneys have an extensive adrenergic innervation. Nerve bundles have been shown on the cortical arterioles, particularly in the space between the afferent and the efferent glomerular arterioles and the adjacent tubules. Direct adrenergic innervation of both the proximal and distal tubules arising from the periarteriolar nerves has also been demonstrated. Sympathetic varicosities containing neurosecretory granules have been shown in direct contact with the basement membrane of proximal and distal renal tubular cells in rats,[1,2] monkeys,[1] dogs,[3] and human fetuses.[4] Dinerstein *et al.*,[5] using histofluorescence techniques, have provided evidence of dopamine-containing neuronal elements at the glomerular vascular poles in canine kidneys.

Radioligand binding studies have shown the presence of both α_1- and α_2- adrenergic receptors in renal tubule plasma membrane preparations of rat.[6] α-Adrenoreceptors have been shown to be increased in spontaneously hypertensive rats as compared to normotensive Wistar–Kyoto rats[7] and in Dahl's salt-sensitive as compared to salt-resistant rats.[8] β-Adrenoceptors (predominantly β_1)[9] and dopamine receptors[10] have also been demonstrated in the rat kidney. The kidneys also receive cholinergic nerves. However, it is not clear whether the cholinergic innervation extends to the afferent and efferent glomerular arterioles and to the renal tubules. Acetylcholinesterase-containing nerve bundles have been shown along the afferent and efferent glomerular arterioles, as well as in the proximal and distal tubules. However, these bundles are destroyed by administration of 6-hydroxydopamine,[11] which selectively destroys adrenergic nerves, suggesting that these are adrenergic bundles containing acetylcholinesterase rather than cholinergic fibers.

The renal sympathetic nerve activity (RSNA) can be influenced by carotid and aortic baroreflexes[12] and by the stimulation of a variety of cardiac mechanoreceptors and chemoreceptors. Pressure elevation inhibited and pressure reduction increased renal nerve activity.[12] Neither carotid nor aortic denervation or vagotomy alone impaired the baroreflex control of renal nerve activity, suggesting a redundancy of afferent input to the RSNA and that aortic and carotid baroreflex influences on RSNA add by occlusive or mutual inhibitory summation.[12]

Stimulation of left atrial receptors caused a decrease in RSNA via an afferent pathway composed of myelinated vagal fibers.[13,14]

Low-pressure cardiopulmonary receptors located in the left side of the heart with vagal afferent fibers regulate the sympathetic impulses to the kidneys in response to changes in intravascular volume.[15,17] Volume

expansion inhibits while volume depletion stimulates RSNA. Skoog *et al.*[18] have shown that hypotensive hemorrhage induced a short-lasting sympathetic excitation, followed within 5–10 min by a powerful sympathetic inhibition and bradycardia in rats. The marked depressor response was due, at least in part, to activation of mechanical sensitive cardiac vagal afferents, as it was reversed by bilateral cervical vagotomy. Stimulation of high-pressure sinoaortic baroreceptors has only a limited role on the inhibition of RSNA induced by volume expansion. Less clear is the role of cardiopulmonary afferent sympathetic pathways in the regulation of RSNA activity. Myocardial ischemia caused by coronary occlusion may enhance RSNA via cardiac sympathetic afferent nerves.[19] Activation of RSNA can also be accomplished by application of bradykinin or potassium chloride on the left ventricular myocardium.[20] This suggests that the chemical composition at the level of cardiac receptors can alter the activity of RSNA. However, the physiopathologic implication of these observations remain largely undetermined. A variety of other afferent somatic visceral or chemosensitive receptors can affect RSNA, and they have been extensively reviewed[21]; their pathophysiologic role is not clear.

Finally, a variety of centrally acting drugs can alter the activity of RSNA; for example, clonidine via activation of central α_2-adrenergic receptors,[22] and β-adrenergic blocking agents decrease RSNA.[23] Vertebral artery infusion of angiotensin II in anesthetized dogs caused an initial increase in arterial pressure and in RSNA activity followed by a decrease, probably as a consequence of sustained elevation of blood pressure.[24]

Bell and Lang[25] have provided evidence that the renal dopaminergic innervation mediating renal vasodilatation is under the influence of various areas of the brain. In fact, electrical stimulation of the hypothalamus or midbrain caused renal vasodilatation. This action was abolished by haloperidol, but not by guanethidine, atropine, or mepyramine.

Myelinated viscerosensory *afferent fibers* are also present in the kidneys. They have been shown in the corticomedullary region at the outer stripe of the outer zone of the medulla, in the periarterial connective tissue, and in the subepithelial connective tissue of the calices, and they are in close relationship with the unmyelinated sympathetic efferent axons.[26,27] The afferent fibers appear to project to the central nervous system signals deriving from the activation of intrarenal mechanoreceptors and chemoceptive receptors.[28–30] The mechanoreceptors are located both in the renal cortex and in the renal pelvis and respond to changes in intrarenal pressure produced by ureteral occlusion, compression of the kidneys, or constriction of a renal artery or vein.[28,29] The chemoreceptors are present primarily in the submucosal layers of the renal

pelvis and have been classified in two types: The first type (R1) is activated by renal ischemia or hypoxia; the second type of chemoreceptor (R2) appears to be activated primarily by alterations in the chemical composition in the renal pelvis and renal interstitium.[30] Central projections of these afferent pathways establish connections with medullary and hypothalamic nuclei involved in cardiovascular and in body sodium volume regulation.[31] Thus, afferent renal nerve stimuli appear to be important modulators of central integrative structures that regulate cardiovascular and volume homeostasis. There is also strong evidence that the afferent renal nerves are involved in renorenal reflexes. For example, activation of chemoreceptors of one kidney in the rat caused contralateral diuresis and natriuresis that was abolished by denervation of the contralateral kidney.[32]

2.2. Physiologic Role of Renal Sympathetic Innervation

2.2.1. Neurogenic Control of Renal Hemodynamics

The effects of the sympathetic nervous system on renal hemodynamics have been extensively studied in both anesthetized and unanesthetized animals, as well as in humans. Maneuvers that result in reflex inhibition of the renal sympathetic efferent activity in anesthetized animals are also associated with renal vasodilation. Increase in carotid pressure,[12] stimulation of left atrial cardiopulmonary receptors,[33,34] coronary artery occlusion,[35] or increase in hepatic portal venous pressure[36] results in reflex decrease in renal sympathetic nerve activity and renal vasodilatation. Less conclusive, however, are the studies performed in unanesthetized conscious animals. Under resting conditions, there is minimal sympathetic activity to the kidneys and no significant renal vasoconstrictive tone both in humans and in animals.[37,38]

Stimulation of high-pressure baroreceptors or low-pressure left-atrial mechanoreceptors exerts an inhibitory effect on RSNA but has little effect on renal blood flow.[39,40] Baroreceptor reflexes stimulated by blood loss of 16% in the conscious dog did not result in any significant change in renal blood flow.[40]

However, pronounced renal vascular responses have been observed in conscious dogs, cats, and monkeys, during naturally elicited fear and excitement.[41–43] Gross and Kirchheim[44] studied the effect of common carotid occlusion and auditory stimulation on RSNA and renal blood flow in conscious chronically instrumented dogs. They observed that occlusion of the common carotid increased blood pressure and efferent RSNA, while renal blood flow remained unchanged, independently of whether renal perfusion pressure was allowed to rise or was kept con-

stant. During acute excitement caused by firing a pistol, blood pressure and heart rate increased, while renal blood flow decreased from 360 to 150 ml/min. Minor excitements caused by shouting or whistling were associated with a 500% increase in RSNA. These data indicate that renal blood flow is not affected by baroreceptor stimulation, despite evident changes in RSNA. However, more intense emotional stimuli can cause renal vasoconstriction and decreased renal blood flow concomitantly with the increase in RSNA.

Similarly, surgical or pharmacologic renal denervation in conscious unanesthetized dogs[45] or normal humans[46] does not result in changes in renal blood flow. Hollenberg *et al.*,[46] while attempting to study the possibility that increased RSNA might be responsible for the reduction in renal perfusion following sodium restriction, infused phentolamine, an α-adrenergic blocking agent, in the renal artery of sodium-depleted normal humans. Phentolamine infusion at rates of up to 3 mg/min did not result in any increase in renal perfusion. These studies suggest that neither RSNA nor circulating catecholamines play a central role in the renal vascular response to salt restriction in normal humans.

More controversial are the studies on the influence of neuroadrenergic stimuli on the glomerular afferent and efferent arterioles. Myers *et al.*,[47] in micropuncture studies in the rat, showed that administration of norepinephrine caused an increase of the efferent, but not of the afferent, resistance. Andreucci *et al.*,[48] however, observed a more profound action of norepinephrine on the afferent glomerular arteriole. Angiotensin II, on the contrary, produced a greater vasoconstriction of the efferent than of the afferent arteriole.[49] During electrical renal nerve stimulation of intensity sufficient to cause a decrease in renal blood flow of 15%, the glomerular filtration rate (GFR) did not change; however, with the administration of an angiotensin II antagonist, the glomerular filtration rate decreased. This suggests that renal nerve stimulation releases angiotensin II, which, in turn, constricts the efferent glomerular arterioles, leading to increased filtration fraction and to maintenance of a normal glomerular filtration rate.[50]

The role of renal dopaminergic neurons on renal hemodynamics is not clear. However, exogenously administered dopamine increased renal blood flow and glomerular filtration rate and induced diuresis and natriuresis in the dog, in the rat, and in humans.[51,52] Intravenous infusion of dopamine (2.6–7.1 μg/kg per min) to seven normal subjects increased the PAH clearance from 507 to 798 ml/min, inulin clearance from 108 to 136 ml/mm, and sodium excretion from 171 to 571 meq/min. Simultaneous hemodynamic measurements showed that the renal changes were accompanied by significant increment in cardiac output, but no significant changes in blood pressure or heart rate.[53]

2.2.2. Neural Regulation of Renal Tubular Sodium Reabsorption

A large body of evidence is available to demonstrate a neurogenic control of renal tubular sodium transport. Claude Bernard made the original observation that section of the greater splanchnic nerve caused immediate diuresis in the anesthetized dog.[54] Stimulation of renal sympathetic nerves or renal arterial infusion of norepinephrine enhanced renal tubular reabsorption of sodium independent of changes in renal hemodynamics.[55,56]

Bello-Reuss *et al.*[57] showed that renal denervation in the anesthetized rat increased urine volume to double its control value and increased urinary sodium excretion sixfold in the ipsilateral site. GFR and renal plasma flow (RPF) remained unchanged. Fractional and absolute sodium and water reabsorption decreased in the proximal tubule, while sodium reabsorption increased in the loop of Henle, distal convoluted tubule, and collecting ducts. There were no changes in GFR, RPF, urinary sodium excretion, or sodium reabsorption in the proximal tubule after sham denervation. These data clearly demonstrated that the diuresis and natriuresis seen after acute renal denervation were caused by a pronounced decrease in sodium and water reabsorption in the proximal tubule and that these changes were unrelated to systemic or intrarenal hemodynamic changes. Similar observations have been made in anesthetized dogs after acute renal denervation[58] and in chronically denervated dogs and rats.[59] Reflex decrease in RSNA in anesthetized dogs produced by left-atrial distention or stellate ganglion stimulation also caused a decrease in renal tubular sodium reabsorption in the absence of changes in renal perfusion pressure, GFR, RPF, or intrarenal distribution of blood flow.[30]

Even more important are the studies on the effects of renal denervation on renal sodium handling in conscious unanesthetized animals. Sadowiski *et al.*[45] have shown that in conscious moderately hydrated dogs, the denervated kidney excreted more sodium and water than the contralateral kidney, but the difference was not as pronounced as in the anesthetized animals. Schneider *et al.*[60] studied the effects of chronic bilateral renal denervation on daily sodium excretion in the conscious dogs. They observed enhanced natriuresis in renal denervated dogs during low-sodium diet (3 meq/day), but not during normal sodium intake (100 meq/day). DiBona and Sawin[61] studied the renal adaptation to normal- and low-sodium diet 8 days after bilateral surgical or pharmacologic renal denervation in the rat. They showed that cumulative sodium balance was positive in rats ingesting a normal-sodium diet, independently of whether they had undergone renal denervation or sham denervation. However, while ingesting a low-sodium diet, the rats with

bilateral renal denervation displayed a pronounced negative sodium balance. These data indicate that intact renal innervation is necessary for normal renal adaptation to sodium restriction in the conscious animals.

Several observations in humans are in agreement with the findings in experimental animals. Wilcox *et al.*[62] studied renal excretion of sodium in five patients with Shy–Drager syndrome. They observed that during 7 days of dietary sodium restriction, urinary sodium excretion remained unchanged in the patients with autonomic failure, whereas it fell rapidly in normal subjects to values comparable with their sodium intake. Gill and Bartter[63] observed that adrenergic blockade produced by treatment with guanethidine in four normal subjects significantly impaired the capacity of the kidneys to retain sodium during low dietary sodium intake (14–19 meq/day), despite a decrease in creatinine clearance and a rise in urinary aldosterone excretion. Taken together, the studies in conscious unanesthetized animals and in human subjects clearly demonstrate that intact renal innervation is essential for the kidneys to increase renal tubular sodium reabsorption during dietary sodium restriction, and that any anatomic or pharmacologic alteration of this innervation may result in significant negative sodium balance. In most mammalian species, the increase in renal tubular sodium and water reabsorption produced by stimulation of efferent sympathetic nerve activity or, to a lesser extent, by circulating catecholamines is mediated predominantly by renal tubular α_1-adrenoreceptors. α_2-Adrenoceptors, on the other hand, do not play an important role in mediating the action of efferent renal sympathetic nerves on sodium and water reabsorption. However, since activation of α_2-adrenoceptors inhibits adenylate cyclase, this may influence the renal tubular response to other hormonal agents that affect sodium and water reabsorption via stimulation of adenylate cyclase.[64]

As opposed to norepinephrine, it appears that dopamine exerts a natriuretic effect. McDonald *et al.*[53] were the first to report increased urinary sodium excretion during intravenous administration of dopamine in normal subjects. Urinary dopamine excretion increased parallel with the natriuresis observed in response to an acute volume expansion with saline infusion, or after increased dietary sodium intake.[65] However, plasma dopamine is suppressed during salt loading in normal subjects.[66] Krishna *et al.*,[67] on the other hand, have shown that plasma dopamine levels increase concomitantly with the natriuretic response caused by headout water immersion as well as by isotonic saline infusion.

The natriuretic effect of dopamine may be due to the combined effects of a direct action on the renal tubule, suppression of aldosterone secretion,[67] and renal vasodilatation.[68] Dopamine can affect other tubular functions besides sodium reabsorption; in particular, it can increase phosphate excretion and decrease potassium excretion.[69]

Alterations in dopamine secretion can be important in the abnormal renal handling of sodium which occurs in certain pathologic states. Harvey *et al.*[70] observed that hypertensive patients failed to display the expected increase in urinary dopamine excretion in response to salt load. Casson *et al.*,[71] in a study of eight patients with chronic glomerulonephritis, also showed no increase in urinary dopamine and no suppression of PRA in response to salt loading. This abnormality may be partially responsible for sodium retention in this condition.

2.2.3. Neural Regulation of Renin Release

Considerable evidence has been accumulated to indicate that renin secretion can be influenced by RSNA as well as by circulating catecholamines. These interactions will not be reviewed in detail here, but several reviews on the subject are already available in the literature.[72,73] Renin secretion can occur with stimulation of RSNA at frequencies that do not cause renal vasoconstriction. This secretion is mediated by β-adrenoreceptors. With greater renal nerve stimulation capable of causing renal vasoconstriction, part of renin secretion is secondary to activation of vascular α_1-adrenoreceptors.

2.3. RSNA in the Pathogenesis of Hypertension

The discovery of efferent and afferent renal nerves and the evidence that they play an important role in cardiovascular regulation have stimulated large interest on the potential role of these nerves in the genesis and/or maintenance of experimental as well as human hypertension.

2.3.1. Afferent Renal Nerves

Renal nerves do not appear to play any significant role in the developmental phase of renovascular hypertension.[74,75] On the other hand, substantial evidence indicates that hyperactivity of the central sympathetic nervous system,[76] of the adrenal medulla, and of RSNA participates in the maintenance of established renovascular hypertension. Denervation of the clipped kidney caused a reduction of arterial pressure in the one-kidney, one-clip and in the two-kidney, one-clip models of renovascular hypertension in the rat, and in the hypertension produced by coarctation of the aorta in dogs.[74] The reduction of blood pressure occurred independently of alterations in glomerular filtration rate, sodium balance, or plasma renin activity, suggesting that this decrease could not be the result of inhibition of efferent RSNA, but rather of afferent impulses. Further support to this notion derives from the studies

of Fink and Brody.[77] These investigators have demonstrated that in rats with either one-kidney or two-kidney hypertension, efferent sympathetic control of renal vascular resistance was inhibited in both the clipped and the unclipped kidney. Furthermore, renal vascular response to renal nerve stimulation was reduced, whereas the response to intrarenal injection of norepinephrine was slightly increased. These data suggest that renal denervation does not decrease blood pressure by eliminating renal efferent influences (since these are depressed), but rather by eliminating afferent renal nerve impulses to the central nervous system. Moreover, these studies suggest that diminished efferent RSNA may attenuate the degree of hypertension by its effects on renal vasculature, sodium retention, and renin release.

The stimulus that triggers increased afferent renal nerve activity during renal ischemia has not been well defined. Katholi[74] has speculated that the chemical mediator of these afferent impulses may be adenosine. This substance is, in fact, released in greater amount during renal ischemia, and when administered intrarenally, it increases afferent renal nerve impulses.

2.3.2. Efferent Renal Nerves

A large body of evidence indicates that increased efferent RSNA is present in several forms of experimental as well as human hypertension and that it may contribute to the maintenance of hypertension by shifting to the right the pressure–natriuresis curve. Indeed, one of the intriguing questions still unanswered in the pathophysiology of hypertension is the mechanism(s) for the alteration in the pressure–natriuresis curve, universally present in all hypertensive states. Some investigators have postulated that this is due to a genetic defect in the ability of the kidneys to excrete a sodium load.[78,79] Several lines of evidence support this contention. First, isolated kidneys from Dahl's salt-sensitive "prehypertensive" rats excrete much less sodium than kidneys from salt-resistant rats.[80] Second, renal cross-transplant studies in three different strains of genetically hypertensive rats have shown that hypertension is transferred with the "hypertensive" kidneys.[81–83] Furthermore, normotensive siblings of hypertensive patients display a delayed excretion of an acute salt load.[84] Moreover, weanling spontaneously hypertensive rats (SHR) excrete less sodium than Wistar–Kyoto (WKY) controls despite similar sodium intake[85]; in adult SHR the ability to excrete sodium and water has been shown to be diminished[86] or unaltered.[87] Finally, Na,K-ATPase activity was found to be higher in 5-week-old SHR than in WKY, but not in 16-week-old adult animals.[88]

On the other hand, direct and indirect evidence is available to sug-

gest that the changes in renal sodium handling in hypertension may be dependent on increased efferent RSNA. First, it has been shown that continuous electrical stimulation of the left stellate ganglion for 7 days produced hypertension in the conscious dog.[89] The rise in blood pressure was abolished by phenoxybenzamine. In these animals sodium excretion did not increase despite the rise in blood pressure, suggesting a shift to the right of the pressure–natriuresis curve probably secondary to an increase in efferent RSNA. Katholi *et al.*[90] have also shown that chronic intrarenal infusion of norepinephrine in conscious dogs caused a sustained rise in blood pressure associated with positive sodium balance. Increased nervous activity has been shown in sympathetic ganglia supplying the splanchnic region[91] and in postganglionic splanchnic fibers[92] of anesthetized prehypertensive SHR. Lundin and Thoren[93] have demonstrated increased RSNA with both multifiber and single-fiber recordings in conscious unanesthetized SHR in comparison with WKY rats; there was also a greater decrease in urinary sodium excretion in concomitance with a more pronounced increase in RSNA in conscious SHR than in WKY. Renal denervation resulted in a delay of the onset of hypertension and attenuated the severity of established hypertension in SHR; this occurred concomitantly with a decrease in fractional reabsorption of sodium.[94] Lundin and Thoren[93] observed that SHR during air stress displayed an exaggerated decrease in urinary sodium excretion in association with a more pronounced rise in RSNA, without any alteration of effective RBF or GFR. The exaggerated sodium retention during stress was abolished by prior renal denervation. Recently, Ricksten *et al*[95] have shown that the exaggerated natriuresis in response to intravenous infusion of isotonic saline in SHR was associated with an exaggerated inhibition of RSNA modulated via activation of cardiopulmonary baroreceptor reflexes. Even in the DOCA–salt hypertension, a classic model of salt-induced hypertension, renal denervation delayed the development of hypertension when performed prior to the start of DOCA–salt treatment and attenuated the degree of hypertension when performed in rats already treated with DOCA–salt for 3 weeks.[96] The decrease in blood pressure was associated with increased natriuresis.

All these data support the concept that increased RSNA may play an important role in the maintenance of several forms of experimental hypertension by shifting the pressure–natriuresis curve to the right, thus causing sodium retention. The efferent RSNA, however, appears to play no role in SHR or DOCA–salt hypertensive rats when the hypertension is well established.[74] Other factors, such as changes in the anatomic structure of the renal vascular bed, may become more important in far-advanced phases of hypertension.

2.3.3. Human Essential Hypertension

A large body of evidence has accumulated in support of a role of the sympathetic nervous system in the genesis and maintenance of essential hypertension in human subjects. This evidence is in large part indirect, with the exception of the studies of Wallin *et al.*[97] These investigators recorded multiunit sympathetic activity in skin and muscle nerves of 24 normal subjects and 21 hypertensive patients. Muscle nerve sympathetic activity occurred in bursts which were suppressed during transient elevations of blood pressure. The inhibitory blood pressure level was higher in hypertensive than in normotensive subjects, suggesting a reduced baroreceptor sensitivity in hypertensive subjects. Probably owing to technical limitations of multifiber sympathetic recordings, no other differences in muscle nerve sympathetic activity were found between these two groups. Conceivably, direct measurements of sympathetic activity to other areas, such as the heart and renal and splanchnic vascular beds, may be more important, since sympathetic activity to the large skeletal muscle vascular bed is not increased or may even be reduced in patients with essential hypertension.[98]

Most of the evidence for increased sympathetic activity in humans is indirect and based on hemodynamic studies, measurement of plasma catecholamines, or pharmacologic interventions. Arterial baroreceptors have higher threshold and reduced sensitivity in patients with essential hypertension.[99] Resetting of baroreceptors has been shown in early essential hypertension, suggesting that it may cause a reflex increase in sympathetic activity and in blood pressure. However, elevation of arterial pressure *per se* causes resetting of baroreceptors within hours,[100] suggesting that the abnormality may be secondary to the increase in blood pressure, and not primary. Young patients with labile hypertension commonly display tachycardia, increased cardiac output, and increased dp/dt, all features suggestive of increased sympathetic nervous system activity.[101]

Since the introduction of sensitive techniques, plasma catecholamine levels have been often used to measure sympathetic nerve activity in humans. A review of 32 studies comparing plasma norepinephrine (NE) levels in hypertensive and normal subjects indicated higher levels in hypertensive patients in 88% of these studies.[102] The difference was more likely to be evident among younger subjects. The increments in plasma concentration of NE during isotonic or isometric exercise were also usually greater in hypertensive than in normal subjects.[103]

Some investigators have found increased plasma levels of epinephrine in essential hypertension, and they have speculated that epinephrine

might stimulate presynaptic inhibitory receptors, thus resulting in increased sympathetic activity.[104]

Recently, the concentration of NE in the cerebrospinal fluid of patients with essential hypertension has been shown to be increased, suggesting hyperactivity of the central sympathetic nervous system in these patients.[105]

Kinetic studies using radiolabeled catecholamines have indicated reduced reuptake[106] or increased spillover of NE[107] in some patients with essential hypertension.

A significant positive correlation between plasma NE levels and blood pressure has been shown in some studies.[108,109] Pharmacologic studies with antiadrenergic agents have also been used to substantiate the hypothesis of a pathophysiologic role of neurogenic factors in essential hypertension. Thus, a significant correlation between the decrease in diastolic blood pressure and the decrease in plasma NE has been shown during administration of pentolinium, a ganglionic blocking agent,[108] or clonidine.[110]

Indirect evidence for increased RSNA have also been shown in patients with essential hypertension.[111,112] Increased renal vascular resistance is commonly present in established essential hypertension. This can be reversed by α-adrenergic blockade[112] or made worse by β-adrenergic blockade.[113]

Recently, renal NE secretion has been found to be increased in many patients with essential hypertension, adding further support to the concept that an abnormal RSNA may play a pathogenetic role in patients with essential hypertension.[114]

2.4. Salt and Neurogenic Factors in Hypertension

Substantial evidence links sodium ingestion to the genesis of hypertension.[115,116] Various investigators have identified two groups of patients with essential hypertension on the basis of their blood pressure response to a sodium load.[117,118] Approximately 60% (salt-sensitive) responded to a sodium load with a rise in blood pressure equal to or greater than 10%, whereas blood pressure remained unchanged in the remaining subjects (salt-resistant). Certain strains of rats mimic the response in humans. Dahl[115] selectively inbred two strains of rats, with one strain becoming hypertensive (salt-sensitive) while the other remained normotensive (salt-resistant) when challenged with high-sodium intakes. Less clear is the role of sodium intake in SHR. In these animals hypertension can develop on a sodium-free diet, but the height to which the blood pressure rises is related to the sodium intake.[119] The mechanisms relating sodium to hypertension remain controversial. It has been postulated that

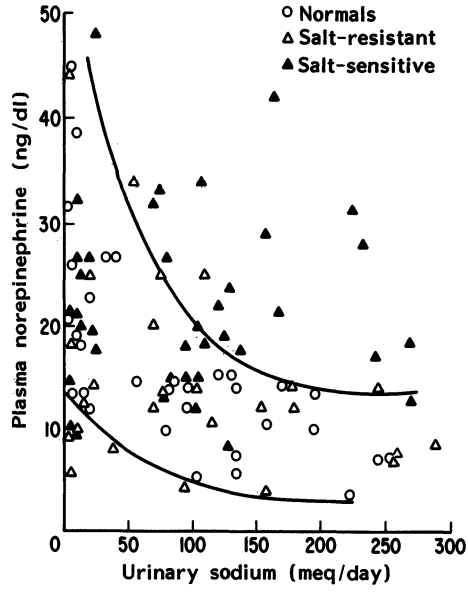

Fig. 1. Correlation between supine plasma norepinephrine level (ng/dl) and urinary sodium excretion (meq/day) in normal subjects (O) and in salt-sensitive (▲) or salt-resistant (Δ) patients. (Reproduced from *Kidney Int.* **21**:371, 1982, with permission.)

a genetic defect involving the ability of the kidneys to excrete a sodium load may be responsible. However, it is also possible that high-sodium intake may activate the sympathetic nervous system.

During ingestion of a high-sodium diet, blood levels of NE, epinephrine, and dopamine were suppressed in normal subjects,[120] so that an inverse relationship between urinary sodium excretion and plasma levels of these amines was evident. In patients with essential hypertension, this inverse relation was not present[118] (Fig. 1). Plasma NE levels were not different between normal subjects, salt-resistant, or salt-sensitive patients while ingesting a low-sodium diet. However, during a high-sodium intake, plasma NE concentration decreased significantly in normal and in salt-resistant subjects, but not in salt-sensitive patients. On the contrary, plasma NE during high-sodium intake increased in the majority of patients. A significant correlation was found between the changes in plasma NE and the changes in blood pressure observed with the two diets. The orthostatic increments of plasma NE were also greater in salt-sensitive patients. These data suggest an abnormal response of the sympathetic nervous system to high-sodium intake as a potential pathogenetic factor in the genesis of hypertension in a subset of patients with essential hypertension. Falkner *et al.*[120] have observed an abnormal sympathetic response during high-sodium intake in young prehypertensive adolescents with a strong family predisposition for hypertension. Similar abnormalities have been observed in SHR,[122] in stroke-prone SHR,[123] and in Dahl's salt-sensitive rats.[124]

The mechanisms responsible for the greater activation of the sympathetic nervous system during high-sodium intake are not clear. Winternitz and Oparil[121] have shown increased NE content in the dorsomedial and anterior hypothalamic nuclei, suggesting a central mechanism. Koepke and DiBona[125] observed that high-sodium intake potentiated the increase in RSNA and the decrease in urinary sodium excretion resulting from air stress in conscious SHR, suggesting a central mediated facilitation of sympathetic neural outflow to the kidney.

Dietz *et al.*,[122] on the other hand, observed that high-sodium intake caused reduced reuptake of NE in the sympathetic end terminals of stroke-prone SHR, pointing to a peripheral mechanism of activation of the sympathetic nervous system. Blaustein[79] has suggested that excessive sodium intake may stimulate the release of a ouabainlike natriuretic factor, which in turn would suppress Na^+–K^+ pump, thus facilitating NE release from the sympathetic end terminals. Finally, high-sodium intake may potentiate neurogenic vasconstriction by increasing vascular reactivity.[126]

Increased dietary intake of potassium appears to antagonize the effect of high-sodium intake on sympathetic activity and on blood pressure.[127]

3. The Renin–Angiotensin System

Renin release is a key event which regulates a major system that controls blood pressure homeostasis and sodium–potassium balance. Renin is an aspartyl protease produced in the juxtaglomerular cells of the kidney. It acts on angiotensinogen, an α_2-globulin from the liver, cleaving a Leu–Val bond in man, to release the decapeptide angiotensin I (ANGI). As ANGI traverses pulmonary, renal, and other vascular beds, it is converted to angiotensin II (ANGII), which is the systemically active component of the system. ANGII is a potent constrictor of vascular smooth muscle, stimulates adrenal aldosterone secretion, enhances thirst and antidiuretic hormone secretion, induces renal vasoconstriction, increases renal tubular sodium reabsorption in low concentrations, and inhibits reabsorption in higher concentrations.[128–132] Lesser-known activities include its ability to modulate catecholamine production from nerve endings and adrenal medulla and to regulate prostaglandin production from a variety of tissues.[130,133] Thus, ANGII regulates blood pressure through a number of different but interrelated mechanisms. In general, ANGII production by the body, and, hence, ANGII activity, are controlled by the amount of the active form of renin that is available. Therefore, investigation of the mechanisms that control biosynthesis, processing,

and release of renin is important in understanding how the cascade of events leading to ANGII production is regulated. In addition, inhibitors of different levels of the cascade are and will be important in defining the physiologic role of the renin system and in treating pathologic states characterized by excess renin.

Overproduction of renin has been strongly implicated in the pathogenesis of high- and normal-renin essential hypertension, which comprises about 80% of patients with high blood pressure. It has definitely been shown to mediate the high blood pressure associated with renovascular disease, malignant hypertension, and hypertension due to some forms of chronic renal failure. Underproduction of renin, on the other hand, results in the syndrome of hyporeninemic hypoaldosteronism, which, in the absence of oliguria, is the most common cause of chronic hyperkalemia and type IV renal tubular acidosis.[134] Diabetics with this syndrome often have high basal and stimulated levels of circulating prorenin with low levels of basal and stimulated active renin, suggesting that a defect in conversion of prorenin to renin results in the syndrome in these patients.[135,136] Indirect evidence in normal humans suggests that conversion of prorenin to renin may play an important role in the physiology of active renin production.[137] Whether renin-dependent forms of hypertension are associated with abnormalities in conversion of prorenin to renin is unknown; however, patients with essential hypertension who respond to propranolol have a decrease in plasma active renin and a rise in prorenin.[138]

This section will focus on posttranslational processing of renin, particularly the relationship between inactive (pro-) renin and active renin, and its role in abnormal states of renin production in man.

3.1. Biosynthesis and Processing of Renin

Current evidence indicates that the renin biosynthetic pathway is similar to that of other enzymes and polypeptide hormones and involves the formation of prepro- and proforms. Mouse submaxillary gland is one of the richest known sources of renin (2–5% of protein) and has served as a model tissue for investigation of renin biosynthesis. Early studies of Poulsen *et al.*[139] demonstrated that the primary translation product of submaxillary gland renin mRNA was a renin immunoreactive protein with a mol. wt. 10,000 larger than renin itself. When added to pancreatic microsomes, a 2000-mol. wt. peptide was cleaved consistent with the signal hypothesis of Lingappa and Blobel[140] and indicates that renin is synthesized as a preprorenin which is converted to prorenin. More recent and detailed studies by Catanzaro *et al.*[141] and Pratt *et al.*[142] demonstrate that mouse submaxillary gland renin is produced and se-

creted as a single-chain form which, when stored in the gland, is slowly (over hours) converted to a two-chain form. Both the one- and two-chain forms, but not prorenin, possess activity, although the specific activity of one-chain renin was fivefold higher than that of two-chain renin. Subcellular fractionation[142] demonstrated that preprorenin is internalized into rough endoplasmic reticulum and hydrolyzed to prorenin within minutes. In the Golgi, prorenin is converted to one-chain renin which can be either secreted or converted to the two-chain form during packaging of renin into granules. The mature granules contain primarily the two-chain form, which is also secreted. Using recombinant DNA techniques, Panthier *et al.*[143] were able to deduce the amino acid sequence of mouse submandibular gland preprorenin. They postulated that the cleavage site of the "presequence" was Cys 19 and that of the "prosequence" occurred after two dibasic peptides Lys 62–Arg 63. Cleavage of one-chain to two-chain renin also occurs after two dibasic peptides Arg 353–Arg 354. This structure agrees with the complete amino acid sequence of pure active mouse submaxillary gland renin determined by Misono *et al.*[144] Pratt *et al*[142] suggested that renin may be secreted by two separate pathways—an early pathway from the Golgi (one-chain renin), which is not known to be regulated, and another, regulated pathway from secretory granules (two-chain renin). Their studies suggested that one-chain and two-chain renin may have different enzyme activities.[145]

Until recently, the low renin concentration in human tissue prevented renin biosynthesis studies in humans. However, Corvol *et al.*[146] were able to study renin production in tissue slices and in cultured cells from a human renin-producing tumor. Pulse labeling in the tissue slices demonstrated that a 55,000-mol. wt. renin-immunoreactive protein was converted to a 44,000-mol. wt. renin. The cultured cells secreted only the 55,000-mol. wt. renin which was inactive and could be activated with trypsin. Corvol *et al.* postulated that the larger-molecular-weight inactive prorenin is secreted by a constitutive pathway in the tumor cells, while active renin is secreted via secretory granules. Characterization studies by Atlas *et al*[147] indicate tumor inactive renin is biochemically similar to renal and plasma inactive renin.

Using the mouse submaxillary gland cDNA as a probe, Imai *et al.*[148] and Soubrier *et al.*[149] simultaneously isolated human kidney renin mRNA (1500 base pairs, BP) and deduced the amino acid sequence of human kidney preprorenin. Its 400 amino acids were 70% homologous to mouse submaxillary gland renin. A schema for the biosynthesis and processing of human renin is depicted in Fig. 2. Imai *et al.* estimated the presegment to be about 20 amino acids in length, with the clip occurring at Cys 20–Thr 21, and the prosegment to be 46 amino acids long, with the clip

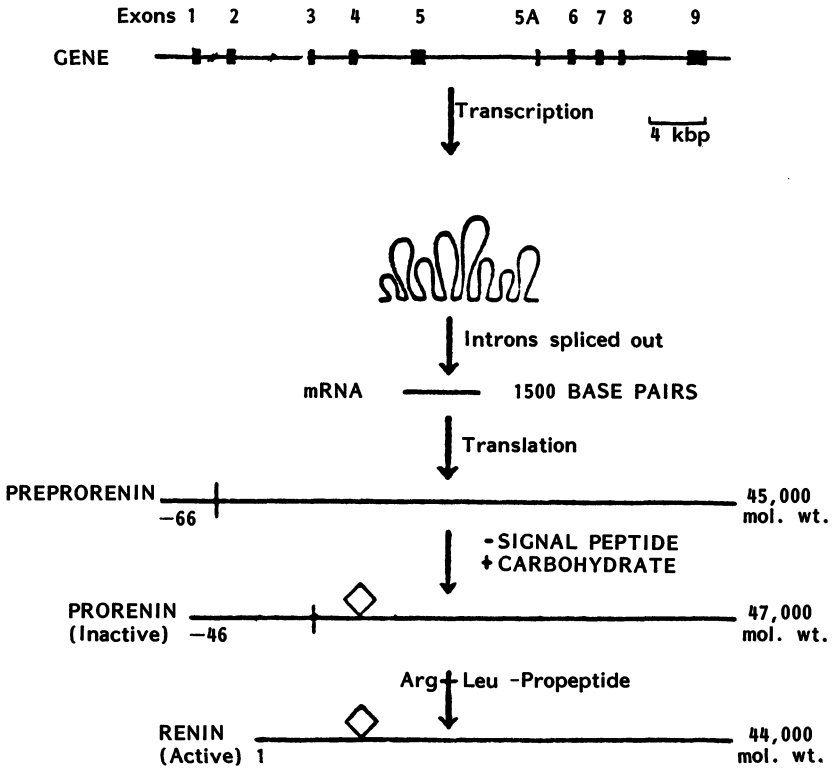

Fig. 2. Schema for the biosynthesis and processing of human renin.

occurring at Arg 66–Leu 67. Recently, a rapid three-step procedure for purification of human renal renin has been developed,[150] and enough material was obtained for N-terminal amino acid sequencing. These studies indicate that the predicted cleavage is correct, and that a leucine residue resides at the amino terminus of pure human renal renin. This indicates that the prosegment is clipped after two basic residues to form active renin, i.e., Lys^{-2}–Arg^{-1}–Leu^{1}.

The clipping of the prosegment after dibasic residues is analogous to that of a number of other hormone systems, including proopiomelanocorticotropin (POMC), proinsulin, and proglucagon.[151] Loh *et al.*[152] have recently purified a paired basic residue-specific proopiomelanocortin-converting enzyme from bovine pituitary intermediate-lobe secretory vesicles. This enzyme was found to be a glycoprotein with a mol. wt. of 70,000, with a pH optimum between 4 and 5 (i.e., active at the intravesicular pH); it could cleave POMC and proinsulin on the carboxyl side of the Arg after a Lys–Arg pair. Cleavage products of POMC included 21,000–23,000 ACTH, α-lipotrophin, β-endorphin, and smaller-

Table I. Some Enzymes That Activate Human Renin

Serine proteases
Trypsin
Glandular kallikrein
Plasma kallikrein
Plasmin
Gamma subunit of nerve growth factor
Acrosin
Tonin
Thiol protease
Cathepsin B, D, H
Acid protease
Pepsin
Renin

molecular-weight ACTHs. Pepstatin A (10^{-6} M) was a potent inhibitor of this enzyme, while leupeptin (10^{-3} M) possessed a partial inhibitory effect, suggesting that the enzyme was an aspartyl protease rather than a serine protease. This raises the question of whether or not the prorenin-processing enzyme is specific to the renin system. Purification of this enzyme from the kidney and comparison with processing enzymes from other systems will be necessary to answer this question.

In the renin system, a number of enzymes have been implicated as the renin-processing enzyme because of their ability to activate inactive renin. Table I lists some enzymes demonstrated to activate human renin. The exact site of cleavage of these proteases on prorenin and whether they exist in high enough concentrations in renin-producing cells of the kidney are unknown. Trypsin activation is a standard technique to detect inactive renin.[153] Trypsin probably cleaves the prosegment after Lys^{-2}–Arg^{-1}. However, it does not completely decrease the molecular weight of human plasma and renal inactive renin to that of active renin. The quantities of trypsin in the kidney are unknown. Glandular kallikrein, particularly renal kallikrein, has been strongly implicated as an *in vivo* activator of renin.[154] Several links exist between the renin and kallikrein systems since angiotensin-converting enzyme is also kininase II, and since renal kallikrein production is regulated by aldosterone.[155,156] Addition of rat urinary kallikrein, but not trypsin, to rat renal cortical slices results in release of active renin which is abolished by trasylol.[157] Early immunohistochemical studies localized kallikrein to renal cortex, and later studies localized it in the tubules abutting against, but not in, juxtaglomerular cells.[158] Further studies suggested that the conformation of inactive renin had to be altered prior to its activation by kallikrein.[159] Compared to trypsin and human plasma kallikrein, glan-

dular kallikrein was shown to be a poor activator of semipurified human plasma inactive renin.[160] Hence, there is no clear evidence that glandular kallikrein is the physiologic activator of renin. Although a number of enzymes in the clotting system, such as plasma kallikrein and plasmin, can activate renin, the physiologic significance of this is unknown. Because of the high concentration of protease inhibitors in plasma, it is unlikely that significant amounts of renin are activated in the circulation. However, during bleeding or inflammation, inactive renin may be activated locally to control blood flow in the local vessels. Whether plasmin or the other serine proteases exist in juxtaglomerular cells is unknown. Cathepsins B and H, extracted from human kidney, have been reported not only to activate kidney inactive renin, but to decrease its molecular weight to a 40,000–45,000 moiety.[161] However, the isoelectric point of cathepsin-activated renin differed from that of natural active renin.[162] Renin has also been demonstrated to activate itself[163]; this is not unusual for a number of naturally occurring enzymes. The major problem with these studies is that they were conducted in impure systems. The availability of pure expressed prorenin promises to resolve a number of issues.

3.2. Regulation of Renin Secretion

Since regulation of renin production controls activity of the renin–angiotensin–aldosterone system, another important question is whether conversion of prorenin to renin is a major mechanism to control renin release, in addition to regulation at the level of transcription. This may offer another approach by which to control the renin–angiotensin system. At present, four well-described mechanisms regulate renin release: (1) baroreceptors in the afferent arteriole (the lack of "stretch" enhances renin secretion), (2) the macula densa, which is an area in the distal tubule sensing sodium reabsorption (decreased sodium reabsorption enhances renin release), (3) the β-adrenergic system (β-agonists such as isoproterenol or dibutyrl cAMP stimulate renin), and (4) ANGII, which is a potent "negative-feedback" inhibitor of renin secretion. Prostacyclin, the vasodilating prostaglandin, appears to mediate the baroreceptor and probably the macula densa signals to renin release.[164,165] Inhibitors of prostaglandin synthesis have been reported to block both mechanisms of renin release, but not β-adrenergic stimulation of renin secretion. Infusion of prostacyclin in humans stimulates renin release.[166] Calcium mediates the effects of angiotensin II. These effects can be blunted by calcium chelators, calcium channel blockers, calmodulin inhibitors, or inhibitors of intracellular calcium movement.[167,168] Vasopressin, potassium, and atrial natriuretic factor directly inhibit renin

release; adenosine, kallikrein, and other factors stimulate renin.[169] The physiologic importance of these factors to overall renin secretion is not clearly established.

Perturbation of the renin system in humans and identification of human inactive renin as the renin biosynthetic precursor prorenin provide indirect evidence to suggest that conversion of prorenin to renin may be a regulatory step in renin production. In normal human plasma, 50–80% of the renin exists in an inactive form, which can be activated and then measured by exposure of plasma to low pH or to trypsin.[170] Ten to fifty percent of the renin in normal human kidney also exists in an inactive form, and a renal arteriovenous gradient of inactive renin has been demonstrated, suggesting the kidney is an important source of circulating inactive renin.[171] If an interrelationship exists between inactive renin secretion and its conversion to active renin, then circulating levels of inactive renin would change reciprocally with active renin. Converting enzyme inhibition (CEI) is a potent stimulus of renin secretion owing to removal of angiotensin II negative feedback on the juxtaglomerular cell. Therefore, the time course of changes in circulating active and inactive renin after CEI has been studied.[172] After equilibration on a 25 meq/day-sodium diet, captopril was given as a single 50-mg oral dose (acute phase), and then administered as 50 mg every 6 hr for 3 days to seven normal volunteers (chronic phase). In the acute phase, supine blood pressure fell 12 ± 2 mm Hg ($p < 0.02$). Active renin acutely increased 12.5 ± 0.9 times the baseline value, peaking at 3–4 hr. Inactive renin, measured by acid activation or trypsin activation, decreased in all subjects to 10% or less of control values from 2 to as long as 6 hr post-CEI and then returned to baseline levels by 8 hr ($p < 0.01$) (Fig. 3).

With chronic CEI, active renin was elevated to 10.8 ± 2.4 times the baseline level, and after 48 hr, inactive renin levels rose to 4.0 ± 0.6 times the base ($p < 0.02$). To determine whether the acute changes in inactive and active renin occurred because of events in the circulation or in the kidney, a single dose of captopril was administered to three subjects with moderate renal insufficiency and hyporeninemic hypoaldosteronism. In contrast to normal subjects, these patients had no change in active and inactive renin levels when given captopril, suggesting the changes observed in the normals were renal-mediated rather than being a plasma phenomenon. These studies support a precursor role of inactive renin when the acute demand for active renin is markedly enhanced. This suggests that the enzyme responsible for processing inactive to active renin is regulated. Conversely, active and inactive renin could be differentially secreted from two pools. If inactive renin was released by a constitutive pathway, then acute stimulation could result in more prorenin being diverted to the regulated pathway of active renin secre-

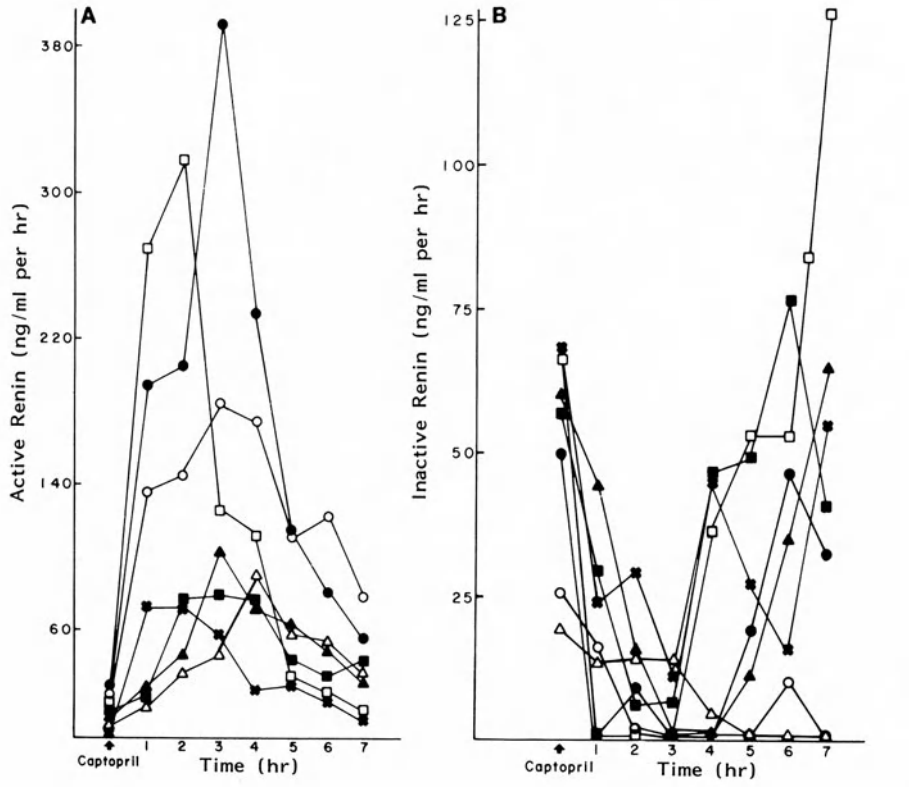

Fig. 3. Effect of converting-enzyme inhibitor (50 mg captopril) orally on plasma renin activity (A) and plasma inactive renin (B) in normal subjects. Each symbol represents an individual subject. Active renin increased 12.5 ± 0.9 times baseline value. Inactive renin dropped to 10% or less of control level. (Reproduced from *J. Clin. Endocrinol. Metab.* **56**:264, 1983, with permission.)

tion and less toward the constitutive pathway, resulting in decreased secretion of inactive renin. On the other hand, both mechanisms could be operating during acute stimulation, i.e., enhanced activity of the processing enzyme and decreased prorenin release through the constitutive pathway. Prior studies questioned this precursor role because active measurements after upright posture or the administration of diuretics demonstrated little change in inactive renin, despite significant increases in active renin (summarized in Ref. 153). In humans, only Derkx *et al.*[173] had previously demonstrated an acute rise in renin following diazoxide and upright tilt. Although significant, the decreases in inactive renin were small. These and other investigators subsequently found responses to CEI in hypertensive humans[174,175] similar to those found in nor-

mals.[172] Two studies are in disagreement with the results of Goldstone *et al.*,[172] probably owing to differences in experimental design.[176,177] However, in the purified dog kidney, Dzau *et al.*[178] demonstrated that CEI enhances the renal production rate of active renin and decreases the production rate of inactive renin.

Subsequent studies have suggested that prostaglandins may play a role in the conversion of inactive renin to renin. Prostaglandin synthetase inhibitors, but not β-adrenergic inhibition, could prevent the drop in inactive renin seen with converting enzyme inhibition.[179] Both, however, lowered the active renin response to CEI. Infusion of both isoproterenol and PGA_1 (a synthetic vasodilator prostaglandin) increased active renin two- to threefold. Inactive renin dropped as active renin rose in response to PGA_1. In contrast, the β-agonist had no effect on inactive renin. The results are consistent with studies in the isolated perfused hog kidney in which trypsin treatment of the perfusate demonstrated a twofold increase in active renin and a 75% decrease in inactive renin during infusion of PGI_2.[180] In humans, isoproterenol has not previously been shown to alter plasma inactive renin levels, despite inducing a rise in active renin.[170] These data suggest that the β-adrenergic system and prostaglandins stimulate renin production at different steps of biosynthesis or secretion. Prostaglandins may preferentially enhance conversion of inactive to active renin, perhaps through regulation of a putative renin-processing enzyme.

A clinical correlation of these observations is the syndrome of hyporeninemic hypoaldosteronism. Half the patients with this syndrome have diabetic renal disease with 3–5 times the normal circulating levels of inactive renin, despite low levels of active renin and aldosterone.[136] The incidence of the high circulating levels of inactive renin correlates with the levels of microalbuminuria, and Luetscher *et al.*[181] postulated that plasma inactive renin levels may serve as a marker of diabetic microvascular disease. In contrast, nondiabetic patients with the syndrome due to such conditions as interstitial nephritis and systemic lupus erythematosus[136] do not have particularly elevated plasma inactive renin levels. Thus, the defect in conversion of prorenin to renin may be specific to diabetes mellitus.

Recent evidence indicates that a prostacyclin deficiency exists in the hyporeninemic hypoaldosterone syndrome.[182] Prostacyclin, a potent vasodilator and renin secretagogue, was markedly reduced, as reflected by measurements of its stable urinary metabolite 6-keto-prostaglandin $F_{1\alpha}$, in patients with hyporeninemic hypoaldosteronism as compared with matched controls with renal insufficiency, and as compared with normal volunteers (see Fig. 4). In contrast, renal prostaglandin E_2 excretion was similar in all three groups. Known stimulants of renal prostacyclin re-

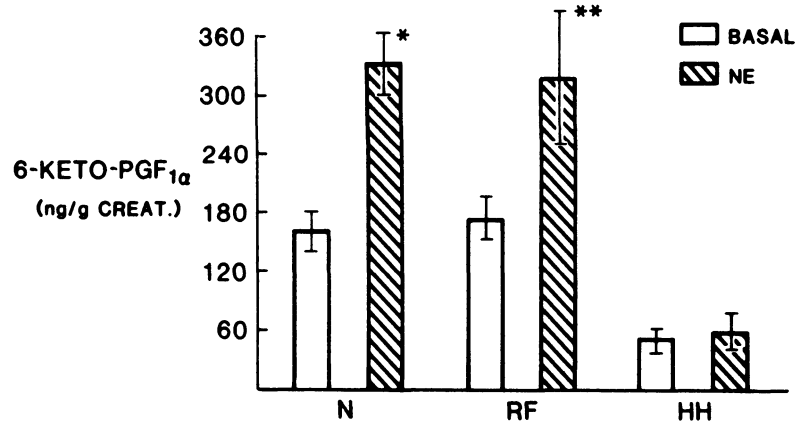

Fig. 4. Effect of a 4-hr norepinephrine infusion on the 4-hr excretion of urinary 6-keto-prostaglandin F_1 (6-keto-$PGF_{1\alpha}$) in normal subjects (N), controls with renal insufficiency (RF), and patients with hyporeninemic hypoaldosteronism (HH). $*p < 0.02$; $**p < 0.05$. (Reproduced from *N. Engl. J. Med.* **314**:1015, 1986, with permission.)

lease, such as low-dose calcium infusion or norepinephrine, did not increase the low basal prostacyclin excretion in the patients. Calcium infusion did not alter blood pressure or renal blood flow in the normal subjects or the controls with renal insufficiency. In contrast, the same dose of calcium produced a significant rise in mean blood pressure and a fall in renal blood flow in patients with hyporeninemic hypoaldosteronism. These results indicate that a deficiency of prostacyclin could explain the low active renin concentration and altered vasomotor tone seen in hyporeninemic hypoaldosteronism. Whether replacement of prostacyclin in these patients will enhance conversion of inactive to active renin remains to be determined.

3.3. Evidence That Inactive Renin Is Prorenin

While these types of *in vivo* studies were being performed, considerable controversy existed as to the nature of inactive renin. Inactive renin was regarded as a putative prorenin because (1) renin-secreting tumors synthesize and secrete large quantities of inactive renin,[183] analogous to polypeptide hormone-secreting tumors, which produce large quantities of prohormone; (2) absolute levels of inactive renin change with perturbation of the renin system, as discussed in Section 3.2; (3) antibodies developed against pure human renal renin cross-react with inactive renin[184,185]; (4) pulse labeling studies in the mouse submandibular gland, which makes large quantities of renin, indicate that renin is synthesized in a prepro- and proform which are inactive[139,141,142]; (5)

antibodies generated against synthetic peptides derived from the "pro" segment of human prorenin react with human inactive renin.[186–188]

Recently, Fritz *et al.*[189] cloned a cDNA sequence coding for human preprorenin from a human kidney cDNA library. This cDNA was expressed in Chinese hamster ovary cells in culture using a mammalian cell expression system. This resulted in the secretion of prorenin into the culture medium. Expressed human prorenin shared a number of biochemical similarities to human renal and plasma inactive renin[190] (Table I). Expressed prorenin was activated similarly with either acid or trypsin treatment. Native human inactive renin and expressed prorenin both demonstrated reversible acid activation, similar molecular weights (47,000 by SDS polyacrylamide gel electrophoresis or gel filtration high-pressure liquid chromatography), and cross-reaction to human prosegment antibody. These data provide strong support for the hypothesis that human inactive renin is prorenin, i.e., the biosynthetic precursor of renin, and help to explain a number of clinical observations involving the measurement of circulating inactive renin.

3.4. Active Renin and Prorenin in Hypertension

3.4.1. Essential Hypertension and Renin Inhibitors

Renin profiling indicates that about 30% of patients with essential hypertension have low renin, 50% have normal plasma renin activity, and 20% have high plasma renin when compared to urinary sodium excretion.[191] It has been suggested that patients with both high and normal levels of renin actually have overactivity of the renin system for their degree of blood pressure elevation. Because of the lack of pure inhibitors of the renin system, this hypothesis has been difficult to prove. Studies with the ANGII analog saralasin are the most suggestive; in states of high ANGII activity, it acts as an antagonist and lowers blood pressure. The degree of lowering is proportional to the basal renin activity. In low-renin hypertension where the ambient ANGII activity is low, saralasin acts as an agonist and can actually enhance blood pressure. β-adrenergic blockers and converting-enzyme inhibitors have effects on blood pressure through other mechanisms besides the renin–angiotensin system and, in general, lower blood pressure regardless of the renin profiling.[192] Specific inhibitors of renin, which are currently being developed, or monoclonal antibodies to renin may be useful tools in determining the renin dependency and, thus, in treating essential hypertension.[193] Specific classes of renin inhibitors that have potential include (1) renin substrate analogs, (2) renin substrate analogs with a reduced bond at the renin cleavage site, (3) statine-containing compounds which

are general acid protease inhibitors, and (4) peptides in the amino-terminal two-thirds of the prosegment of prorenin which interact with the active site of renin (reviewed in Ref. 193). The development of an orally active renin inhibitor is currently being aggressively pursued.

Except in some acute studies, circulating prorenin levels generally parallel active renin levels. Both increase during chronic low-salt intake and chronic inhibition of converting enzyme; both decrease during chronic high-sodium intake.[194] In essential hypertension, the prorenin levels are in the normal range, but tend to parallel active renin and are significantly lower in patients with low-renin essential hypertension.[170] Low levels of both active renin and prorenin can be found in patients with primary aldosteronism. The blood pressure response to propranolol treatment appears to determine the prorenin response in patients with essential hypertension such that there is an inverse relationship between changes in pressure and changes in prorenin. This work was interpreted to suggest that in patients who responded with a decrease in blood pressure, blockade affected both overall renin production and conversion of prorenin to renin, while in the nonresponders, only overall production was affected. Thus, another potentially useful approach to treating blood pressure may be to inhibit posttranslational processing of renin, i.e., at the step involving conversion of prorenin to renin.

3.4.2. Known Renin-Dependent Forms of Hypertension

Renovascular disease is a common (3–5% of all patients with hypertension) renin-dependent form of hypertension. However, there are two stages of this disease. The initial stage represents the two-kidney Goldblatt model of hypertension, which develops after clamping of one renal artery in a normal animal. Blood pressure and renin levels increase. The blood pressure is well controlled with ANGII antagonists or antibodies to renin[195] or improved if the clamp is removed. Patients in this stage respond well to surgical intervention. In contrast, during the later stage of renovascular hypertension, which may take many years to develop, renin levels tend to decrease, and patients become less responsive to surgical intervention. This stage resembles the one-kidney Goldblatt model of hypertension, in which the uninvolved kidney is removed following clamping of the renal artery of the opposite kidney. This form of hypertension tends to be highly volume-dependent and probably develops with chronic damage from the effects of hypertension on the uninvolved kidney. The rise in renin in response to ANGII inhibitors (saralasin or converting-enzyme inhibition) was initially thought to be useful in differentiating these forms of renovascular hypertension,[196] but these observations have not been confirmed. Currently, renal vein

renin sampling under proper conditions[197] still seems to be the most useful predictor of surgical response. Prorenin levels have been reported to be in the normal range in renovascular hypertension.[170]

Renin-secreting tumors represent a much rarer form of renin-dependent hypertension. They are interesting because the highest levels of circulating prorenin ever reported have been in patients with renin-secreting tumors. They are suspected because of hypertension, hypokalemia, and high plasma renin activity in the absence of renal artery disease. Wilms' tumors, hypernephromas, and hemangiopericytomas have been reported to secrete renin; in addition, nonrenal tumors (ovary and pancreas)[183,198,199] have been associated with renin secretion. These tumors are analogous to other peptide-secreting tumors in that they release larger amounts of the inactive proforms as well as the active forms of the hormones, indicating that variable degrees of hormone processing by these tumors are present. The high prorenin levels represent a marker for renin-secreting tumors. Surgical removal of the tumor usually results in a decrease in blood pressure and decrease in plasma levels of active renin and prorenin.

Other forms of renin-dependent hypertension include malignant hypertension, which is secondary to microvascular renal ischemia, some forms of chronic renal disease, and maldevelopment of the kidney such as the Ask-Upmark kidney.

3.4.3. Pregnancy-Induced Hypertension

In normotensive pregnant women, plasma renin activity is increased largely due to the increase in angiotensinogen production. The plasma concentration of active renin, a measurement of renin independent of substrate, however, is in the normal nonpregnant range or only modestly elevated and generally decreases after delivery.[200] In contrast, during the first trimester of pregnancy the plasma inactive renin concentration rapidly increases to about 5 times the value in nonpregnant women, then declines slowly throughout the remainder of pregnancy and falls quickly to the normal nonpregnant range after delivery.[200] The source and physiologic significance of the high circulating prorenin levels in pregnancy are unknown. In the first trimester, prorenin levels change with altered dietary sodium intake. This response is similar to that seen in nonpregnant subjects and implies a renal source of the elevated prorenin levels in pregnancy. However, the uteroplacental unit, particularly the chorionic membranes and uterine smooth muscle, contain and secrete relatively large quantities of prorenin,[201–204] and amniotic fluid concentrations are 2–5 times the levels found in plasma of pregnant women.[205] Recent studies have demonstrated an arteriovenous gradient of prorenin, but no active renin, in blood obtained from normotensive preg-

nant women at the time of cesarean section.[206] These data suggest that the uteroplacental unit contributes to the elevated prorenin levels in pregnancy at term.

In pregnancy-induced hypertension (PIH), inappropriate vasoconstriction in response to ANGII, NE, etc., has been demonstrated, suggesting that excess amounts of a circulating vasoconstrictor or loss of a vasodilator contributes to the enhanced pressor responsiveness. Since the hypertension corrects upon delivery of the fetus and placenta, a "toxic" substance may arise from the uterine–placental unit, which is smaller than normal and which often demonstrates ischemic changes in PIH. The nature of this substance is unknown. Active renin levels in maternal blood tend to be in the normal range or slightly decreased in patients with PIH. In samples obtained at delivery, there was no increase in prorenin levels in PIH patients compared to normal pregnant women.[207] However, chorionic tissue and fetal (cord artery and vein) levels of active renin, but not prorenin, tended to be higher in samples obtained from women with PIH compared to normal.[208] Whether enhanced conversion of prorenin to renin in the chorion is a marker of placental ischemia is an intriguing question. This could represent another example where renin processing plays an integral role in the development of a clinical disease state involving altered blood pressure regulation.

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8

Immunologic Aspects of Renal Disease

William G. Couser

1. Introduction

In this chapter, I shall present an overview of the advances made in understanding immune disease mechanisms during 1984–1985, with particular attention to new directions, ideas, and processes that have significant promise for the future. In taking this approach, I shall inevitably exclude isolated observations of significant merit that do not blend easily into the currents of the field in general. For similar reasons, included here will be studies of less significance that are reviewed because they provide links, or potential links, between larger areas of relatively greater importance.

In the second part of this chapter I shall review new studies related to the major clinical entities considered in the category of immunologic renal disease, again with an emphasis on evolving concepts rather than cataloguing every study of potential interest that was published during the interval covered. Whenever possible, I have linked the observations on disease mechanisms with the clinical entities that probably result from them.

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2. Mechanisms of Immune Glomerular Injury

2.1. Glomerular Immune Deposit Formation

The presence of granular immune complex deposits by immunofluorescence and electron microscopy remains the most common immunopathologic finding in immune renal disease. Since the observations made in the late 1970s that several prototypical immune complex diseases resulted from local immune complex formation within the glomerulus rather than from circulating immune complex trapping,[1] observations continue to be made that further expand our understanding of what these deposits are and how they form. The following summary is organized on the basis of morphologic sites of immune deposit formation, since deposits at different sites in the glomerulus probably form by somewhat different mechanisms. Salant *et al.* have provided a further rationale for this approach by demonstrating that the site of deposit formation is a critical determinant of the mediators activated and consequently of the lesions produced.[2] Thus, deposits of the same antigen–antibody complex produce a noninflammatory terminal complement-dependent lesion in a subepithelial site, whereas in an endothelial distribution effector cells are activated and a proliferative inflammatory lesion results.[2]

2.1.1. Subepithelial Immune Complex Deposits

2.1.1.1. Fixed Glomerular Antigens. Subepithelial immune complex deposits are characteristic of idiopathic membranous nephropathy as well as membranous glomerular lesions associated with lupus, various drugs, malignancy, and several other diseases. The mechanism responsible for the development of these granular subepithelial deposits has been best studied in the active and passive Heymann nephritis models of membranous nephropathy in rats (reviewed in Ref. 1). Studies in these models have produced a volume of recent information to clarify the nature of the mechanisms of deposit formation involved. Kerjaschki and Farquhar earlier found that the antigen responsible for formation of subepithelial immune complex deposits in the rat is a glycoprotein expressed on the surface of the visceral glomerular epithelial cell, as well as on proximal tubular cell brush border membranes.[3] Subsequent studies utilizing polyclonal and monoclonal antibodies to isolated kidney tubular microvillus membranes identified the antigen as a molecule of mol. wt. 330,000 (gp 330), which could be localized by immunoperoxidase techniques to clathrin-coated microdomains in proximal tubular brush border membranes and to several glomerular epithelial cell structures, including the endoplasmic reticulum, Golgi components, micro-

tubular bodies, and coated pits at the cell surface.[4] Both polyclonal and monoclonal antibodies to gp 330 stained both the glomerular epithelial cell membrane and proximal tubular epithelial cell membrane antigens.[3,4] Both rat and rabbit antibodies to gp 330 reproduce the subepithelial deposits characteristic of Heymann nephritis, or membranous nephropathy, when injected intravenously into rats, although they have not been demonstrated to transfer the disease in the sense of causing significant proteinuria.[4,5] Of particular interest is the fact that immunoprecipitation autoradiography performed with antibody eluted from kidneys of rats with Heymann nephritis also stained a gp 330 band in glomerular digests, suggesting that gp 330 may be the nephritogenic antigen in actively immunized animals.[3] gp 330 is also present in the immune deposits which appear to begin in coated pits of the glomerular podocyte.[4] gp 330 appears structurally related to maltase, another membrane glycoprotein, but is antigenically distinct.[5]

Although the studies of Kerjaschki and Farquhar on gp 330 have greatly advanced our understanding of the pathogenesis of membranous nephropathy in the Heymann models, controversy persists regarding the exact identity of the antigen involved. There is also evidence that more than one antigen may be important. Bhan and colleagues describe three monoclonal antibodies reactive with brush border antigens, including one apparently reactive with gp 330 and one reactive with another 110-kd antigen of the podocyte cell membrane.[6] However, Makker and Singh have reported isolation of a glycoprotein weighing 600,000 (gp 600) which can induce Heymann nephritis and can be resolved into several subunits, including gp 330 and gp 70.[7] gp 600 and gp 70 are circulating antigens and raise again the question of whether circulating antigens and immune complexes participate in the pathogenesis of Heymann nephritis, as was believed prior to 1978. Abrass has found a circulating tubular antigen that can apparently localize directly in glomeruli by an undefined mechanism.[8] Thus, the search for the "real" Heymann antigen goes on in several laboratories, and the final answer is not yet in. It seems certain that the gp 330 antigen is a major factor in this process. However, it also seems probable at this point that more than one "nephritogenic" antigen–antibody system is probably involved in Heymann nephritis. This suspicion is supported by studies such as those of Kamata *et al.* documenting antibody in glomerular deposits reactive both with gp 330 and a 95-kd antigen.[9] However, while controversy persists regarding which, and how many, antigens participate in the pathogenesis of Heymann nephritis, the fact that membranous nephropathy in this fascinating model is initiated by antibody binding to some intrinsic glomerular epithelial cell membrane antigen has been well established since 1983.

An important question to be answered in the future is whether the observations made in Heymann nephritis are relevant only to rats or, in fact, define the mechanism of idiopathic membranous nephropathy in humans, a lesion that looks identical to the Heymann models. With regard to species other than the rat, Neale *et al.* have reported the spontaneous development of an atypical membranous lesion in rabbits mediated by antibody to another antigen localized at the base of the glomerular podocyte.[10] A Heymann nephritis-like lesion has also been reported in rabbits injected with a guinea pig antibody to proximal tubular brush border, although a glomerular antigen was not identified in that study.[11] Assmann and colleagues, using a pronase-digested renal tubular antigen, showed that antibody to this antigen produced a membranous lesion in normal mice and contained antibodies reactive with the 330- and 95-kd antigens present in rat glomeruli, but that the deposits in mice were produced primarily by the antibody to the 95-kd determinant.[12] Thus, membranous nephropathy due to antibody binding to glomerular epithelial cell antigens can apparently be produced, albeit with some difficulty, in species other than the rat. Suspicion is therefore now very high that membranous nephropathy in humans is probably an autoimmune disease mediated by a similar mechanism. However, there are as yet no published studies that determine whether a circulating or deposited antibody in human membranous nephropathy is reactive with the human glomerular epithelial cell.

A second area of major progress in understanding subepithelial immune deposit disease is also related to studies of Heymann nephritis. While the studies reviewed above clarify why antibody is deposited in a subepithelial distribution in Heymann nephritis, they do not account for the distinct granularity of the deposits formed which might be expected to simply line the epithelial cell membranes or accumulate in coated pits. In an elegant series of studies, Andres and colleagues have defined how the glomerular deposits develop. Earlier they demonstrated that injection of rabbits with antibody to an antigen expressed on alveolar capillary endothelial cell plasma membrane (angiotensin-converting enzyme) induced local immune complex formation on the endothelial cell surface followed by a loss of antigen (antigen modulation) as the complexes were capped and shed into the circulation.[13] They then utilized rabbit oocytes to examine what happened when cells that were surrounded by a basement membrane-like structure were exposed to antibody and found that exposure of oocytes to antibody directed against a cell membrane protein (again angiotensin-converting enzyme) resulted in formation of granular deposits in the zona pellucida through a patching and shedding phenomenon which required divalent antibodies.[14] Finally, they turned to the glomerulus and utilized rat glomerular epithelial cells in culture with

both polyclonal and monoclonal antibodies to gp 330 to demonstrate that antibodies bound to the cell surface, then clustered into aggregates to form patches and caps with loss of antigenic reactivity in areas free of complexes.[15] This antibody-induced change in antigen distribution required divalent antibody capable of forming immune complex lattices and is probably associated with changes in cellular cytoskeletal elements related to cell contraction. Thus, the ongoing evolution of our knowledge of the pathogenesis of membranous nephropathy illustrates well the application of basic techniques in biochemistry and cell biology to understanding an important disease mechanism in humans. These studies of Heymann nephritis have defined an entirely new process of immune deposit formation, i.e., immune complex nephritis induced by the binding of antibodies to antigenic structures expressed on resident glomerular cell membranes. Similar studies are now in progress of the consequences of antibody binding to glomerular endothelial and mesangial cells (see Section 2.1.2.1).

Before leaving the Heymann nephritis studies, it is worth noting that other aspects of this model have also been of interest to investigators in the recent past. Thus, Abrass has shown, in actively immunized rats with Heymann nephritis, that impairment of the mononuclear phagocyte system produces worse disease independently of antibody and complement levels, another bit of evidence in favor of a circulating factor in the pathogenesis of this model.[16] The group in Buffalo has continued studies of the consequences of antibody deposition along the proximal tubular brush border in this model and has shown impaired proximal tubular function which persists after antibody disappearance and apparent morphologic recovery of injured proximal tubular epithelial cells.[17,18] The model may thus serve as a useful probe for the effects of cell membrane antibody binding on tubular transport function. Finally, persistent proteinuria in Heymann nephritis leads, as it does in membranous nephropathy in humans, to progressive glomerular sclerosis and renal failure.[19] Heymann nephritis may therefore prove a useful model for studies of the mechanisms involved in slowly progressive glomerular disease.

2.1.1.2. Exogenous Antigens. Most studies of subepithelial immune complex deposits induced with exogenous antigens have concentrated on the mechanisms by which the glomerular immune complex deposits form. Border and colleagues have expanded on their original observations that subepithelial deposits in chronic BSA-serum sickness induced with cationized BSA resulted from initial "planting" of the cationic antigen by charge interaction with glomerular anionic sites to demonstrate in perfusion studies that a cationic antigen is required for this process and that the deposits form on a local basis.[20] Unfortunately, the role of

this mechanism versus autoantibody binding to a glomerular epithelial cell antigen in the pathogenesis of human membranous nephropathy remains unresolved, and polycations have no apparent effect on the latter process.[21]

This concept of subepithelial immune complex formation due to cationic antigens has been extended into the clinical arena by Vogt and colleagues, who utilized antibody specific for several cationic and anionic extracellular proteins derived from nephritogenic streptococci to document glomerular localization of only the cationic antigens in glomerular immune deposits of 8 of 18 patients with early poststreptococcal glomerulonephritis.[22] This group has also pioneered several experimental studies of the role of antigen size and charge in the formation of glomerular immune complex deposits (reviewed in Ref. 23). It should be emphasized, however, that demonstration of putative antigens in glomerular deposits does not by itself provide compelling evidence that they participate in causing the disease. Proteins may be trapped nonspecifically in diseased glomeruli, particularly if a mechanism such as charge interaction exists to facilitate this process. However, the impressive array of lesions that can be readily induced by cationic antigen immunization or perfusion has provided important new insights into what types of antigens may be nephritogenic in human disease.

Studies have also been carried out of the role of antibody charge in deposit formation. Using cationic and anionic fractions of IgG antibody to both glomerular epithelial cell and GBM antigens, Madaio *et al.* found increased deposition of the cationic antibody fraction, a finding interpreted as an effect of the glomerular charge barrier on rate of antibody deposition, particularly at a subepithelial site.[24] Adler *et al.* found a similar, but less dramatic effect.[21] These findings accord with data obtained by eluting antibody from kidneys of lupus mice, which is also primarily cationic, perhaps reflecting the influence of the charge barrier, or perhaps the anionic charge of the deposited DNA antigen.[25] Thus, there appears to be an effect of charge on glomerular deposition of antibody as well as antigen.

An obvious question arising from the above observations is whether antibody rather than antigen localization on a charge basis might be the event that initiates *in situ* immune complex formation in situations where the responsible exogenous antigen is anionic and therefore not capable of electrical interaction with glomerular anionic sites—for example, serum sickness nephritis induced with native BSA or lupus nephritis involving DNA-containing immune complexes. DNA has been shown to exhibit an affinity for glomerular collagen, primarily type V, which may in part account for its localization.[26] However, the capacity of antibodies made cationic to initiate local deposit formation has been well established by Agodoa *et al.* using cationized antibodies to human serum albumin per-

fused into a rat kidney prior to administration of antigen which resulted in extensive subepithelial immune complex deposits.[27] This mechanism probably accounts for the local formation of subepithelial complexes of native BSA and anti-BSA reported following sequential perfusions with these reagents by Fleuren *et al.* in 1980,[28] a study that first demonstrated the *in situ* mechanism of subepithelial immune deposit formation involving an exogenous antigen.

Another probable new mechanism of local glomerular immune complex formation related to charge phenomena has also been recently recognized. A variety of inflammatory stimuli including intravascular immune complex formation can lead to release and glomerular binding on an electrical basis of platelet and neutrophil-derived cationic proteins, including platelet factor 4 and others.[29–31] Glomerular bound cationic proteins may then facilitate localization, or planting, of anionic antigens, resulting in enhanced local immune complex formation. Prior infusion of polycations clearly enhances glomerular immune deposits induced by injection of anionic antigen followed by antibody to it or of native BSA containing immune complexes.[31] Whether this finding is a consequence of enhanced anionic antigen localization or enhanced deposition of preformed immune complexes is not clear. However, suggestions that a similar phenomenon may occur *in vivo* derive from several studies by Cavallo *et al.* demonstrating that a loss of glomerular anionic sites appears to precede development of detectable glomerular immune deposits in murine lupus nephritis.[32]

Little has been added recently to the data reviewed by Glassock and Cohen in Volume 3 of this series regarding the role of circulating immune complex trapping in this process. Gallo, Lamm, and colleagues have shown that covalently bound immune complexes of appropriate size and charge may deposit, at least transiently, in a subepithelial distribution.[33,34] This group of investigators have continued their studies to demonstrate that cationic immune complexes can bind to heparan sulfate–proteoglycan anionic sites[35] and that this binding occurs independently of Fc receptor interactions.[36] It still seems probable that local mechanisms predominate in deposit formation at a subepithelial site.[1] Not surprisingly, the recent intensive study of mechanisms of subepithelial immune complex formation has also led to new insights into how these deposits can cause glomerular injury, as reviewed in Section 2.2.

2.1.2. Mesangial and Subendothelial Immune Deposit Formation

2.1.2.1. In Situ Immune Complex Formation. Less work was done on this important topic during the past 2 years than was done with subepithelial deposits, but similar principles presumably apply. Thus, Andres *et al.* have been able to induce glomerular injury in the rabbit by the

ingenious maneuver of treating rabbits with captopril to enhance the expression of angiotensin-converting enzyme on glomerular endothelial cells followed by injection of heterologous antibody to angiotensin-converting enzyme.[37] Again, a capping-and-shedding phenomenon similar to that described with epithelial cells was observed, resulting in antigen modulation and a relatively transient presence of deposits which are rapidly shed into the circulation.[37] The subsequent development of subepithelial deposits in this model is also of interest and may represent a relocation of immune deposits from subendothelial to subepithelial areas.

Observing that mesangial cell membranes contain an antigenic epitope recognized by antithymocyte antibody, Yamamoto and Wilson carried out similar studies with an antibody to a mesangial cell membrane antigen which produces an initial mesangiolysis followed by a mesangial proliferative glomerulonephritis that is mediated by complement activation.[38] Thus, autologous antibodies to plasma cell membranes of all three resident glomerular cells have now been shown to induce immune deposit formation and glomerulonephritis with features similar to those seen in several human renal diseases. Whether this new mechanism is operative in humans or not remains to be investigated. Antibody reactivity with endothelial cell membrane antigens has been reported in patients with lupus nephritis.[39]

2.1.2.2. Circulating Immune Complex Trapping. With regard to subendothelial and/or mesangial deposits of exogenous antigen–antibody systems, the factors that regulate the trapping of preformed immune complexes in these situations have been extensively reviewed previously, and new information is limited. Hebert and colleagues have provided more data on the importance of the erythrocyte CR1 receptor in binding large preformed immune complexes and have shown that complement depletion reduces this binding and therefore results in an accelerated rate of removal of immune complexes from the circulation.[40] These studies add another factor to those which have already been defined to influence immune complex removal and may be of particular relevance in primate systems. Although anionic charge sites have been identified in the mesangium, charge appears to play a less important role than complex size in influencing mesangial trapping of preformed complexes.[41]

The technology for measuring individual immunoglobulins and complement components in circulating immune complexes has advanced during this period,[42,43] as has the ability to analyze complement fixation by immune complexes.[44,45] However, little new information supporting the utility of immune complex assays in renal disease has emerged. Interpretation of such studies has also been complicated by recent recognition that material reactive in the Raji cell assay for immune com-

plexes may represent antibody to nuclear components of the Raji cell rather than immune complexes,[46] and more recently by evidence that immune complex reactivity measured by some solid-phase C1q assays probably represents antibody reactive with a neoantigen on bound C1q rather than soluble immune complexes.[47] The issue of the respective role of circulating immune complex trapping versus *in situ* immune complex formation in the pathogenesis of immune complex nephritis remains unresolved and probably is inherently unresolvable in human disease.[1] Experimental data to date demonstrate that preformed immune complexes can produce mesangial and subendothelial deposits but rarely produce subepithelial ones, that deposits at all these sites can also develop on a local basis, and that tissue injury in experimental systems has so far been demonstrated only with *in situ* mechanism of deposit formation.[1]

2.1.3. Anti-GBM Antibody Disease

Another example of *in situ* immune complex formation due to a fixed glomerular antigen is anti-GBM nephritis. Recent studies of immune glomerular disease have concentrated more on the mechanisms of immune complex nephritis than on the traditional model of anti-GBM, or nephrotoxic, nephritis. The major topic of research interest in this area has been the nature of Goodpasture's antigen and the ultimate development of more specific assays for anti-GBM antibody. The literature in this area is extensive, confusing, and conflicting. Wieslander *et al.* have made two helpful contributions. Using ELISA techniques, they localized reactivity of anti-GBM antibody in Goodpasture sera to the noncollagenous proteins of the GBM and also pointed out that patients with other forms of glomerulonephritis, including SLE, polyarteritis nodosa, and IgA nephropathy, may demonstrate antibody reactivity with collagenous domains in GBM.[48] Further analysis of Goodpasture's antigen obtained from collagenase digestions of GBM has revealed that anti-GBM antibody from these patients reacts primarily with a protein with a molecular weight of about 26,000 which is also present in aggregates weighing about 48,000.[49] Fish and colleagues have also reported that 10 different human anti-GBM antibodies studied in two-dimensional gel electrophoresis reacted primarily with 25–27 and 45–50 kd determinants in digests of human GBM.[50,51] Abolition of reactivity following antigen reduction suggested that some GBM antigenic determinants may be hidden or "masked."[52] Hudson and colleagues have further localized the reactive antigenic epitopes to the M2 monomer fragment (mol. wt. 32,000) of the globular domain of type IV collagen.[53] Wilson and Dixon have reported similar findings.[54] Several authors agree

that there is some heterogeneity in antigenic reactivity of anti-GBM antibody from different patients with anti-GBM nephritis.[54–57] For example, some anti-GBM antibodies react with fetal and infant kidneys, while others react only with adult glomeruli.[57]

Knowledge of the overall biochemical composition of GBM is advancing, with known components including type IV and V collagen, laminin, entactin, amyloid P component, and heparin–sulfate proteoglycans. Kerjaschki *et al.* have characterized a 140-kd protein containing 4.5% sialic acid and termed podocalyxin as the major sialoprotein and negatively charged structure of the glomerular epithelial cell.[58] Another 62-kd component of the cell membrane of epithelial and endothelial cells has also been identified and termed podendin.[59] It is noteworthy that diseases mediated by antibodies to most of these entities have not been reported in humans, and immunization with purified non-GBM antigens rarely leads to significant glomerulonephritis.[60] Kanwar has contributed an excellent scholarly review of the structural–functional relationships between these various GBM components and the pathophysiology of proteinuria.[61]

2.2. Mediation of Immune Renal Injury

In addition to advances in understanding new mechanisms by which immune complex deposits may form in glomeruli, reviewed in Section 2.1, significant advances were also made in 1984–1985 in clarifying the mechanisms by which these deposits cause glomerular and interstitial injury. Many of these observations have been reviewed elsewhere.[1,62] At least five distinct mediation pathways are now established for injury to the glomerulus.

2.2.1. Injury Induced by Antibody Alone

First, studies in the isolated perfused rat kidney (in which complement and circulating inflammatory cells are absent) have now shown that deposition of anti-GBM antibody IgG alone can cause a marked increase in glomerular protein filtration.[63] Since heavy proteinuria is not a common feature of clinical anti-GBM disease, the relevance of this observation relates primarily to documenting that structural alterations in the filtration barrier induced by interaction with antibody alone may markedly alter barrier function, a phenomenon that may occur as well with nonantibody nephrotoxins in diseases such as minimal-change nephrotoxic syndrome.

2.2.2. Injury Induced by Terminal Complement (C5b-9) Membrane Attack Complexes

A major topic of research activity recently has been the role of the complement C5b-9, or membrane attack complex, in immune renal injury.[62] Until recently the nephritogenic role of complement in renal disease was believed to be an indirect one, involving only neutrophil chemotaxis through release of C5a or neutrophil adherence via CR1 receptors. In 1980 we found that proteinuria in the passive Heymann nephritis model of membranous nephropathy (mediated by antibody binding to a glomerular epithelial cell antigen, as described earlier) could be abolished by generalized complement depletion, despite the fact that neutrophils could be shown to play no role in mediating this lesion. A similar mechanism is operative when membranous nephropathy is induced with a planted exogenous antigen. We hypothesized that this new role for complement might involve the C5b-9 portion of the complement system (reviewed in Ref. 62). Recent studies have verified this hypothesis. Thus, C6-deficient rabbits with a membranous lesion induced by repeated immunization with cationized BSA have a marked reduction in proteinuria compared to normocomplementemic controls[64]; C6 depletion abolishes proteinuria without altering antibody deposits in both the passive Heymann nephritis[65] and an exogenous antigen-induced model of membranous nephropathy in rats[66]; and C5b-9 neoantigens and terminal complement components are present by immunofluorescence in immune deposits in experimental models with complement-dependent glomerular lesions but are absent in lesions of equivalent severity that are not complement-dependent.[67,68] Others have also identified C5b-9 neoantigen deposits in the Heymann nephritis models.[69,70] Of interest, this mechanism is not confined to noninflammatory lesions such as membranous nephropathy. Thus, nephrotoxic nephritis induced by anti-GBM antibody, a lesion with inflammatory cell involvement, is markedly attenuated in C6-deficient rabbits compared to normocomplementemic controls.[71] Thus, this direct complement effect is probably of equal or greater importance than the effect of C5a-neutrophil chemotaxis in mediating immune renal injury. That the mechanism is operative in human disease as well is strongly suggested by several recent studies that demonstrate C5b-9 neoantigen deposition in a variety of immune human renal diseases, including lupus nephritis, membranous nephropathy, IgA nephropathy, anti-GBM nephritis, poststreptococcal nephritis, and others.[72,73] It is noteworthy that C5b-9 neoantigen deposition is also prominent in structural lesions that do not contain immune deposits, particularly areas of sclerosis, hyalinosis, interstitial inflammation, and vascular

degeneration.[72,73] The pathogenetic significance of these deposits is unclear. We have demonstrated that damaged kidney cells activate complement, form cell-membrane-bound C3 convertase, and assemble C5b-9 on cell surfaces.[74] Whether this secondary activation of C5b-9 by injured cells contributes to further cell injury or impedes repair is currently unknown.

Of more interest is the question of how antibody-activated assembly of the C5b-9 complex leads to glomerular injury in the absence of inflammatory cells. This question has not been answered, but clues are emerging. Most glomerular C5b-9 deposits appear to be associated with cell membranes.[62,75] Lysis of nucleated cells by C5b-9 is difficult. However, membrane insertion of sublytic quantities of C5b-9 now appears to be a potent activator of some cellular metabolic processes. Thus, Hansch and colleagues have documented increased release of arachidonic acid, PGE_2, and thromboxane from macrophages exposed to sublytic concentrations of C5b-9.[76] Lovett and colleagues have shown that glomerular mesangial cells behave similarly in response to C5b-9 and release increased quantities of an interleukin-1-like cytokine.[77] We have recently shown that C5b-9 stimulates mesangial cells to release large quantities of the reactive oxygen species, hydrogen peroxide and superoxide anion.[78] We now believe that the nephritogenic effect of C5b-9 may involve alterations in the metabolism of resident glomerular cells such that they become effector cells which lead to glomerular dysfunction and proteinuria.[62]

2.2.3. Injury Mediated by Neutrophils

A third effector mechanism is neutrophil-mediated glomerular injury. Generally, this occurs where complement activation is also involved and is presumed to involve C5a generation, although the relative importance of C5a chemotaxis versus CR1 receptor immune adherence has not been studied. Neutrophils have until recently been assumed to induce glomerular injury through release of proteases which digest normal GBM. However, evidence for this has been indirect, including the capacity of neutrophil-derived proteases to digest GBM *in vitro* and the presence of GBM fragments in the urine in neutrophil-mediated injury. However, another response of activated neutrophils is to undergo a respiratory burst and release reactive oxygen metabolites, including hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-). The capacity of these reactive oxygen species to induce tissue injury is well established in nonrenal systems. Recently, Rehan and colleagues have carried out a series of studies that establish reactive oxygen species as a major mechanism by which neutrophils damage glomeruli. Neutrophil activation

has been induced by three mechanisms: intrarenal infusion of phorbol myristate acetate,[79] cobra venom factor to activate complement and generate C5a,[80] or anti-GBM antibody.[81] In each case infusion resulted in a glomerular neutrophil infiltrate and mild proteinuria which could be abolished by neutrophil depletion. Proteinuria was also significantly inhibited by administration of catalase (which degrades H_2O_2), whereas superoxide dismutase (which converts superoxide anion to H_2O_2) was of little benefit. These results suggest that H_2O_2 is the major ROS involved in neutrophil-mediated glomerular injury. The mechanism by which H_2O_2 is nephrotoxic has now been studied by Dr. Richard Johnson in our laboratory, who has documented that perfusion of kidneys with low concentrations of H_2O_2 or neutrophil myeloperoxidase (MPO) does not cause glomerular injury. However, when MPO, a highly cationic neutrophil enzyme, is first localized in the kidney by binding to glomerular anionic sites, perfusion of H_2O_2 with a halide results in proteinuria and severe glomerular injury associated with halogenation of the GBM.[82] Thus the H_2O_2–MPO–halide system may be a major mechanism of neutrophil-induced glomerulonephritis, presumably through generation of hypohalous acids and perhaps singlet oxygen. The capacity of mesangial cells to produce ROS was mentioned earlier, and macrophages have similar properties.

2.2.4. Injury Mediated by Macrophages

A fourth effector mechanism, independent of complement, is antibody-induced glomerular injury mediated by macrophages. Holdsworth and colleagues have extended their studies of macrophages as effector cells of glomerular injury by demonstrating that glomerular injury in cell-depleted rabbits could be reconstituted with mouse peritoneal macrophages.[83] Glomerular macrophage infiltrates appear to induce resident glomerular cell proliferation in the mouse.[83] These authors also documented a dramatic effect of intravenous methylprednisolone in reducing glomerular macrophages and macrophage-mediated renal injury in two different models, whereas steroids had little effect on injury induced by neutrophils.[84,85] Baud *et al.* have presented data to suggest that macrophage adherence to glomeruli may depend on glomerular lipoxygenase products.[86] Evidence for a role for macrophages in glomerular fibrin deposition is discussed in Section 2.2.7. However, a major question raised by the growing evidence for macrophage participation in glomerular injury is whether these cells are attracted only by immune adherence mechanisms or whether they reflect participation of a sensitized T-cell process in the mediation of glomerulonephritis (see Section 2.2.5). Holdsworth *et al.* further studied this question utilizing mono-

clonal antibodies to rat T lymphocytes in a macrophage-dependent model and documented a glomerular infiltrate of helper T cells preceding the macrophage infiltrate as well as abrogation of injury utilizing cyclosporin A, which selectively depletes helper T cells.[87] These data add to a growing literature that suggests that sensitized cells, independently of antibody, may play a role in the pathogenesis of glomerulonephritis. It should be noted that recent studies of mononuclear cell participation in human glomerulonephritis utilizing monoclonal antibodies have given somewhat inconclusive results. Hooke *et al.*[88] and Ferrario *et al.*[89] found a significant increase in glomerular macrophages in several forms of proliferative glomerulonephritis but were unable to detect significant numbers of T cells in glomeruli of such patients, although significant numbers of both monocytes and T lymphocytes were found by Stachura *et al.* in idiopathic crescentic glomerulonephritis.[90]

A second area of interest related to the macrophage is the observation of Schreiner and colleagues that Ia-positive mononuclear cells are present within the mesangium of the rat. Following up on earlier observations that a portion of resident glomerular mesangial cells express Ia antigen and therefore can presumably function as antigen-presenting cells, these workers have now shown that Ia antigen expression by cells in the mesangium can be modulated in glomerular disease.[91,92] Thus, Ia expression is increased in anti-GBM nephritis and decreased early, followed by a later increase in aminonucleoside nephrosis. Some of the increased Ia expression occurs on leukocyte common antigen positive cells derived from the circulation in both models.[91,92] These observations raise the intriguing possibility of a local cell-mediated immune reaction within the mesangium contributing to glomerular disease. However, studies of Ia antigen in human renal disease have so far not revealed significant mesangial Ia reactivity.[93]

2.2.5. Injury Due to Sensitized Lymphocytes

A fifth immune mechanism of glomerular injury involves immune cells sensitized to components in or of the glomerulus independently of antibody deposition. Although long sought but never well documented, convincing evidence that this mechanism exists has now been provided by the ingenious studies of Bolton *et al.* Bolton capitalized on the fact that the antibody response in chickens can be abrogated by bursectomy to study the effect of immunization with various GBM antigen preparations in animals that could mount only a cellular immune response. Animals so immunized developed a proliferative and crescentic glomerular lesion which was independent of anti-GBM antibody production or deposition and must therefore have been cell-mediated.[94] The lesion

is characterized by an increase in resident mesangial cells, macrophages, and lymphocytes[95] and has recently been reported to be transferable with sensitized cells alone.[96] Bhan *et al.* have also extended their earlier studies of cell-mediated immunity in experimental glomerulonephritis to demonstrate that systemic interaction of sensitized cells with specific antigens may also lead to a glomerular mononuclear cell infiltrate.[97] The potential role of cell-mediated immune mechanisms in renal disease has recently been reviewed by Bolton.[98] These mechanisms may be of particular importance in the pathogenesis of idiopathic rapidly progressive glomerulonephritis and glomerulonephritis in vasculitis where glomerular immune deposits are not often seen. Additional evidence for cellular mechanisms of immune injury derived from studies of macrophage-mediated lesions is reviewed in Section 2.2.4.

2.2.6. Injury Induced by Resident Glomerular Cells

A novel concept related to effector cells in the glomerulus has emerged in the past 2 years. There is mounting evidence now that resident glomerular cells themselves may be nephritogenic. Much of this evidence has derived from the studies of Lovett, Sterzel, and colleagues, who have shown that rat glomerular mesangial cells can produce neutral proteases capable of degrading normal GBM[98,99] and an interleukin-1-like cytokine.[100,101] The latter observation has recently been confirmed by others.[102] Release of these potential inflammatory mediators, as well as of prostanoids and reactive oxygen species, by mesangial cells can be elicited by exposure to the terminal complement complex C5b-9 and by other immune stimuli within the mesangium.[77,78] The technology now exists to examine this concept in much more detail utilizing pure cultures of all three glomerular cell types. Other observations on the mesangial cell of potential importance in the study of immune renal injury include new understanding of the changes in cell phospholipid metabolism associated with increased prostaglandin production in response to vasoactive agents[103] and appreciation of the expression of Thy 1.1, an antigen on several immune cells and neural tissue, on rat mesangial cells.[104] A cytotoxic effect of certain low-molecular-weight fibrin degradation products on the mesangium has also been documented.[105]

2.2.7. Injury Due to Coagulation Mechanisms

Earlier studies of the mechanism of fibrin deposition in human crescentic glomerulonephritis by Hoyer *et al.* showed deposits of fibrin-related antigen without Factor VIII suggesting a thrombin-independent

mechanism or impaired fibrinolysis compared to clearance of Factor VIII. In a careful study of an experimental model of crescentic glomerulonephritis in rats, Silva *et al.* have confirmed the latter mechanism by showing that early fibrin deposits are associated with endothelial damage before crescent formation and that Factor VIII is present in crescents during early phases but is cleared more rapidly than fibrin, which appears to be poorly cleared from areas of crescent formation.[106] An additional observation of interest with respect to glomerular fibrin deposits in crescentic glomerulonephritis is that of Holdsworth and Tipping, who used a model of glomerulonephritis in rabbits to demonstrate that macrophage depletion reduced glomerular fibrin deposition and that macrophages grown from isolated glomeruli in these animals exhibited procoagulant activity, suggesting that macrophages may contribute to the fibrin deposits seen in several severe forms of proliferative glomerulonephritis.[107] It is of interest that enhanced circulating mononuclear cell procoagulant activity has also been noted in association with severe proliferative lesions in lupus nephritis.[108]

2.3. Interstitial Nephritis

Although the most common immune renal diseases in humans are the glomerular diseases, several forms of interstitial nephritis are probably immunologically mediated as well. It is particularly noteworthy that the major advances in applying basic cellular immunology to studies of the kidney have occurred in models of interstitial nephritis in which a cell-mediated component is much more easily demonstrable. The relevance of these observations to humans must be considered in light of the fact that they have been made largely in models of interstitial nephritis associated with antibody to tubular basement membrane (TBM), a mechanism relatively uncommon in human interstitial disease. Nonetheless, these recent studies of interstitial nephritis have resulted in a number of new and potentially important observations that may be applicable to other forms of renal disease. Neilson and colleagues have pioneered the work in this field. Studies by this group prior to 1983 are summarized in an Editorial Review.[109] In continued studies of anti-TBM interstitial nephritis in mice, this group has shown that the interstitial lesion can be transferred with immune T lymphocytes,[110] that susceptibility to development of interstitial nephritis is defined by a unique effector T-cell mechanism that recognizes renal tubular antigen only within the context of specific immune response genes,[111] and that tubular antigen-specific T-cell lines that are not themselves effector cells can induce an effector cell population in naive spleen cells *in vitro*.[112] They have also shown that the effector T-cell response producing interstitial

nephritis can be largely inhibited by adoptive transfer of suppressor T cells that are functionally restricted by immune response gene products.[113] In an elegant series of studies, this group has utilized monoclonal antibodies to rabbit TBM to isolate a chaotropic noncollagenous glycoprotein of mol. wt. 48,000 that appears to be the nephritogenic TBM antigen localized along the lateral margin of TBMs.[114] Thus, understanding of the nature of the nephritogenic TBM antigen has rapidly developed to a level almost comparable with knowledge of the GBM ection antigen reviewed earlier.

In other studies antiidiotypic immunity has been shown to inhibit the development of anti-TBM interstitial nephritis, and failure of this regulatory response has been linked to a nonspecific T-suppressor-cell system.[115] A particularly interesting recent observation is the spontaneous development of an interstitial nephritis in kdkd mice that is purely cell-mediated but develops because of the apparent absence in this strain of a suppressor T cell normally present in other mice.[116] Mampaso and Wilson have also focused on the immune mechanisms in anti-TBM nephritis in rats and have described a model characterized early by antibody-complement-neutrophil-mediated injury followed by a later mononuclear cell phase with a predominance of helper T cells.[117] Bannister and Wilson have defined the kinetics of antibody deposition in this model as well as the role of complement in mediating another model of anti-TBM nephritis.[118] These workers have also shown disease suppression with antiidiotypic antibody and disease transfer with immune cells.[119] The expanding understanding of the role of antibody idiotypes and antiidiotypic antibody in a variety of renal and other autoimmune diseases has recently been reviewed by Colvin and Olson[120] and is discussed again under pathogenesis of lupus nephritis (see Section 5.1.2.2a). Another study of some interest regarding possible immune pathogenetic mechanisms in interstitial nephritis, by Nath *et al.*, demonstrates that the interstitial nephritis that develops with glomerulosclerosis in the remnant rat kidney is accompanied by extensive peritubular interstitial deposits of C3 and C5b-9. When the dietary acid load was reduced by sodium bicarbonate supplementation, a marked reduction in interstitial complement deposition and histologic change was observed, a finding the authors attribute to a reduction in cortical ammonia production, which may facilitate alternative complement pathway activation and secondarily tubulointerstitial injury.[121] These studies of the immunologic mechanisms of tubulointerstitial nephritis are at the forefront of applying the techniques of modern immunology to the study of immune renal disease and can hopefully be ultimately extended to the glomerulus, where definition of cellular nephritogenic mechanisms has been considerably more difficult.

3. Cinical Aspects of Immune Renal Disease

3.1. Introduction

Not surprisingly, few of the experimental observations reviewed earlier have yet been translated into clinical practice, although the rapidity with which understanding of immune mechanisms of renal disease is advancing encourages optimism for the future. Unfortunately, when patients present with signs and symptoms of renal disease, the processes that initiated these diseases are often well established. Thus, the major challenge in the clinical arena remains establishing an accurate diagnosis before irreversible structural changes have occurred and identifying treatment modalities that are effective in reducing further damage when applied sufficiently early in the course of the disease. The major factors that complicate interpretation of the literature on this subject are the heterogeneity of the recognized glomerular diseases, some of which almost certainly include several disease entities, variables such as immunogenetic factors that cannot be stratified for in designing therapeutic trials, and variations among individual patients in the way that a disease is expressed or a given dose of a particular drug is handled.

Perhaps the major ongoing advance in this area is increased appreciation of the immunogenetic factors that influence disease severity and prognosis in a number of glomerular diseases.[122] Knowledge of these factors is sufficiently advanced in several diseases to allow them to be considered clinically in determining prognosis and intensity of therapy, and certainly has reached the point where they should be controlled for in treatment trials.

3.2. Diseases That Present as Acute Glomerulonephritis

3.2.1. Postinfectious Nephritis

The topic of epidemic poststreptococcal nephritis has been updated by Rodriguez-Iturbe.[123] The sporadic form of this disease has become relatively uncommon now in developed countries, although epidemic outbreaks still occur elsewhere.[123] Most investigation is centered on the continued search for the nephritogenic streptococcal antigen. Lange *et al.* believe this to be "endostreptosin," an anionic cytoplasmic protein antigen of mol. wt. 45,000 derived from Group A streptococci. Antibody to endostreptosin stains material deposited in glomeruli of patients with early streptococcal nephritis, although it is not clear that this material is present in the characteristic subepithelial "humps" seen in this disease.

Recent studies document that elevations of antibody titers to endostreptosin appear more specific for streptococcal nephritis than antibodies to other streptococcal antigens.[124] In contrast, Friedman *et al.* have detected streptococcal antigens in circulating immune complexes from patients with poststreptococcal nephritis and have identified a unique antigen in such patients that appears to resemble an extracellular protein of mol. wt. 49,000 previously demonstrated to be present in glomerular deposits[125] and probably similar to endostreptosin. The studies of Vogt and colleagues[22] identifying a cationic extracellular protein in biopsies of patients with poststreptococcal nephritis were mentioned earlier under mechanisms of subepithelial immune deposit formation (Section 2.1.1.2). Finally, Mosquera and Rodriguez-Iturbe have reviewed the evidence that neuraminidase produced from nephritogenic streptococci may lead to loss of sialic acid from autologous IgG, thereby rendering the IgG cationic and immunogenic.[126] Thus, most of the studies of how glomerular immune deposits form in poststreptococcal nephritis are currently influenced by the experimental data reviewed earlier suggesting that subepithelial deposits induced by exogenous antigens probably form *in situ* and may involve participation of relatively cationic antigens.

The progression of poststreptococcal nephritis from a diffuse proliferative to a crescenteric lesion with renal failure is documented in one case associated with an infection at a renal biopsy site, emphasizing again the fact that the poststreptococcal nephritis can cause rapidly progressive glomerulonephritis (RPGN) and is not always reversible.[127]

Other studies of postinfectious glomerulonephritis include a report of eight more patients with diffuse proliferative glomerulonephritis associated with remote visceral (nonstreptococcal) infections, emphasizing the prominence of glomerular monocytes as a possible (but nonspecific) clue to this diagnosis.[128] Also of note is the frequency of nephrotic syndrome (50%) and resemblance of this lesion histologically to membranoproliferative glomerulonephritis (MPGN) in other patients.[128] In another case, crescentic RPGN was reported in a renal allograft following a mycotic aneurysm of a coronary artery.[129] In the glomerulonephritis associated with many remote visceral infections, immunofluorescence is negative, raising questions regarding the mechanisms by which the infections produce severe glomerular disease.[128,129]

Shunt nephritis is reviewed in a report of serial biopsies in a patient after shunt removal that documents loss of active disease histologically within 5 months.[130] Two papers review the renal complications of bacterial endocarditis, emphasizing the spectrum of glomerular lesions seen from focal necrotizing nephritis to diffuse proliferative nephritis, the latter apparently more common with right-sided lesions; the predomi-

nance of *Staphylococcus aureus* as the infecting organism; and the overlap with the glomerular lesions associated with drug abuse.[131,132] It is noteworthy that with passage of time, more patients with the nephropathy of drug abuse appear to be developing amyloidosis.[133] Presumably, this is a consequence of repeated exposures to bacterial infection.

A case of RPGN associated with syphilis, in which identification of treponemal antigen and antibody to it in glomeruli was achieved, represents a rare case of postinfectious RPGN in which an etiologic agent has apparently been identified.[134] A study from Cameroon implicates a filarial parasite antigen from *Onchocerca volvulus* in the pathogenesis of some immune complex-mediated cases of nephropathy in the tropics.[135] Malarial antigen has now been shown to appear in glomeruli independently of antibody, localizing by mechanisms that have not been defined.[136] Studies of urinary schistosomiasis from the Sudan suggest that proteinuria in this disorder may be more related to penetration of ova through the bladder mucosa than to renal disease, although renal biopsies were not performed.[137,138]

Particular attention has focused on renal biopsies in patients with AIDS and the question of whether a specific viral nephropathy accompanies this disease. Up to 10% of AIDS patients have been reported to have nephrotic syndrome, and 50% to be proteinuric.[139–141] Renal involvement appears to confer a worse prognosis but is associated with a variety of glomerular, tubular, and interstitial lesions presumably related to immune abnormalities, infectious complications, drug exposure, and other factors.[139–141] The rather high incidence of proteinuria and of either minimal-change nephrotic syndrome or focal glomerulosclerosis suggests that some AIDS-related immune abnormality may initiate this lesion.[139–142] Similar lesions occur with intravenous drug abuse,[140] and the rapid loss of renal function seen in this subset of patients with nephropathy and AIDS also resembles the clinical course of patients with the nephropathy of drug abuse. However, although focal sclerosis is the most common lesion seen, no renal lesion specific for AIDS has yet been identified. The subject of renal disease in AIDS has been well reviewed recently by Seek *et al.*[143]

The exact relationship between various infectious agents associated with glomerulonephritis and the pathogenesis of the glomerular disease remains unclear and is almost certainly more complex than the usual analogy drawn with chronic BSA–serum sickness in rabbits. This point is underscored by a careful study of 12 patients with typical serum sickness that failed to document glomerulonephritis in any.[144] To my knowledge, glomerulonephritis induced by immunization with a foreign serum protein alone has never been reported in humans.

3.2.2. IgA Nephropathy

More was written about IgA nephropathy during 1984–1985 than any other single glomerular disease, and important new information has emerged regarding both the pathogenesis and clinical features of this lesion. Iga nephropathy is the most common primary glomerular disease seen in Asia, Australia, and parts of Europe.[145] Turning first to pathogenesis, the mesangial localization of IgA-containing immune complexes has now been achieved experimentally by a variety of maneuvers,[146–148] including passive administration of IgA-containing immune complexes, active immunization with dextran, oral immunization with protein antigens and mercuric chloride,[149] induction of hepatobiliary disease, bile duct ligation, and spontaneously with age in ddY mice.[150] It is now known that the increased serum polymeric IgA levels often seen in IgA nephropathy are associated with abnormalities of immune regulation, including increased IgA production by peripheral blood lymphocytes,[151,152] decreased IgA suppressor cell activity,[153] and increased IgA helper cell activity, abnormalities also seen in many healthy relatives of IgA patients.[154] Mesangial IgA deposits are largely polymeric (mucosal in origin), anionic,[155] and contain more IgA_1 than IgA_2,[156] although some IgA_1 epitopes may be "masked" by the configuration of the antibody deposited at subepithelial or intramembranous sites.[157] Other components of the deposits include C4 binding protein, beta 1H, and C3d, suggesting classical complement pathway activation,[158,159] and perhaps some herpes viral antigens.[160] IgA is presumed to be present in immune complex form, although the antigenic specificity of the IgA antibody remains unestablished, except that it is not reactive with renal tissue, is reactive with an antigen present in mesangial deposits of some other patients with IgA nephropathy, and may have reactivity with an antigen in tonsillar tissue of patients with IgA nephropathy.[161] The possibility of increased glomerular deposition of circulating IgA complexes is favored by the demonstration of increased circulating levels of IgA complexes in patients with IgA nephropathy[162] and documentation of defective splenic clearance function[163] and of impaired Fc and C3b receptor function,[163,164] as well as by the finding of electron-dense deposits in some extraglomerular vascular structures.[165] A respiratory as well as gastrointestinal site of mucosal antigen challenge is favored by the finding of frequent glomerular IgA deposits in patients with pulmonary disease,[166,167] as well as the clinical association with respiratory and gastrointestinal viral infections. Finally, there appear to be important genetic factors that influence disease susceptibility, as evidenced by the low incidence in blacks,[168] high incidence in several Asian populations and

American Indians,[169] association with C4 null alleles,[170] and reports of familial cases of IgA nephropathy.[171] Attempts to document an association between IgA nephropathy and HLA markers have given different and conflicting results in different patient populations, although several recent reports suggest an increased relative risk in patients with HLA DR4.[171,172]

A number of excellent clinical reviews of IgA nephropathy have appeared recently.[173–188] All support the usual concept of the disease as one of young people aged 15–30, with a predominance of males and a clinical picture dominated by hematuria, often recurrent, following viral upper respiratory and gastrointestinal infections. With time, a somewhat poorer prognosis is being appreciated, with progression apparent in about 50% of patients in some series.[176,180] Prognostic factors are emerging more clearly now and include hypertension, proteinuria in excess of 1.0 g/day, initial impairment in GFR, IgA deposits on the capillary wall, sclerotic glomeruli, crescents, and microscopic hematuria in the absence of macroscopic hematuria.[173,174,180,182,185,188–191] The evidence that macroscopic hematuria may be a good prognostic sign is curious, since this clinical event is well associated with crescent formation.[192] However, decreased renal function during macroscopic hematuria has been attributed by others to reversible tubular obstruction and necrosis.[193]

The spectrum of disease attributed to an IgA mechanism has also expanded well beyond focal nephritis with mesangial IgA deposits. A number of cases of RPGN with crescents have been reported, usually associated with extensive, lupuslike deposits of IgA on both the subepithelial and subendothelial surfaces of the capillary wall.[173,174,179,184,188,194] Although steroid-resistant nephrotic-range proteinuria is common in these patients with crescentic disease, another clinical entity has now emerged of steroid-responsive nephrotic syndrome associated with mesangial IgA deposits and normal renal function.[183,189,195–197] In contrast to patients with nephrotic syndrome and extensive capillary wall deposits of IgA, who do poorly, these patients usually do not have hematuria, have deposits confined to the mesangium, and appear to have minimal-change nephrotic syndrome with some, probably incidental, deposits of IgA trapped in the mesangium. They usually respond well to steroids, although relapses may occur.[189] It seems unlikely that the IgA deposits are pathogenic in these cases, and they should probably be viewed as cases of minimal-change nephrotic syndrome rather than as part of the spectrum of IgA disease.[197]

Despite its frequency and intensive study, no consensus has emerged that any form of therapy is useful in most cases of IgA nephropathy except for control of blood pressure.[176] A second report of a controlled trial of phenytoin, 300 mg/day, in 74 patients from Spain confirmed a

reduction in levels of IgA and IgA immune complexes as well as a reduction in hematuria in treated patients, but did not demonstrate a beneficial effect on the renal lesion.[198] Anecdotes of patients with IgA-induced RPGN responding to both pulse steroids[184,187] and plasma exchange[194,199,200] have appeared. Dipyridamole, 300 mg/day, has been reported by Japanese workers to stabilize renal function.[187] Also, serum from IgA patients treated with danazol, a heterocyclic steroid compound that can raise complement levels, has been shown to have enhanced capacity to solubilize deposited IgA immune complexes[201] and reported to reduce proteinuria in six of nine patients treated.[187] Other agents undergoing treatment trials in IgA nephropathy include oral administration of broad-spectrum antibiotics to reduce the frequency of gastrointestinal infections, and eicosopentanoic acid (fish oil) to reduce glomerular production of vasoconstrictor prostaglandins and thromboxane. At the present time there is no treatment of established benefit for the patient with slowly progressive, noncrescentic IgA nephropathy, other than blood pressure control.

It is widely accepted that IgA nephropathy is a monosymptomatic form of Henoch–Schönlein purpura, a concept encouraged by the extensive overlap in both clinical and immunologic findings as well as renal pathology in these two diseases.[146,147,176,202] Immunofluorescence deposits of IgA may be found in skin biopsies of patients with IgA nephropathy as they are in Henoch–Schönlein purpura, but this finding is rather uncommon.[203] It is therefore likely that most of the pathogenetic observations reviewed above in IgA nephropathy apply as well to Henoch–Schönlein purpura.[146]

3.2.3. Rapidly Progressive Glomerulonephritis

3.2.3.1. Introduction. I have emphasized the clinical utility of separating patients with RPGN into categories based on the underlying immunopathogenetic mechanisms.[204,205] Most patients with crescentic nephritis associated with immune complex deposits will have an underlying postinfectious or collagen-vascular disease. Patients with linear deposits of IgG have anti-GBM nephritis with or without pulmonary hemorrhage, and a substantial majority (about 80%) of the non-anti-GBM patients have no significant immunoglobulin deposits, or idiopathic RPGN. RPGN in postinfectious and collagen vascular diseases is reviewed in Sections 3.2.1 and 5.

3.2.3.2. Anti-GBM Nephritis. Advances in defining the nature of Goodpasture's antigen are reviewed in Section 2.1.3. Apparently, nonspecific linear staining can be enhanced by neutralizing capillary wall anionic sites.[206] The etiology of anti-GBM antibody production remains

obscure, although attention still centers on exposure to various pulmonary toxins, including hydrocarbons.[207,208] Whether these exposures are immunogenic or simply permissive in facilitating pulmonary localization of anti-GBM antibody produced in response to other antigenic stimuli remains unclear. An ELISA assay for anti-GBM antibody has recently been described,[209] but the radioimmunoassay performed by Dr. Curtis Wilson in La Jolla remains the only readily available anti-GBM assay of proven reliability.[54]

The importance of pulmonary toxins in development of alveolar hemorrhage is graphically illustrated by Donaghy and Rees, who reported pulmonary hemorrhage in 37 of 37 smokers with anti-GBM disease compared to only 2 of 10 patients with anti-GBM antibody who had no smoking history.[210] Rees *et al.* have also clearly identified genetic factors as major predictors of the risk and severity of anti-GBM nephritis. Patients with HLA-DR2 have a 36-fold increased risk, with HLA-B7 a fivefold increased risk, and the presence of HLA-DR2 and B7 together confers a significantly worse prognosis.[211] These immunogenetic factors clearly need to be considered in interpreting the results of subsequent treatment trials.

The conviction that intensive plasma exchange combined with steroids and cyclophosphamide is of therapeutic benefit in anti-GBM disease persists based on a more rapid reduction in anti-GBM antibody levels (about 2 months compared to 11 untreated) and response rates of about 70% in patients with Goodpasture's syndrome and 30% in older patients.[212,213] However, over 70% of patients with this disease require dialysis when they present, and the response rate in this group approaches zero.[212,213] Similar results with plasmapheresis have been reported from the United States.[214] A study of 17 patients found the major indicators of a poor prognosis to be the presence of more than 50% crescents and a presenting serum creatinine exceeding 6 mg/dl.[214] This study documents a more rapid disappearance of anti-GBM antibody in the group treated with plasma exchange and suggests a beneficial clinical effect, although the study is inconclusive in that regard owing to small numbers of patients and more severe histologic changes in the group that received immunosuppression alone.[214] Walker *et al.* reported somewhat more encouraging results, with 5 of 11 patients who were oliguric or requiring dialysis improving and three recovering renal function to a serum creatinine of 3 mg/dl or less.[215] Several of these patients received antiplatelet agents and anticoagulants as well.[215] All these studies encourage continued use of plasma exchange with immunosuppression as the treatment of choice for early anti-GBM nephritis and emphasize the importance of early diagnosis. Unfortunately, a truly controlled study of any treatment in this disease will probably never be done owing to

the limited number of patients available for study. Although the disease is usually self-limited, rare examples of recurrences years later have been reported.[216] Anti-GBM nephritis can occur *de novo* in renal allografts,[217] particularly in patients with Alport's syndrome, whose glomeruli appear to lack a nephritogenic GBM antigen.[218] Usually anti-GBM antibody deposition does not lead to graft failure.[217,218] Leatherman *et al.* have written an excellent review of the spectrum of diseases that may produce alveolar hemorrhage as a primary manifestation of disease, including many with renal involvement.[219] They emphasize that not all combinations of glomerulonephritis with pulmonary hemorrhage represent Goodpasture's syndrome.

3.2.3.3. Idiopathic Rapidly Progressive Glomerulonephritis. Most authors now accept this entity of RPGN without immune deposits as the most common underlying lesion in patients with RPGN of no established etiology. The pathogenesis of the disease is unknown. The evidence reviewed earlier for cell-mediated mechanisms of glomerular injury, as well as the prominence of glomerular monocytes in these patients,[90,220] may provide a clue to this process. The fact that vague extrarenal signs and symptoms are common and that a similar glomerular lesion may appear in various forms of renal vasculitis encourages speculation that idiopathic RPGN is a form of vasculitis confined to the glomerular capillaries (see Section 5.1).[221] The disease seems to occur with greater frequency in the elderly[222,223] and has recently been reported in association with penicillamine therapy in rheumatoid arthritis,[224] a phenylbutazone-induced vasculitis,[225] and legionnaires' disease,[226] as well as in several cases of monoclonal gammopathy, where treatment of the hematologic disorder may improve renal function.[227,228] An increased incidence of hydrocarbon solvent exposure has also been noted in these patients with primary proliferative glomerulonephritis.[229] Biawa and colleagues have also reported a 20% incidence of nonrenal carcinomas in patients with idiopathic RPGN over age 40.[230] Idiopathic RPGN can occur in childhood as well.[231] In children, a nonstreptococcal etiology is a poor prognostic factor, and over 50% of patients with crescents in 50% of glomeruli progressed to end-stage renal disease despite treatment.[231] The clinical behavior and response to therapy of idiopathic RPGN with and without immune complex deposits appears very similar.[232–234] In the immune complex type, an increased relative risk (15-fold) has been reported for patients carrying the BfF properdin factor phenotype in addition to HLA DR2 and the D-related antigen MT3, again suggesting the importance of immunogenetic factors in disease expression.[235]

Most attention in the area of RPGN has focused on therapy. Bolton continues to report the efficacy of methylprednisolone pulse therapy in idiopathic RPGN, describing improvement (more than 30% decrease in

serume creatinine or discontinuation of hemodialysis) in 18 of 21 patients, with 11 of 12 requiring dialysis able to discontinue it.[233] Bolton reports similar success rates in patients with over 50% crescents and with oliguria.[233] Moreover, 85% of responding patients remain off dialysis after a mean follow-up of 32 months.[233] Similar results have been reported by others.[236] While these results are clearly superior to historical controls treated with oral steroids and immunosuppressive agents, they have not been derived from appropriate controlled prospective studies and must therefore be regarded as anecdotal.

Roughly comparable (and equally anecdotal) results have been reported in non-anti-GBM antibody-mediated RPGN treated with plasma exchange (plus steroids and immunosuppression).[213,234] Response rates of over 70%, including 13 of 19 oliguric patients and seven of nine azotemic patients, have been reported by the group at Hammersmith in England.[213,234] These data have been well summarized by Balow *et al.*[232] and by Glassock *et al.*[236,237] The group treated with plasma exchange was heterogeneous and included patients with Wegener's and other forms of small-vessel vasculitis, making conclusions regarding the efficacy of plasma exchange in idiopathic RPGN alone difficult. However, there is no compelling evidence that plasma exchange in non-anti-GBM RPGN is more efficacious than steroid pulse therapy, and both the cost and complication rate of plasma exchange seem higher.[233] Most authors currently regard plasma exchange as the treatment of choice for anti-GBM nephritis and steroid pulse therapy as the treatment of choice for idiopathic RPGN.[204,205,236,237] However, neither of these treatment modalities have been shown to be effective when major prognostic factors, such as severity of histologic disease, renal function, and possible immunogenetic variables, have been adequately controlled for.

4. Diseases That Commonly Present as Nephrotic Syndrome

4.1. Nephrotic Syndrome—Physiology and Consequences

The mechanisms of altered glomerular permeability to protein differ in different glomerular diseases—for example, loss of the glomerular charge barrier in minimal-change nephrotic syndrome (MCNS)[238] and development of nonselective pores in the glomerular capillary wall in membranous nephropathy.[239] However, the consequences of protein loss are similar in all diseases—hypoalbuminemia, salt and water retention, hyperlipidemia, and lipiduria, often accompanied by coagulation problems and other metabolic disorders. Several studies have examined

the relationship between the renin–angiotensin system, plasma volume, and sodium retention in the nephrotic syndrome and generally confirmed previous findings that patients with MCNS tend to have elevated plasma renin and aldosterone,[240–243] retain sodium,[244] and have variable blood volumes, often despite low serum albumin levels.[241–243] The correlation between serum albumin levels, plasma volume, and sodium retention has generally been poor.[240–243] Other glomerular lesions have less tendency to activate the renin–angiotensin system and usually demonstrate less sodium retention.[240–243] Increased urinary kallikrein excretion has also been reported consistently in the nephrotic syndrome and may reflect intrarenal hemodynamic or hormonal factors.[245] Dorhout Mees *et al.* have reviewed this confusing and conflicting literature and concluded that sodium retention in patients with nephrotic syndrome secondary to reduced colloid oncotic pressure and consequent hypovolemia is rare, and sodium retention more likely reflects a primary renal event, probably glomerular dysfunction manifested by a reduced Kf, filtration fraction, and ability to excrete sodium.[246] The latter mechanism has been shown to be operative in acute glomerulonephritis.[246] Strauss *et al.* reached similar conclusions regarding an intrarenal defect in discussing the treatment of nephrotic edema.[247] Support for this idea is provided by a study of MCNS that correlates the decrease in GFR with reduction in total length of the glomerular epithelial cell slit pores as a result of foot process fusion, again suggesting that the reduced GFR may reflect glomerular dysfunction rather than a decrease in intravascular volume.[248] Elevated levels of ADH do appear to correlate with reduced plasma volume in the nephrotic syndrome and may contribute to water retention in such patients.[249] Studying rats with membranous nephropathy, Kaysen *et al.* showed that increased albumin catabolism played little role in albumin homeostasis in well-fed animals in which albuminuria was determined primarily by dietary protein intake.[250]

The mechanism of hyperlipidemia was studied in 20 nephrotic patients by Appel *et al.*, who found that increased cholesterol levels were inversely related to plasma albumin and oncotic pressure but not to viscosity, and that high-density lipoprotein cholesterol was normal or low, suggesting that hypoalbuminemia is the major stimulus to hepatic lipoprotein synthesis.[251] With regard to coagulation abnormalities in the nephrotic syndrome, increased urinary excretion and decreased plasma activity of prothrombin (Factor II) were measured in nephrotic patients.[252] Levels of fibrinopeptide A (produced by fibrinogen cleavage by thrombin) and a product of fibrinogen degradation by plasmin as indices of intravascular coagulation and fibrinolysis are elevated in the nephrotic syndrome.[253] Nephrotic plasma was shown to stimulate arachidonic acid metabolism by platelets, a hyperactivity that may contribute

to aggregation and thrombosis.[254] The latter two findings could contribute to the hypercoagulability and thrombotic complications commonly seen in nephrotic patients. The pathophysiology of nephrotic hypercoagulation has been reviewed in depth by Llach, including evidence for reduced zymogen factors, increased levels of Factors V, VIII, and fibrinogen, decreased antithrombin III and antiplasmin, increased levels and aggregation of platelets, and increased levels of β-thromboglobulin.[255] The clinical spectrum of renal vein thrombosis and other thrombotic complications of the nephrotic syndrome are also discussed. Alon and Chan have reviewed studies of calcium metabolism in the nephrotic syndrome indicating that hypocalcemia results not only from reduced albumin levels, but also from a decrease in gastrointestinal calcium absorption due to losses of vitamin D metabolites 25 $(OH)D_3$ and $1,25(OH)_2D_3$ in the urine.[256] Hypocalcemia may lead to elevation in serum parathyroid hormone levels and bone disease in some patients.

4.2. Minimal-Change Nephrotic Syndrome

The major focus of research in this disease continues to be on pathogenesis, which, unfortunately, remains entirely obscure despite the abundance of patients for study and improved understanding of factors that regulate glomerular permselectivity. It is still believed that the disease results from a generalized loss of negative charges on the glomerular capillary wall, a phenomenon that may be demonstrable in extrarenal cell membranes as well.[238] The concept that the disease is an immunologic one involving a disorder of T-cell function (Shalhoub hypothesis) persists, supported by documentation of a variety of cellular immune defects in these patients, including impaired delayed hypersensitivity reactions to standard skin tests,[257] depressed graft-versus-host reactions,[258] and reduced T-colony-forming capacity and T-colony-stimulating factor release.[259] Lymphocyte response to mitogens is reduced in most patients with the nephrotic syndrome including MCNS,[260] and peripheral T lymphocytes from patients with MCNS release a vascular permeability factor *in vitro* which is inhibited by plasma from these same patients.[261] Nagata *et al.* reported some increase in glomerular T cells and monocytes reactive with several monoclonal antibodies in MCNS, although such cells were not at all numerous.[262] Unclear from all of these studies, as well as previous ones in the literature, is what role, if any, these various abnormalities in immune cell function play in the pathogenesis of the disease, as well as whether they are primary phenomena or secondary to the nephrotic syndrome. Case reports of MCNS induced by hypersensitivity reactions to nonsteroidal antiinflammatory drugs continue to appear,[263,264] usually associated with interstitial ne-

phritis. Piroxicam has produced a similar lesion,[265] as has therapy with recombinant leukocyte A interferon.[266,267] Along with other disorders known to be associated with MCNS, such as Hodgkin's disease and perhaps AIDS, these associations are providing clues to the pathogenesis of MCNS which have not yet been recognized. In Japanese adults HLA DQw3 appears to confer increased susceptibility in MCNS.[268]

There have been relatively few significant contributions to the clinical literature on MCNS except those related to therapy. The occurrence of MCNS in AIDS is commented on in Section 3.2.1, under viral etiologies of glomerular disease, and two cases of development of membranous nephropathy late in the course of MCNS have been reported.[269] Recurrence of biopsy-proven and steroid-responsive MCNS 4–25 years after development of remission in childhood is described in 16 patients by Pru and colleagues.[270] Recurrences were precipitated in some patients by upper-respiratory-tract infections and in others by pregnancy.[270] MCNS appears to be considerably more frequent in Asian children.[271] Tejani has analyzed the morphologic changes in repeat biopsies of 48 children with steroid-sensitive MCNS and frequent relapses; he documented progression to focal sclerosis in 45% and IgM nephropathy (see Section 4.3) in 27%, with persistent proteinuria and/or renal functional deterioration in 65% of all patients.[272] Based on this and several other similar studies published in the past, as well as on evidence that there is a diffuse loss of the glomerular charge barrier in focal glomerular sclerosis as well as in MCNS,[238] the author believes that the entities of MCNS, nephrotic syndrome with mesangial cell proliferation (mesangial proliferative glomerulonephritis) or IgM deposits (IgM nephropathy) (see Section 4.3), and focal glomerulosclerosis (FGS) (see Section 4.4) probably represent points on a spectrum of disease severity in which a common underlying (but undefined) mechanism leads to a loss of glomerular capillary wall charge resulting in increased glomerular permeability. The decrease may be relatively "mild" (steroid responsive or MCNS), more severe accompanied by mesangial changes and increased resistance to steroids (mesangial proliferative glomerulonephritis, IgM nephropathy), or quite severe with uniform steroid resistance and progressive FGS. Others prefer to view these variants of idiopathic nephrotic syndrome as separate disease entities.[273]

4.3. Mesangial Proliferative Glomerulonephritis and IgM Nephropathy

The presence of both mesangial hypercellularity and mesangial IgM deposits in patients with clinical and morphologic features otherwise similar to MCNS has been associated with reduced steroid responsive-

ness, a tendency to develop progressive glomerular sclerosis, and perhaps an increased rate of recurrence of nephrotic syndrome in renal allografts.[274] Two studies have compared patients with mesangial IgM deposits with or without focal mesangial hypercellularity to patients with pure MCNS without mesangial deposits and failed to demonstrate differences in clinical presentation, response to treatment, or outcome.[275,276] Ji-Yun *et al.* confirmed a reduced incidence of complete steroid responsiveness in patients with idiopathic nephrotic syndrome and mesangial proliferation, but failed to define any clinical or morphologic features associated with IgM deposits that would suggest that they are pathologic or markers of a separate disease entity.[277] The latter authors review their own extensive work on increased mesangial trapping of macromolecules in nephrotic glomeruli as a possible explanation for the increased incidence of mesangial IgM deposits in patients with the nephrotic syndrome compared to normals. Progression of MCNS with mesangial proliferation and IgM deposits to focal sclerosis is documented in two studies and reviewed in these papers.[278,279] Thus, the current literature tends to support the usefulness of both diffuse mesangial cell proliferation and perhaps IgM deposits as markers of disease more likely to resist steroid therapy and progress to renal failure, but does not provide compelling reasons to view these patients as having separate disease entities. A lesion similar to IgM nephropathy, but characterized by C1q deposits, mesangial proliferation, and steroid-resistant nephrotic syndrome, has been described by Jennette and Hipp, and probably represents another variant in this same disease spectrum.[280]

4.4. Focal Glomerulosclerosis

Perhaps the most significant development in this area is the mounting evidence developed by Brenner and colleagues that the focal sclerotic lesion is (1) hemodynamically mediated by increased intraglomerular pressures, and (2) a common lesion underlying progressive renal disease of multiple etiologies (reviewed in Ref. 281). It is beyond the scope of this chapter to review this concept in detail, except to say that similar mechanisms may be operative in development of the focal sclerotic lesions that appear in patients with steroid-resistant MCNS. Two studies of the morphology of FGS are of interest. Striker *et al.* note that the composition of sclerotic material is similar to that of normal mesangial matrix, suggesting that it derives from mesangial overproduction or impaired degradation of matrix components.[282] Similar conclusions have been reached in studying matrix proteins in sclerotic lesions of diabetes and light-chain nephropathy.[283] However, synechial lesions contain interstitial type III collagen associated with disruptions in Bowman's cap-

sule, suggesting that some interstitial component may also contribute to these lesions.[282] Schwartz and Lewis have characterized a cellular lesion overlying sclerotic areas (scars) marked by epithelial cell proliferation.[284] This lesion is seen in patients with more severe nephrotic syndrome and a shorter interval from onset of disease to renal biopsy. While the cellular events that lead to sclerosis, and the mechanisms that trigger these events in the nephrotic syndrome, remain to be elucidated, progress is clearly being made in this area. The location of sclerotic lesions has also been shown to have prognostic value. Ten of sixteen patients with sclerosis in a hilar distribution progressed to renal failure, whereas none of 11 patients with more peripheral lesions did so.[285] In studies of one patient with recurrent nephrotic syndrome and FGS in a transplant, serum infused into the renal artery of a rat caused a mild, but significant increase in urinary albumin excretion, while serum from other nephrotic patients did not do this, suggesting a possible humoral mediator of altered glomerular permeability in this disease.[286] Recurrence of the nephrotic syndrome in patients with FGS undergoing renal transplantation is still being reported[287] and appears to develop in about 20% of all patients. Significant risk factors include young age (less than 6 years), rapid progression to renal failure (less than 3 years), presence of diffuse mesangial hypercellularity in the original kidney biopsy, and a closely matched living-related-donor kidney.[273] With all these factors present, recurrence rates may approach 80%. Recurrence in one allograft does not necessarily predict recurrence in a second one.[288]

4.5. Treatment of MCNS–FGS

Steroids remain the mainstay of therapy for this group of diseases; optimal doses and schedules are reviewed in previous editions of *Contemporary Nephrology*. It should be noted that up to 23% of relapses in frequently relapsing patients and 10% in steroid-dependent patients may remit spontaneously within 10 days, and some delay in restarting steroid therapy may be warranted in this group of patients who are so prone to steroid toxicity.[289] The pharmacokinetics of prednisone in nephrotic patients has been examined and shown to differ between nephrotics and controls, with higher free and lower total steroid concentrations for a given dose in the nephrotics.[290] A controlled study in 89 patients comparing treatment with three intravenous methylprednisolone pulses followed by low-dose (0.5 mg/kg) prednisone for 6 months compared to high-dose prednisone (1 mg/kg) for 4 weeks showed that the pulse–low-dose prednisone group responded sooner but also relapsed earlier than controls, but apparently had significantly fewer steroid side effects.[291]

More attention has been directed at the use of cytotoxic drugs or

other agents in patients with steroid-resistant nephrotic syndrome or steroid toxicity. Cyclophosphamide is generally regarded as being beneficial only to prolong remissions and reduce steroid toxicity in steroid-responsive patients. For example, Tejani *et al.* report inducing remissions with cyclophosphamide in frequently relaping steroid-sensitive nephrotic children and show that this occurs in 100% of children with pure MCNS on biopsy, about 60% of patients with mesangial IgM deposits, but only 1 in 15 patients with histologic evidence of FGS.[292] Another study from India in children reported similar results and concluded that an 8-week course of cyclophosphamide (2 mg/kg per day) was effective in inducing prolonged remission in about 70% of steroid-dependent or frequently relapsing but steroid-sensitive patients, particularly if they were over 8 years of age.[293] However, Geary *et al.* report that 12 of 20 steroid-resistant children with FGS had a complete or partial remission in response to cyclophosphamide (2.5 mg/kg for 6 weeks or more), and only one progressed to end-stage renal disease, whereas seven of eight nephrotics resistant to cyclophosphamide progressed to end-stage renal disease.[294] If confirmed, these results would support a more vigorous approach to treating the steroid-resistant patient with MCNS than is usually taken at the present time. Feehally *et al.* document that cyclophosphamide-induced abnormalities in cellular immunity in MCNS resolve within 6–12 months.[295] Steinberg points out that cyclophosphamide used for less than 3 months has minimal long-term toxicity and notes several alternatives to daily oral cyclophosphamide administration that may have less toxicity (see Treatment of Lupus Nephritis, Section 5.1.2.2c).[296] However, there is currently no published experience with intravenous cyclophosphamide regimens in idiopathic nephrotic syndrome.

Nitrogen mustard given as two 4-day courses 2–4 weeks apart in frequently relapsing children decreased the number of relapses from 2.76 to 0.88 per year and induced complete remission in 10 of 17 partial responders but in no nonresponders.[297] However, this drug is quite toxic and did not produce lasting remissions. Two publications from the Mayo Clinic suggest that meclofenamate, a nonsteroidal antiinflammatory agent, may be of use in some steroid-resistant patients with FGS. One patient with recurrent nephrotic syndrome and FGS in a renal allograft was treated with meclofenamate (300 mg/day) and achieved an 80% reduction in urine protein excretion with relatively stable renal function.[298] Proteinuria returned when the drug was discontinued. This led to a prospective study in 16 patients with steroid-resistant FGS and 12 with membranous nephropathy. Ten of seventeen FGS patients had a reduction in proteinuria of 40% or more with minimal long-term effects on GFR.[299] Since progression in FGS is clearly related to the level of

proteinuria, this effect may predict a capacity of this drug to preserve renal function. However, about a third of responders had to discontinue the drug because of significant side effects. Nevertheless, the results suggest that this approach warrants further study in steroid-resistant, severely nephrotic patients. The mechanism of this effect is unclear, although nonsteroidal antiinflammatory agents alter glomerular hemodynamics, decrease GFR, probably alter capillary wall permeability, and reduce inflammation.[300] One case of recurrent FGS in a transplant responsive to plasmapheresis and dipyridamole has been reported.[301] If all else fails, a technique for performing percutaneous renal infarction to resolve refractory nephrotic syndrome has been described by Abrass *et al.*[302]

4.6. Membranous Nephropathy

New information continues to appear on this common cause of nephrotic syndrome in adults. With regard to pathogenesis, the studies documenting an autoimmune response to a glomerular epithelial cell membrane antigen as the underlying mechanism in a rat model of this disease that closely mimics the human condition were discussed earlier (Section 2.1.1.1). There is a high suspicion that a similar mechanism is operative in humans as well. The etiology in most cases is unknown. Membranous nephropathy continues to be reported in association with both gold and captopril therapy.[303,304] It is of interest that captopril induces a monocyte-mediated suppressor cell effect[305] similar to that reported in idiopathic membranous nephropathy. Both gold and captopril induce other autoimmune manifestations in animals, including production of antibodies to tubular epithelial cell antigens.[306] Membranous nephropathy appears to be a strongly HLA-linked disease associated with HLA-DR3 and B8 in a French study[307] and DR2 and MT1 in two studies from Japan.[307–309] DR3 has also been implicated as a risk factor for patients developing membranous nephropathy on gold therapy.[310] In addition to these immunogenetic factors, several familial cases of membranous nephropathy have now been reported.[311] The relative frequency of membranous nephropathy as a *de novo* lesion in transplanted kidneys[312,313] and its apparent association in some cases with anti-GBM disease[314] also strengthen the suspicion of an autoimmune pathogenesis. Other recently reported associations include those with prostatic carcinoma (one case),[315] enterococcal endocarditis,[316] and hyper-high-density lipoproteinemia.[317] The association of membranous nephropathy with hepatitis B antigen in children has again been confirmed.[318] Antigen-positive patients had a tendency to hypocomplementemia and more subendothelial and mesangial immune deposits,

thus somewhat resembling mild cases of membranoproliferative glomerulonephritis. Whether this association is pathogenetic or simply marks an immunologic defect in these patients remains unclear.

A fascinating portrayal by Bonsib of the consequences of this disease as visualized in acellular glomeruli using scanning electron microscopy reveals the presence of diffuse shallow pits and "pinholes" in the GBM in early membranous nephropathy and a reticular pattern of GBM "spike" formation in later stages of the disease.[319] With regard to pathogenesis, removal of cells from the GBM also removed immune complex deposits in idiopathic membranous nephropathy, whereas many of these deposits remained in patients with lupus membranous nephropathy, suggesting a possible different mechanism of deposit formation more related to the epithelial cell membrane in patients with the idiopathic form of the disease.[320] Shemesh *et al.* have published an elegant study of the pathophysiology of glomerular barrier function in membranous nephropathy suggesting the presence of a population of nondiscriminating large pores which may account for much of the loss of barrier function, whereas a diffuse decrease in capillary permeability, apparently related more to loss of epithelial slit pores than to density of subepithelial deposits, correlated with a reduction in GFR.[239] The relationship between these physiologic abnormalities and the structural lesions described earlier by scanning electron microscopy remains unclear.

Three studies have looked at the long-term clinical course of membranous nephropathy. A Finnish study of 67 patients revealed renal survival at 5, 10, and 15 years of 94, 83, and 69%, respectively, with no differences between steroid-treated and untreated patients.[321] Similar results were reported in 104 Japanese patients, with 50% having a GFR of less than 80 ml/min at 15 years but a lesser percent progressing to renal failure.[322] About 50% of a British group of 64 patients did not progress, while the remainder progressed slowly, doubling the serum creatinine in a average period of about 30 months.[323] All studies confirm the significance of male sex, persistent proteinuria exceeding 2.0 g, and elevated creatinine at the time of presentation as poor prognostic factors. Age has also been a predictor of outcome in some studies[323] but not others.[324]

Finally, one major new study of therapy in membranous nephropathy has appeared since the publication of Volume 3 of *Contemporary Nephrology*. Ponticelli *et al.* studied 67 adults with idiopathic membranous nephropathy randomized to receive treatment with either methylprednisolone, 1 g intravenously for 3 days followed by oral prednisone, 0.4–0.5 mg/kg for 1 month (cycle A) followed by chlorambucil, 2 mg/kg per day for 1 month (cycle B).[325] Cycles A and B were repeated three times over 6-six month period. A control group received symptomatic treatment

only. At the end of a mean follow-up period of 31 months, 23 of 32 treated patients were in complete or partial remission versus 9 of 30 controls ($p < 0.001$). Treated patients also had no change in renal function, whereas the mean value of 1/plasma creatinine had fallen to about 0.8 in the control group at 2 years.[325] The increased number of remissions, as well as preservation of normal renal function in treated patients, suggests that this regimen may be superior to the beneficial effect of 3 months of alternate-day steroids reported by the United States Collaborative Study of Adult Nephrotic Syndrome in 1979. The effect of steroids alone has also been suggested by more recent studies.[326] Unfortunately, however, the selection of untreated patients as the control group makes it impossible to determine whether the benefit described was due to the longer course of steroid treatment, the use of methylprednisolone pulses, or the addition of chlorambucil to the regimen. Several earlier uncontrolled studies have suggested a beneficial effect of adding a cytotoxic drug to oral prednisone in membranous nephropathy. However, the utility of this approach must be confirmed with appropriate prospective controlled studies. The option of utilizing a second drug warrants consideration in that group of patients with the risk factors defined earlier who have some evidence of renal functional deterioration within 2 years of the onset of disease.

4.7. Membranoproliferative Glomerulonephritis

Several reviews of this group of diseases have appeared recently.[327–330] There are clearly two disease entities present in this category: Type I MPGN has immune deposits and classical complement pathway activation similar to that seen in lupus, but with more striking membranous changes in the biopsy and less inflammation than usually seen in patients with diffuse proliferative lupus nephritis. Type II MPGN (dense-deposit disease) is often associated with alternate complement pathway activation and presence of the autoantibody C3 nephritic factor, but is not a glomerular immune deposit disease. The nature of the dense deposits and their relationship, if any, to persistent complement activation remain unclear. Type I disease remits spontaneously in less than 10% of patients and progresses to renal failure in about 50% within 8–10 years, particularly in patients with persistent nephrotic syndrome, crescent formation, or sclerotic glomeruli.[328,331] Focal, as opposed to diffuse, duplication of basement membrane has also been suggested to be a good prognostic indicator.[329] A lesion similar to type I MPGN is seen in lupus, shunt nephritis, cryoglobulinemia, nephrotic syndrome with hepatitis B infection, malarial and schistosomal nephropathy, heroin nephropathy, sickle cell nephropathy, transplant nephropathy, and some cases of lym-

phoma and leukemia.[327] Several observers have suggested that patients with clinical and pathological manifestations similar to type I MPGN but with additional subepithelial deposits be regarded as a separate entity (type III MPGN), but there is little reason to believe that such patients in fact have a different disease.[331] Patients with dense-deposit disease are less common, younger, more prone to nephritic episodes, and have a somewhat worse prognosis.[328,330]

Recent attention has focused on treatment of MPGN. Long-term (2 years) courses of alternate-day prednisone (2–2.5 mg/kg) may reduce proteinuria, improve renal histology, and perhaps preserve renal function in some pediatric patients with type I MPGN when initiated early in the disease.[331–334] However, these effects may be accompanied by significant steroid toxicity, including hypertension. More encouraging results have been obtained using the platelet inhibitor dipyridamole, 225 mg/day, and aspirin, 975 mg/day, in a prospective, randomized, double-blind, placebo-controlled trial in patients with type I MPGN, which showed an average decline in GFR of 1.3 ml/min per year in treated patients compared to 19.6 ml/min per year in controls.[335] Fewer treated patients also reached end-stage renal disease. Side effects of this treatment program were minimal. In another prospective trial of 47 type I patients and 12 type II patients, a combination of dipyridamole with cyclophosphamide and coumadin for 18 months revealed no beneficial effect of this regimen during that period of follow-up,[336] a result that differs from an earlier report of benefit with warfarin and dipyridamole in MPGN.[337] However, several patients in the former study had relatively advanced renal failure at the time of initiation of therapy.[336] Thus, aspirin and dipyridamole appears to be the regimen of most established benefit and least toxicity for type I MPGN at the present time. The use of these agents has been reviewed by Donadio.[338] All these trials contain too few type II patients to reach any conclusions regarding therapeutic benefit in that disease. Patients with crescentic MPGN have been reported to respond to steroid pulse therapy.[233] Three patients with type I MPGN have also been reported to maintain stable renal function when treated with long-term plasmapheresis alone.[339]

5. Glomerular Involvement in Systemic Immune Diseases

5.1. Vasculitis

The classification of vasculitis from a renal perspective can be separated into large- and small-vessel varieties. The glomerular lesion of vasculitis is a focal segmental necrotizing glomerulonephritis, usually

without immune deposits, which may be mild or can be severe with crescents and RPGN.[221,340] In large-vessel disease (polyarteritis nodosa), the major renal lesion is ischemia, and the presence of segmental necrotizing glomerulonephritis suggests a concomitant small-vessel involvement or "overlap syndrome."[221] Diseases with systemic necrotizing vasculitis of small vessels and renal involvement include lupus nephritis, Henoch–Schönlein purpura, cryoglobulinemia, Wegener's granulomatosis, and a group of patients with an idiopathic form of small-vessel vasculitis usually referred to as hypersensitivity vasculitis if skin lesions are present or microscopic polyarteritis if only organ involvement is seen. Balow has provided an excellent review of the current classification and terminology of renal vasculitis.[221]

Although the pathogenesis of vasculitis has always been presumed to involve deposition of circulating antigen–antibody complexes, particularly when associated with hepatitis B infection as often occurs in polyarteritis nodosa and cryoglobulinemia,[341] the focal necrotizing glomerular lesions are usually free of significant immune complex deposits.[221,340,342] The possibility of a cell-mediated component to these lesions is suggested by a recent study demonstrating that vascular smooth muscle cells from mice with autoimmune disease can release interleukin-1, express Ia antigen, and stimulate a mononuclear inflammatory cell infiltrate that destroys these cells.[343] Moreover, lymphocytes sensitized *in vitro* to microvascular smooth muscle can produce a vasculitis when transferred to naive recipients.[344] However, the immune mechanisms in vasculitis are still very poorly understood, as emphasized in one study where both vascular and glomerular immune deposits were carefully excluded in 20 patients with vasculitis.[342]

5.1.1. Large-Vessel Vasculitis

The role of abdominal angiography in the diagnosis of polyarteritis has been emphasized,[345] and the findings are well illustrated in a recent review.[341] Balow has reviewed the data favoring the use of steroids and cyclophosphamide in the treatment of polyarteritis nodosa.[221,346] Crescentic glomerulonephritis may occur in polyarteritis nodosa[347] and appears to respond to steroid pulse therapy similar to the way idiopathic RPGN responds.[233]

5.1.2. Systemic Necrotizing Vasculitis of Small Vessels

5.1.2.1. Renal Vasculitis. Several excellent recent reviews have concentrated on patients with focal necrotizing glomerulonephritis with multisystem involvement but renal disease as a major manifesta-

tion.[221,340,342,348–350] Serra *et al.* made the diagnosis of vasculitis in these patients based on either histologic evidence of vasculitis or the presence of segmental necrotizing glomerulonephritis with clinical evidence of systemic disease, such as fever, weight loss, and malaise, but without histologic evidence of vasculitis.[340,350] These criteria emphasize the importance of the necrotizing glomerular lesion, essentially vasculitis of the glomerular capillaries, in diagnosis, a point often not appreciated in interpretation of renal biopsies, which usually do not reveal typical extraglomerular vascular lesions. In Serra's series, patients with extraglomerular evidence of vasculitis histologically were compared to patients with only glomerular disease and found to have identical clinical features and a similar prognosis.[340,350] The patients with renal vasculitis did poorly, with a mortality of about 30% within 2 months, 46% at 1 year, and 62% at 5 years despite therapy, figures considerably worse than those reported for vasculitis in general.[340] Renal disease usually presented with minor hematuria and proteinuria, but renal failure was the most common cause of a poor outcome.[340,350] Patients presenting with predominant pulmonary involvement are also well described.[351] Parfey *et al.*[348] and Weiss and Crissman[349] emphasize the importance of vasculitis as a cause of segmental necrotizing glomerulonephritis and again point out the lack of influence of the presence of extraglomerular vascular lesions in the kidney on clinical features and outcome, as well as the relatively poor prognosis of this form of renal vasculitis. Treatment of renal vasculitis involves use of steroids and probably cyclophosphamide,[221,346] with steroid pulse therapy probably effective in cases with extensive crescents and RPGN.[233]

Recognition that segmental necrotizing glomerulonephritis without immune deposits is virtually synonymous with vasculitis, even in the absence of other histologic evidence of vascular involvement, raises obvious questions regarding whether patients with the no-deposit form of idiopathic RPGN discussed earlier represent a vasculitic syndrome rather than a separate disease entity. At the moment, this issue is unresolved, although it seems increasingly likely that many patients with idiopathic RPGN have a small-vessel vasculitis of the renal capillaries and should be treated for vasculitis with cytotoxic agents, particularly if a segmental necrotizing glomerulonephritis is present. Other patients, however, do not have an underlying segmental necrotizing glomerular lesion.[204,231]

5.1.2.2. Systemic Lupus Erythematosus.

5.1.2.2a. Pathogenesis. Several significant new observations were made in 1984–1985 on both pathogenetic mechanisms and treatment of lupus nephritis. Central to the pathogenesis of renal disease in lupus is the DNA–anti-DNA immune complex system. Although the stimulus for autoantibody production remains unclear, a variety of immunoregula-

tory disturbances have been reported and were reviewed by Steinberg *et al.*[352] Recent studies, summarized well by Schwartz and Stollar[353] and Madaio,[354] suggest that production of lupus antibodies is more restricted than would be predicted by a generalized B-cell-activation mechanism. Human and murine monoclonal antibodies have been utilized to define ligand-binding properties, genetic markers, and primary structure of lupus autoantibodies.[353] These studies indicate that a relatively restricted and genetically related network of B lymphocytes accounts for anti-DNA antibody production, but that the antibodies produced are reactive with a variety of antigenic determinants on bases, nucleotides, and oligonucleotides in single-stranded or denatured DNA (but not expressed in native DNA) that may be present in a variety of tissue structures.[353,354] Thus, a single monoclonal antibody may react with multiple polynucleotides, depending on the configuration of the nucleic acid backbones, which vary with base composition. DNA may not be the preferred antigen for some "anti-DNA" antibodies, and some such antibodies detected with idiotypic probes do not have anti-DNA reactivity.[355] This polynucleotide reactivity of anti-DNA antibodies leads to cross-reaction with a variety of structures, including cardiolipin, intermediate filaments such as vimentin,[356] platelet antigens, and membrane proteins of Raji cells.[46] Thus, a single antibody molecule may cause multiple serologic as well as clinical manifestations owing to reactivity with a small antigenic epitope recurrent in a variety of molecules. In addition, anti-DNA antibodies from many humans appear to share a "public" idiotype, a serologically defined structure of the variable region of the antibody molecule, suggesting a common genetic origin.[357] Apparently, normal B cells have the capacity to produce antibody with DNA reactivity when stimulated by B-cell activation or the lpr gene which encodes for a murine form of lupus nephritis.[358,359] B-lymphocyte function has been noted to correlate with activity of renal disease by biopsy in lupus.[360] Although it now seems unlikely that an altered DNA provides the primary immunogenic stimulus that leads to anti-DNA antibody production, more evidence linking these antibodies to bacterial antigens is emerging.[361] Cross-reactive anti-DNA antibody idiotypes have been identified in glomerular deposits in lupus.[362] The importance of genetic factors in the production of these antibodies is illustrated by the association between SLE and HLA DR2 and DR3 antigens, homozygous deficiencies of early complement components, and a T4 epitope defined on helper T cells,[363] as well as by the presence of familial cases. Reactive anti-DNA antibody idiotypes are also present in healthy family members.[355,364] Lupus has also been reported with high frequency in patients with diabetes induced by autoantibodies to insulin receptors.[365]

Exactly how all these new data on the nature of the autoantibody

in lupus relates to the development of immune complex nephritis is an area of ongoing investigation. Some anti-DNA antibodies appear to react directly with structures in glomeruli[366]; anti-endothelial-cell membrane antibodies have recently been reported in SLE[39]; low-molecular-weight DNA is present in free and immune complex form in SLE[367,368] and may bind directly to GBM collagen[26] but correlates poorly with nephritis.[367] These observations suggest that a local, or *in situ*, mechanism of glomerular immune complex formation may be operative in SLE.

Although some studies still correlate certain types of complement-fixing immune complexes with clinical activity in SLE[369–372] and document impaired reticuloendothelial Fc function apparently related to B-cell activity and disease activity,[373,374] two observations now make interpretation of much of the data on immune complexes in SLE difficult. The first is that lupus sera have antibody reactivity with the cell membrane of Raji cells, the cell line used in one standard immune complex assay system.[46] The second is that lupus patients have now been shown to possess an IgG antibody reactive with a C1q neoantigen expressed by C1q bound to polystyrene as used in the standard C1q solid-phase assay for immune complexes.[47] Thus, previously employed immune complex assay systems in SLE may be directly measuring autoantibody reactivity rather than levels of circulating complexes. Circulating inhibitors of C3 convertase formation[375] or antibodies to the convertase[376] in SLE may inhibit C3b generation and thereby interfere with immune complex solubilization and clearance. Gabrielli *et al.* reported that immune complex deposits at the dermal–epidermal junction in lupus correlated with circulating immune complex levels, hypocomplementemia, and impaired ability to solubilize preformed immune complex.[377]

5.1.2.2b. Clinical lupus nephritis. The revised American Rheumatism Association criteria for diagnosis of lupus and the World Health Organization (WHO) classification of renal lesions have been reviewed by Glassock and Cohen in Volume 3 of *Contemporary Nephrology*. As of 1983, evidence for the efficacy of any therapy except steroids was marginal, and the utility of accurately characterizing renal involvement in SLE beyond clinical parameters of renal function and protein excretion could be debated. Schwartz has written an excellent review of the current classification of renal lesions in SLE and the utility of the renal biopsy in assessing renal involvement.[378] Central to this issue is the recent addition to the histologic classification of SLE of "activity" and "chronicity" indices, defined on the basis of histologic criteria by the group at the NIH. Understanding of these indices is essential to interpreting new treatment data reviewed below. The activity index is derived by scoring from 1 to 3^+ the degree of glomerular cell proliferation, leukocyte exudation, karyorrhexis and fibrinoid necrosis, cellular crescents, hyaline

deposits, and interstitial mononuclear cell infiltration.[379] The chronicity index represents a similar 1 to 3^+ assessment of glomerular sclerosis, fibrous crescents, tubular atrophy, and interstitial fibrosis.[379] Austin *et al.* have documented the predictive value of these indices in identifying patients with proliferative forms of lupus nephritis (class III–IV) who have a poor prognosis.[379] The utility of the WHO classification, as well as the activity and chronicity indices, in defining prognosis in SLE is also supported by the study of Banfi *et al.*[380] Magil *et al.* have reassessed the importance of a variety of clinical, laboratory, and pathologic features as prognostic indicators in 35 female patients with diffuse proliferative lupus nephritis and found the serum creatinine on presentation and the extent of extraglomerular immune deposits (primarily TBM) by morphometric analysis to be the best predictors of a poor renal outcome.[381] Moreover, when the number of intraglomerular monocytes detected by nonspecific esterase staining was combined with the serum creatinine, strong predictive value was obtained.[381] Taken together, these findings provide additional new support for the value of data obtained from the renal biopsy in assessing prognosis in SLE.

The phenomenon of transition from one WHO class to another, usually from worse to better, is documented in 50% of rebiopsied patients by Lee *et al.*[382] The importance of race (black worse than white), age (young worse than old), and immunogenetic factors in disease expression was analyzed by Hochberg *et al.*[383] A French study suggests that the prognosis of renal disease in children with lupus is probably similar to that in adults.[384] The importance of atherosclerotic disease as a cause of late mortality in SLE is emphasized by Rubin *et al.*[385] Korean patients appear to have more severe lupus renal disease.[386]

Other noninvasive measures touted as useful in assessing disease activity in proliferative lupus nephritis include the development of a low filtration fraction[387] and a positive gallium scan.[388] An analysis of glomerular barrier function in nephrotic patients with SLE by Friedman *et al.* reveals a reduced Kf and development of a subpopulation of large protein-permeable pores, which decrease in size with therapy and may provide another useful index of disease activity and adequacy of treatment.[389] Tubular dysfunction, particularly distal renal tubular acidosis and tubular proteinuria, continues to be noted in some patients with lupus nephritis.[390,391]

The membranous form of lupus nephritis (WHO class V) has been studied in Malaysia, where the prognosis was related to the degree of proliferation seen but was generally good in patients with pure membranous lesions.[392] The utility of so-called "fingerprint" deposits as a predictor of subsequent SLE has been advocated in patients who present with membranous nephropathy without clinical or serologic evidence of

lupus.[393] Moreover, crescents can occasionally develop in patients with membranous nephropathy and lupus and assure a poor prognosis.[394]

Lupus induced by quinidine[395] and by hydralazine[396] is reported and reviewed, with several of the latter patients noted to be slow acetylators and to develop an immune complex glomerulonephritis with crescents and mild but reversible changes in renal function. The spectrum of drug-induced lupus is well reviewed by Cush and Goldings, who also note a low but definite incidence of significant renal involvement.[397]

Although patients with lupus represent less than 2% of the population of patients with end-stage renal disease, Correia *et al.* analyzed 24 such patients and noted that complications on dialysis develop commonly in patients with an acute deterioriation of renal function immediately prior to starting dialysis, whereas patients with slowly progressive disease apparently do well.[398] Because of the significant incidence of recovery of renal function after starting dialysis in lupus, a delay of 1 year is recommended before performing renal transplantation.[398] Renal transplantation in lupus nephritis appears to be uncomplicated by clinically significant recurrent disease.

5.1.2.2c. Treatment of lupus nephritis. As noted in Section 5.1.2.2b, in 1983 there was little convincing evidence from prospective controlled studies that the addition of cytotoxic drug therapy to steroids significantly improved the renal survival of patients with diffuse proliferative lupus nephritis. Survival rates in lupus have increased progressively from about 28% prior to 1950 to over 70% at 5 years in the 1980s, and this change has occurred apparently independent of newer forms of therapy. These studies are well summarized by Coggins[399] and Donadio.[400] In 1983, the first of several recent studies from the NIH group appeared analyzing data from 107 patients, most with diffuse proliferative lupus nephritis, treated with various single- and two-drug regimens since 1969.[401] This study concluded that immunosuppressive (cyclophosphamide or azathioprine) drug therapy was not helpful in patients with a chronicity index on renal biopsy of less than 1 and did not improve results obtained with steroids alone for patients with chronicity indices of greater than 4, but appeared to reduce the probability of losing renal function in a subset of patients with a chronicity index between 1 and 4.[401] However, this group was small and had been defined only retrospectively by pathologic criteria of uncertain reproducibility. However, another study to support the benefit of a second drug was that of Felson and Anderson.[402] These workers utilized conventional power analysis to make the important point that if the risk of an adverse outcome (such as end-stage renal disease) is 25% or less, a reasonable figure in lupus nephritis today, any study would require 200 patients to have an 80% chance of demonstrating a benefit of therapy at the 5% ($p < 0.05$) level.[402] To overcome the

limited number of patients included in all published treatment trials of lupus nephritis, these workers pooled the data from eight prospective, randomized clinical trials of prednisone versus prednisone and a second drug in 250 patients with lupus nephritis and reported that patients receiving second drugs had significantly less deterioration of renal function, a lower incidence of end-stage renal disease, and a reduced likelihood of death from renal failure compared to patients treated with steroids alone.[402] However, the overall mortality in the two groups was not different. The hazards of such pooling are obvious, and the results were significant only when cyclophosphamide and azathioprine studies were combined, but not for either drug alone. However, the magnitude of the difference when examined this way is impressive. A later study from the NIH group of 62 patients receiving a second renal biopsy an average of 44 months after initiation of therapy reported a linear increase in the chronicity index in steroid-treated patients, whereas no change was found in the group receiving steroids plus cytotoxic agents, a finding interpreted as evidence that a second drug may reduce the development of chronic structural damage in these patients.[403] However, no evidence for a beneficial effect in preserving renal function was presented. Finally, the most recent paper from this group notes a reduced probability of maintaining stable renal function after 5 years in patients treated with steroids alone.[404] Moreover, when patients with a chronicity index greater than 1 were analyzed, a statistically significant difference in the probability of renal failure could be demonstrated when patients treated with prednisone alone were compared to patients treated with intravenous cyclophosphamide.[404] However, this difference did not hold for the second drug group as a whole and was of only marginal significance ($p = 0.05$) when only the patients treated with prednisone *concurrently* with the more recently introduced intravenous cyclophosphamide protocol were analyzed.[404] The validity of the conclusion that cyclophosphamide is useful in lupus nephritis can only be confirmed if more patients are observed for a longer period of time.

The complications of cytotoxic drug therapy are also well documented in this study including major infection (17%), herpes zoster (33%), cancer (17%), and premature ovarian failure (71%) in the oral cyclophosphamide patients.[404] The complication rate is lower, however, in patients receiving cyclophosphamide intravenously in monthly pulse doses.

At the present time, I believe that a fair summary of this data would be to say that renal biopsy probably is useful as a prognostic indicator and aid in selecting therapy in patients with lupus who have significant renal involvement, including a reduction in GFR and/or proteinuria in excess of 1.0 g/day; cyclophosphamide *may* be of benefit in preserving function in a subset of patients with evidence of active disease and mild

chronic changes; and intravenous cyclophosphamide administered on a monthly schedule appears to be as efficacious and somewhat safer than daily oral cyclophosphamide. The details of therapeutic protocols utilizing cyclophosphamide and steroids have been provided by Balow and Austin.[405] Obviously, these results require confirmation by other studies, and use of a cytotoxic drug should be considered only in very well-studied patients with appropriate indications.

Pulse steroids continue to be advocated as a way of initiating high-dose steroid therapy in crescentic lupus nephritis patients, some of whom may present with acute renal failure.[406] Earlier studies have shown that pulse steroids restore maximal GFR more rapidly than oral steroids but do not improve the long-term renal function or prognosis in lupus nephritis. Strober *et al.* have reported improved serologies and a reduction in proteinuria and serum creatinine in 10 patients with severe lupus nephritis unresponsive to prednisone and azathioprine who were treated with 10–14 days of total lymphoid irradiation.[407] However, this therapy is associated with significant side effects. Encouraging anecdotal results have also been reported with the defibrinating agent ancrod, which remains an experimental drug.[408] At the time of this review, the results of an ongoing prospective controlled trial of plasmic exchange in diffuse proliferative lupus nephritis have not yet been reported. The studies of anti-DNA antibody idiotypes reviewed above, as well as the successful suppression of murine lupus nephritis by treatment with an antiidiotypic antibody to DNA, encourage optimism that more specific immunotherapy for this disease may be forthcoming in the near future.[409]

5.1.3. Henoch–Schönlein Purpura

Most of what was discussed in Section 3.2.2 on IgA nephropathy applies as well to the nephritis of Henoch–Schönlein purpura (HSP), and a number of the papers reviewed in that section deal with both diseases.[146,147,170] Roth *et al.* report kidney biopsies during the initial episode of HSP in nine adults demonstrating focal nephritis (seven) and a more severe diffuse proliferative nephritis (two).[410] The high incidence of antecedent events, particularly infections and drug exposure, is emphasized. The impression that steroid therapy ameliorated extrarenal manifestations but was of little benefit to the renal lesion accords with most of the literature on this disease.[410]

5.1.4. Cryoglobulinemia

Cryoglobulins may be type I (monoclonal), type II (monoclonal, usually IgM rheumatoid factor reactive with polyclonal immunoglobu-

lin), or type III (mixed, two polyclonal immunoglobulins usually containing an IgM rheumatoid factor). Low levels of types II and III cryoglobulins are present in various collagen–vascular diseases. However, in essential cryoglobulinemia, cryoglobulins are present in higher concentrations (>20 mg/ml), and renal disease accompanied by cutaneous vasculitis and arthralgias is common.[411] Evidence of hepatitis B infection is common, and reduced levels of hemolytic complement and C4 may be present with normal levels of C3, a phenomenon usually due to *in vitro* activation of complement by the cryoglobulin in the test tube.[411,412] Vasculitic renal involvement and cryoglobulinemia associated with hairy cell leukemia has been noted.[413] The glomerular lesion in cryoglobulinemia is usually similar to that of type I MPGN rather than the segmental necrotizing glomerulonephritis seen in most vasculitides, and extensive immune deposits with a characteristic appearance by electron microscopy are seen.[411] Nephrotic syndrome with nephritic features is common. There is increasing evidence that plasma exchange alone may be the treatment of choice for patients with glomerulonephritis and renal failure.[414,415]

5.1.5. Wegener's Granulomatosis

A comprehensive review of 85 patients with Wegener's granulomatosis studied for up to 21 years was provided by Fauci *et al.* in 1983 and emphasizes the efficacy of the prednisone-and-cyclophosphamide regimen used to induce remission in 93% of patients.[416] Littlejohn *et al.*[417] and ten Berge *et al.*[418] have reviewed 17 and 12 patients respectively, emphasizing the importance of renal involvement in prognosis. A marked increase in the ratio of helper to suppressor T cells was noted in periglomerular and interstitial cell infiltrates, suggesting the possibility of a cell-mediated reaction occurring in the kidney in Wegener's granulomatosis, although patients exhibited a poor delayed hypersensitivity reaction to DNCB, perhaps because of concomitant renal failure.[418] Weiss and Crissman provided a detailed analysis of renal pathology in Wegener's granulomatosis, emphasizing the segmental necrotizing glomerular lesion, frequent evidence of glomerular thrombosis, and lack of immune complex deposition.[419] A case discussion emphasizing the differential diagnosis and clinical approach to patients with Wegener's granulomatosis has appeared recently,[420] and the potential severity of pulmonary hemorrhage has been emphasized.[421] Prednisone and cyclophosphamide in a regimen similar to that discussed by Fauci remain the mainstay of therapy for Wegener's granulomatosis, achieving an overall 10-year survival of 80% in all cases, although the risk of renal failure is about 33% at 10 years and is higher in patients with crescents

and RPGN or with proteinuria in excess of 2.5 g/day.[421] Data from the Mayo Clinic suggest that an improved response may be seen in patients also treated with trimethoprim–sulfamethoxazole, although the mechanism of this effect is unclear.[422] The capacity of prednisone and cyclophosphamide to occasionally reverse rather severe renal involvement requiring dialysis is illustrated dramatically in one report.[423] Hind *et al.* have provided evidence that measurement of C-reactive protein may provide a useful laboratory parameter of disease activity and response to therapy in Wegener's granulomatosis.[424]

5.2. Glomerulonephritis in Renal Transplants

Recurrence of the original glomerular disease in the transplant is well described but is probably of less clinical significance than once believed. Graft failure may result in patients with focal glomerular sclerosis and the risk factors reviewed above, or significant levels of anti-GBM antibody. Rapidly progressive (<3 years) membranous nephropathy may recur in grafts to produce nephrotic syndrome but is more common *de novo* and usually does not impair graft function. Diseases such as IgA nephropathy and types I and II MPGN recur morphologically but rarely cause graft loss.[425] No significant effect of glomerulonephritis on allograft survival was found by Cats *et al.*[426] Anti-GBM disease may also occur *de novo* in the allograft, as membranous nephropathy does.[427] The most common cause of nephrotic syndrome in transplant patients is "transplant glomerulopathy," a lesion marked by early endothelial and mesangial cell swelling followed by lobulation of glomeruli with GBM changes similar to those in MPGN. IgM and fibrin deposits are seen by immunofluorescence, and endothelial damage is prominent by electron microscopy.[428,429] The lesion is presumably rejection-related and carries a relatively poor prognosis.[428,429]

5.3. Hemolytic–Uremic Syndrome and Thrombotic Thrombocytopenic Purpura

These two diseases, collectively referred to as thrombotic microangiopathies, are probably part of a spectrum of disease resulting from endothelial cell injury due to a variety of different mechanisms. I have therefore not attempted to separate the literature on the two diseases. Current understanding of hemolytic–uremic syndrome (HUS) has been recently reviewed.[430,431] The clinical overlap between HUS and thrombotic thrombocytopenic purpura (TTP) is extensive. Several different

forms of HUS probably exist, including the classical childhood form that follows episodes of bloody diarrhea, a postinfectious form following a variety of bacterial infections when endotoxemia may be important, a hereditary form,[432–434] some cases with a probable immune pathogenesis associated with glomerular deposits and sometimes reduced complement levels, a form associated with systemic illnesses such as lupus,[435] cancer,[436] and various drugs, particularly cancer chemotherapeutic agents[437–439] and cyclosporin,[440] and forms related to pregnancy and oral contraceptives. The etiology of HUS in all these subsets of patients is uncertain.[430] Platelets appear to have a defect in aggregation even when present in normal numbers, and some circulating factor appears to render platelets insensitive to the antiaggregatory effect of prostacyclin.[441–443] A platelet aggregatory factor inhibited by normal IgG has also been reported,[443] and plasma fibronectin levels may be low associated with glomerular fibronectin deposits.[444] Abnormalities of prostacyclin metabolism, including a lack of a plasma factor required for endothelial cell synthesis of prostacyclin, presence of an inhibitor or diminished serum binding of prostacyclin, or increased prostacyclin degradation, have all been proposed.[431,445,446] An exciting recent observation is the association between the classical childhood form of HUS and verotoxin-producing *Escherichia coli,* often of the 0157-H7 strain.[447,448] Detection of fecal verotoxin seems diagnostically useful in these patients, although the role of verotoxin in disease pathogenesis remains unclear.

The childhood form of HUS generally requires only supportive therapy, even when renal failure sufficient to require dialysis is present, and mortality is less than 10%. However, in other forms of the disease, often associated with arterial rather than glomerular microangiopathy, in older patients, or when clinical manifestations of TTP are present, more specific therapy is routinely employed.[449] No controlled study has established the efficacy of any form of therapy, but dramatic responses have been reported with both fresh plasma infusion[450,451] and plasma exchange,[451,452] and both these treatments are now commonly employed. The rational for the apparent benefit of this approach is as hazy as the understanding of the pathogenesis of HUS–TTP, but possibilities include supply of a factor that stimulates prostacyclin release by endothelial cells, inactivation or removal of a platelet-aggregating factor or changes in Factor VIII–Von Willeband factor multimers that may trigger platelet aggregation, as well as removal or inactivation of an as-yet-unidentified factor that initiates the endothelial cell damage.[451] Hopefully, advances in understanding the pathogenesis of these disorders will soon catch up with what appears to be significant improvement in their treatment since the introduction of plasma infusion and plasma exchange therapy.

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9

Acute Renal Failure and Toxic Nephropathy

H. David Humes and Vo D. Nguyen

1. Introduction

Acute renal failure is a common clinical syndrome. This syndrome can be caused by prerenal functional hemodynamic processes, intrarenal structural injury, or postrenal obstructive disorders. Prerenal acute renal failure, or prerenal azotemia, results from a persistent, significant decline in renal blood flow (RBF), which leads to a decline in the rate of glomerular filtration (GFR) and rising levels of blood urea nitrogen (BUN) and plasma creatinine. Usually this decline in renal perfusion is a component of a generalized process of poor tissue perfusion, but selective declines in RBF, and hence GFR, may develop disproportionately to blood flow to other tissues. A variety of drugs, most notably agents that inhibit prostaglandin synthesis, have been demonstrated to produce nephrotoxic side effects by an ability to promote selective declines in RBF and GFR.[1] With regard to intrarenal structural processes, several factors make the kidney especially susceptible to toxic injury. The high rates of delivery of compounds to the kidney, concentration of drugs in tubule lumens and interstitium, and transcellular transport of toxins by

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the kidney make the renal tubular cells especially vulnerable to toxic injury.[2] The high metabolic demands for the normal transport activities of renal tubular cells and the virtual absolute requirement for oxidative metabolism as an energy source by proximal tubular cells make the renal tubular cells also keenly susceptible to ischemic injury.[3] Both toxic and ischemic insults have the ability to cause substantial renal structural damage to produce acute renal excretory failure.

Toxic and postischemic acute renal failure results from a complex, and still incompletely understood, interplay between cellular, nephronal, and hemodynamic events.[4] It is now generally accepted that the occurrence of persistent acute renal failure correlates with the presence of renal tubular cell injury.[5,6] The loss of normal renal tubule cell function, loss of continuity of the tubular epithelium, and formation of debris from injured tubules all contribute critically to the derangements in nephron function that occur during acute renal failure. But since nephrons function as units in series, injury localized to limited nephron segments may lead to substantial renal failure if the tubular obstruction from cellular debris, or backleak of glomerular filtrate, or secondary compensatory alterations in glomerular hemodynamics is sufficient to compromise function of the whole nephron. The structural heterogeneity of the kidney and the complex interplay of vascular, nephronal, and cellular events in the pathophysiology of acute renal failure pose substantial difficulty in delineating the direct toxic potential of a variety of compounds associated with nephrotoxic acute renal failure. In this regard, recent work has provided a better understanding of the pathophysiology of two important drugs with well-known nephrotoxicity, radiographic contrast agents and cyclosporine. This chapter will review these newer insights and correlate this new understanding to the clinical features of these two nephrotoxic disorders.

Increased understanding of the events involved in the pathogenesis of ischemic acute renal failure has also emphasized the importance of the associated renal tubular cell injury. Intranephronal obstruction of tubule lumens with debris from damaged cells and backleak across damaged epithelial surfaces have been shown to play significant roles in producing the reductions in GFR seen in ischemic acute renal failure.[4] The potential contribution to loss of renal function by sublethally injured tubules has also been emphasized by microperfusion and micropuncture studies demonstrating marked functional abnormalities in such tubules.[4] Very recent studies have highlighted the importance of the balance between energy consumption and energy production in determining the maintenance of renal tubular cell viability.[7,8] Important roles have also been suggested for phospholipase activation and phospholipid degradation, for free-radical production and lipid peroxidation, and for al-

terations in calcium metabolism in ischemic or hypoxic renal tubule cell injury. This chapter will also summarize the most recent data detailing the role for these metabolic processes in evolving hypoxic cell injury. It is hoped that as these cellular processes responsible for renal cell injury become better understood, rational approaches to prevent renal tubule cell injury which results in toxic and postischemic acute renal failure can be developed and clinically applied.

1.1. Radiographic Contrast Agent-Induced Acute Renal Failure

A significant increase in the incidence of radiocontrast-induced acute renal failure has occurred recently. The increased incidence in radiocontrast-related acute renal failure over the past several years is attributable both to an increased physician awareness and to greater use of these agents for an increasing number of radiologic procedures, including intravenous pyelography, angiography, and computerized tomography (CT), rather than to an increased nephrotoxic potential of the contrast agents.[2] The intravenous agents most commonly in use are triiodinated derivatives of benzoic acid, including the sodium and meglumine salts of diatrizoate, iothalamate, metrizoate, and ioxitalamate. These compounds are ionic species that dissociate from their cation at physiologic pH and exist as a charged anion. A new group of nonionic radiocontrast agents have recently been introduced and include metrizimide, iohexol, and iopamidol.[9] Owing to the nonionic nature of these newer agents, they do not have an accompanying cation and, therefore, the same iodine load can be delivered in a solution with much lower osmolality compared to the older ionic agents. An advantage of these newer nonionic contrast agents over the older ionic contrast agents to lower the risk of radiocontrast nephrotoxicity has yet to be demonstrated.

Since the kidney is the principal excretory organ for contrast media, the potential exists for nephrotoxicity to occur with their use. In patients without identified risk factors (discussed in Section 1.1.1) the incidence of contrast-induced acute renal failure appears to be less than 2%.[2] Acute renal failure has been observed following urography, angiography, and CT. Because of the larger doses required, angiography may have a slightly higher incidence of nephrotoxic reactions compared to urographic and CT scanning procedures.

1.1.1. Clinical Features

Nephrotoxicity following the administration of radiocontrast agents may range in severity from asymptomatic, nonoliguric transient renal

dysfunction to oliguric, severe acute renal failure requiring dialysis. Patients with mild, nonoliguric acute renal failure have transient abnormalities. Serum creatinine usually peaks 3–5 days after exposure and returns to baseline within 10–14 days. Patients with severe nephrotoxicity develop oliguria within the initial 24 hr after the contrast study. Oliguria usually persists for 2–5 days. Serum creatinine levels in these individuals reach a peak in 5–10 days and return to baseline within 14–21 days. Only a small percentage of patients develop acute renal failure requiring dialysis.

The urinalysis in patients with radiocontrast-induced acute renal failure is usually nonspecific. The urine osmolality is near isotonicity, although the specific gravity may be extremely high owing to the property of radiocontrast agents to raise urine specific gravity. During the oliguric phase, the urinary sodium concentration and the fractional excretion of sodium (FE_{Na}) in these patients, in contrast to most other causes of nephrotoxic or ischemic acute renal failure, can be extremely low, with FE_{Na} less than 1.0%.[10] A persistent nephrogram is commonly seen in radiographs taken 24 hr after the contrast study.[2]

Although the incidence of nephrotoxic complications with the use of these agents is relatively low, several risk factors that increase the chance of developing nephrotoxicity have been identified. The classic risk factor commonly referred to is multiple myeloma. Several retrospective studies have reviewed the incidence of radiocontrast-induced acute renal failure. Of approximately 450 patients collectively evaluated, the incidence of acute renal failure was less than 4%,[2] an incidence only slightly greater than that of the patient population at large. All these studies were retrospective and probably detected only patients with severe, oliguric acute renal failure. Since no prospective study has been reported, the true incidence of contrast-induced acute renal failure in myeloma is not known and may be higher than that estimated from retrospective studies.

Substantially greater risk factors are preexisting renal insufficiency and diabetes mellitus. Recent reports have clearly shown that 50–75% of patients who developed substantial nephrotoxicity after contrast administration had renal insufficiency, with levels of serum creatinine between 2 and 3 mg/dl.[11] This association may result from the increased drug delivery per functional surviving nephron in diseased kidneys.

An additional important risk factor is diabetes mellitus.[11–13] In patients with diabetes and normal renal function, with serum creatinine less than 1.5 mg/dl, the incidence of contrast-induced nephrotoxicity is low. Patients with diabetes and serum creatinines between 1.5 and 2.0 mg/dl have a significantly greater risk. Between 50 and 75% of diabetic

Table I. Clinical Features and Risk Factors of Radiocontrast-Induced Acute Renal Failure

Clinical features
Rapid onset with oliguria
Fractional excretion of sodium less than 1%
Persistent nephrogram
Risk factors
High risk
Preexisting renal insufficiency
Diabetes mellitus
Moderate risk
Dehydration
Multiple myeloma
Previous contrast nephrotoxicity
Mild risk
Large contrast load
Advanced age

patients with moderately severe renal failure, with serum creatinines between 2 and 4 mg/dl, developed significant worsening of renal function after contrast studies. Although most patients reverted to baseline renal function, a significant number developed an irreversible component of renal failure. Nearly 100% of patients with diabetes mellitus and severe renal insufficiency (serum creatinine greater than 4.5 mg/dl) developed acute renal failure after radiocontrast administration. It also appears that patients with renal insufficiency caused by diabetic nephropathy are at greater risk than patients with renal insufficiency from other causes. Furthermore, patients with juvenile-onset diabetes mellitus appear to be more prone than patients with adult-onset diabetes mellitus to the development of acute renal failure secondary to radiocontrast administration.

Dehydration also increases the risk of developing nephrotoxicity[13] in patients who already have an increased susceptibility from preexisting renal insufficiency, diabetes mellitus, or multiple myeloma. Dehydration has not been clearly defined as a risk factor in patients with normal renal function. Patients with a history of prior contrast-related acute renal failure have been demonstrated to have repeated episodes of nephrotoxicity following reexposure to contrast agents[13] and are, therefore, at greater risk for developing this complication. Large doses and repeated administration of radiocontrast agents also are more likely to cause acute renal failure in patients at risk. The clinical features and risk factors are summarized in Table I.

1.1.2. Pathogenesis

Several mechanisms have been suggested to explain the pathophysiology of this disorder. Changes in renal hemodynamics may play a role. Radiocontrast agents clearly produce a biphasic hemodynamic response in the kidney, with an initial vasodilation followed by a more prolonged phase of vasoconstriction.[2] The vasoconstrictive effect in normal animals is transient in nature, lasting only a few minutes, but in volume-depleted animals this decline in RBF persists for as long as 20–30 min.[14] Although this magnitude and duration of RBF decline is not sufficient under normal conditions to produce ischemic tubular cell injury of the degree to result in persistent declines in renal excretory function, this vasoconstrictive response may aggravate a toxic process produced by the radiocontrast agent.

Tubule obstruction by proteinaceous casts has been postulated as a cause of this disorder. The older contrast agents were shown to precipitate Bence Jones proteins *in vitro*.[2] Although the present agents do not have a similar effect *in vitro*, they can precipitate Tamm–Horsfall mucoprotein,[2] a protein found in the distal nephron and the major constituent of urinary casts. These observations suggest that radiocontrast agents can induce tubule cast formation and intrarenal obstruction, leading to acute excretory failure. Recent *in vitro* experiments utilizing suspensions of rabbit proximal tubule segments have demonstrated that diatrizoate interacts with cellular debris arising from tubule cell injury to form a denser and more precipitable product than usually found.[15] These data indicate that contrast agents precipitate renal cell membrane components intraluminally and promote intratubular cast formation with resultant intrarenal obstruction.

Radiocontrast agents may also produce acute renal failure secondary to direct detrimental effects on renal tubule cell viability. Histologically, acute renal failure following contrast administration is associated with vacuolization, degeneration, and sloughing of proximal tubule cells.[2] Diatrizoate and iothalamate can directly alter sodium transport across transporting epithelia.[2] Enzymuria, an indirect sign of renal cell injury, has been described in this disorder.[2] The most direct evidence for this possible pathogenetic mechanism arises from recent *in vitro* experiments. Diatrizoate has been shown to be directly toxic to rabbit proximal tubule segments in suspension.[16] This toxicity was demonstrated by the measurement of several quantitative metabolic parameters and morphologic evaluation. The magnitude of injury produced by this contrast agent was both dose and time dependent. Further studies demonstrated that *N*-methylglucosamine, or meglumine, which is a cationic compound added to radiocontrast dye solution, also had mild to moderate toxicity on renal

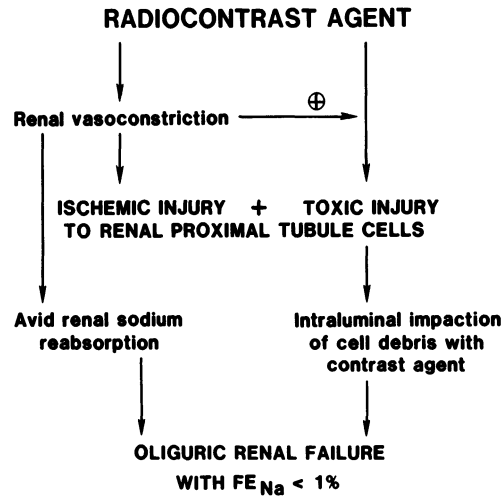

Fig. 1. Pathogenesis of radiocontrast-induced acute renal failure.

proximal tubule segments.[16,17] Of substantial interest was the additional finding that diatrizoate potentiated the magnitude of injury induced by a brief period of hypoxia which alone produces a reversible degree of injury. The presence of diatrizoate during this brief hypoxic period resulted in severe irreversible injury to the tubule preparation.

These results suggest that the pathogenesis of radiocontrast-induced acute renal failure may be related to several factors, including renal vasoconstriction, intratubular obstruction from proteinaceous debris, and direct cell injury. In fact, a possible scheme can be derived from these data to explain the pathogenesis of radiologic contrast agent-induced acute renal failure. This scheme is depicted in Fig. 1. Radiocontrast agents have a modest direct toxic effect which is both time and concentration dependent on renal tubule epithelial cells. If a simultaneous ischemic insult occurs to the kidney which is potentiated by the renal vasoconstrictive effect of the contrast agent, the simultaneous toxic and hypoxic injury to renal epithelia may be of sufficient magnitude, depending on concentration and time of exposure to the contrast agent and length of time of ischemia, to produce renal excretory failure and the clinical syndrome of acute renal failure.

This scheme provides a reasonable explanation for the clinical events observed in radiocontrast-induced acute renal failure, as detailed in Table I. Because acute renal failure arises from a simultaneous toxic and ischemic insult of substantial additive magnitude, the onset of renal excretory failure will be abrupt and often associated with oliguria. Because of the rapid development of tubule injury and the propensity of the contrast agent to precipitate intraluminally with other proteins and

membrane components, a major intraluminal and intrarenal obstructive process will develop during the period of time in which the iodinated contrast agent is transiting through the tubule lumen. Consequently, if the acute injury is severe, the contrast agent will not have the ability to transit through the nephron and egress into the collecting system of the kidney. A persistent nephrogram without a urographic phase will result. Finally, because a major component of this pathogenetic process is related to an ischemic process, the urine being formed during the developing injury phase of this process and prior to the full development of renal cell injury and intratubular obstruction will arise from a hemodynamically compromised kidney. The urine emanating from the kidneys before excretory failure develops will, therefore, be nearly sodium free because of the sodium avidity of the poorly perfused kidney. A low FE_{Na} (less than 1%) will be found in the early diagnostic phase of this disorder. The clinical features of this disease process with the acute onset of oliguric acute renal failure, the low FE_{Na}, and the persistent nephrogram are, thus, all explainable by this pathogenetic scheme.

This scheme also provides reasonable explanations for the role of risk factors in this disease process, such as dose, dehydration, preexisting renal insufficiency, and diabetes mellitus. Since the intraluminal concentration of diatrizoate achieved after its administration is near toxic levels under normal circumstances, any process that increases plasma, and therefore intraluminal, concentration of this agent or increases proximal renal tubule cell exposure time to this compound has the potential to increase the risk of nephrotoxic complications. A higher dose of this dye will increase the concentration and exposure time of the renal proximal tubule cell to this compound. Volume depletion results in avid sodium and water reabsorption along the proximal tubule. Since contrast agents are not reabsorbed by the nephron, the increase in fluid reabsorption during dehydration will increase the intratubular concentration of the dye along the proximal tubule. Since contrast dyes are excreted primarily by glomerular filtration, renal insufficiency results in a longer plasma half-life of the radiocontrast agent and a greater exposure time to renal epithelial cells. Furthermore, with renal dysfunction, the number of functioning nephrons declines. The load per nephron of excreted contrast dye rises proportionately. Potentially toxic levels of this compound at the nephronal level may, therefore, be achieved more readily in patients with preexisting renal disease.

The most important clinical risk factor for radiocontrast-induced acute renal failure is diabetes mellitus. A high incidence of this complication occurs in patients with both diabetes mellitus and renal insufficiency. The majority of these patients have a high incidence of macrovascular and microvascular disease and compromised blood flow to most

organ systems, including the kidneys. In these patients large segments of renal tissue are likely to be poorly perfused and susceptible to ischemic injury. This fact, coupled with the known vasoconstrictive effect of contrast agents, sets the stage for the additive deleterious effects of these nephrotoxins and hypoxia after administration of the contrast agent.

1.1.3. Treatment

The approach to radiocontrast-induced acute renal failure is, first, prevention by identifying patients at risk and modifying clinical settings that increase the risk of this complication. In this regard, careful determination of the risk and benefits from the proposed contrast study should be made, especially in high-risk patients who have renal insufficiency and diabetes mellitus. Volume depletion and volume-depleting maneuvers should be avoided in high-risk patients. The usual dehydrating preparation for an intravenous pyelogram consisting of cathartics and fluid restriction should be avoided and supplemented with reasonable fluid administration to maintain euvolemia in patients at greater risk, even though these measures may interfere with optimal renal visualization. Patients undergoing angiography and CT should be well hydrated both prior to and during the procedure, since the volume state of the patient does not influence the quality of these studies. Doses exceeding 0.88 mg iodine per kilogram of body weight should be avoided in patients with renal insufficiency.[11] Meglumine-containing solutions should also be avoided in patients with any risk factor. Patients with a previous episode of radiocontrast-induced acute renal failure should be regarded as at risk, and contrast procedures should be avoided unless absolutely necessary. Multiple contrast studies within 24–48 hr should also be avoided.

Besides treatment directed toward prevention of this drug-induced complication by identifying patients at risk and following the recommendations detailed earlier, a number of studies have also suggested that mannitol given either prior to or immediately following a contrast study may decrease the incidence of contrast-related acute renal failure in high-risk patients with preexisting renal insufficiency or diabetes mellitus.[18] Administering 12.5 or 25 g of mannitol both before and just after the contrast procedure may be a reasonable approach to help prevent radiocontrast-induced acute renal failure. Since mannitol is an osmotic diuretic, this maneuver will diminish sodium and water reabsorption in the proximal tubule and dilute the concentration of contrast agent exposed to the luminal membrane of the proximal tubule cell, thereby diminishing the likelihood of toxic consequences. Fluid overload, however, must be carefully avoided during this manuever. The final hope of preventing this disease process is to identify less nephrotoxic com-

pounds for contrast studies. The newer nonionic radiocontrast agents have been developed with this advantage in mind. Definitive experimental and/or clinical support for a modification of nephrotoxic potential of these new compounds has yet to be established.

1.2. Cyclosporine Nephrotoxicity

Cyclosporine is a potent new immunosuppressive agent that selectively inhibits T-cell function. Specifically, it acts on the inductive phase of cellular immunity by a relatively selective inhibition of lymphokine generation by helper T cells. This selective immunosuppressive effect allows cyclosporine to be used without generalized myelosuppression, which gives it a clear advantage over more conventional immunosuppressive therapy.[19] Numerous studies have now demonstrated the superiority of cyclosporine over azothioprine in preventing graft rejection and prolonging graft survival following kidney, heart, liver, and bone marrow transplantation. Cyclosporine is also associated with a lower incidence of bacterial and fungal infections in the posttransplant period.[20] Because of the drug's capacity to suppress immune responses, a large variety of clinical and experimental trials are also underway to assess the therapeutic potential of cyclosporine in a number of autoimmune diseases, including diabetes mellitus type 1, rheumatoid arthritis, multiple sclerosis, anterior and posterior uveitis, primary biliary cirrhosis, pulmonary sarcoidosis, and childhood nephrotic syndrome.

1.2.1. Clinical Features

Nephrotoxicity is the most frequent and clinically most important complication associated with cyclosporine use.[21] A cyclosporine-related decline in GFR with elevated levels of BUN and serum creatinine concentration occurs in nearly all treated patients, including transplant recipients and those with autoimmune diseases. The concern over cyclosporine nephrotoxicity, however, is paramount in renal transplantation when it confounds the diagnosis of renal allograft rejection.

In renal transplantation, a decline in renal excretory function may occur at three distinct time points during cyclosporine therapy corresponding to an acute, subacute, or chronic form of drug-induced nephrotoxicity. An acute decline may occur immediately following or during the first week after transplantation. This acute form of nephrotoxicity has been reported to occur more frequently in patients who have received cadaveric renal allografts with prolonged warm ischemia time or with prolonged (24 hr or more) machine perfusion preservation or in patients in whom surgical completion of the renal vascular anastomosis

of the transplanted organ required 45 min or more.[22–24] Not all centers, however, have observed this association.[25] Other perioperative complications, including urinary tract obstruction, administration of nephrotoxic antibiotics or radiocontrast agents, and postoperative acute tubular necrosis (ATN), are additional possible risk factors for acute cyclosporine nephrotoxicity. This delayed graft function in the presence of cyclosporine therapy has resulted in a significant decrease in 3-month graft survival compared to grafts without delayed function, as well as significant increases in the number of dialyses required or time needed for the recipient patient, compared to azathioprine-treated patients, to recover from ATN during the posttransplant period.[23,24]

A subacute form of cyclosporine nephrotoxicity is frequently seen in the first few months following renal transplantation. This form of toxicity is characterized by a mild to moderate, but nonprogressive, reduction in GFR and an increase in serum creatinine concentration rarely above 2.5 mg/dl. This alteration may occur as early as 2 weeks after therapy is initiated. Reduction of cyclosporine dose usually results in reversal of the decline in renal function.

Finally, a chronic form of cyclosporine nephrotoxicity appears to develop in a subpopulation of renal transplant recipients. This form of toxicity is characterized by slow but progressive declines in renal excretory function, as reflected by slow progressive elevations in BUN and serum creatinine concentration exceeding 3 mg/dl. Dose reduction may reverse a component of the decline in renal function, but in some circumstances the lower dose required to improve renal function may not provide sufficient immunosuppression. Controversy presently exists as to whether chronic nephrotoxicity is a distinct entity in the renal transplant, since chronic rejection has clinical and histologic features similar to those used to define this type of nephrotoxicity.

Similar forms of the three types of cyclosporine nephrotoxicity have been observed in other clinical settings, including heart, liver, and bone marrow transplantation, and in autoimmune diseases.[24] Acute declines in renal function with a fall in GFR and increases in BUN and serum creatinine concentration often occur in the immediate postoperative period following heart and liver transplants. Subacute declines in GFR are almost always observed in all clinical settings in which the drug is used. The decrease in GFR usually develops within a few weeks after cyclosporine therapy is begun, produces a mild to moderate alteration in renal excretory parameters with the serum creatinine concentration rarely rising above 1.5 times baseline values, is reversible with dose reduction, and is not progressive over a follow-up period of 1–4 years. In contrast, in the clinical setting of heart transplantation, a cyclosporine-related chronic progressive decline in renal function, leading in some patients

to renal insufficiency requiring dialytic support, has been described.[26] This renal process is characterized histologically by diffuse interstitital fibrosis and focal glomerulosclerosis. Morphologic alterations consisting of areas of interstitial fibrosis, tubular atrophy, and mild interstitial cell infiltration have also been described in patients treated for a long term with cyclosporine for autoimmune uveitis.[27] The severity of the morphologic abnormalities did not correlate with the average or cumulative dose of cyclosporine. Elevations of serum creatinine levels greater than 50% above baseline values correlated with more severe histologic alterations. The highest serum creatinine concentration in this group of patients followed on average for 2 years was 2.4 mg/dl.[27]

Besides a decline in GFR, other forms of cyclosporine-related renal abnormalities have been described, including hyperkalemia,[28,29] renal magnesium wasting with hypomagnesemia,[30] and hypertension.[31,32] These other forms of toxicity are rarely of sufficient magnitude to cause major clinical complications and are readily treatable.

1.2.2. Pathogenesis

In general, two major processes may result in acute or subacute declines in renal excretory function.[1,2] First, functional hemodynamic alterations that lead to a decline in renal blood flow (RBF) can produce a consequential fall in GFR because of the dependence of GFR on RBF. Additionally, structural injury to renal tubule epithelial cells from toxic or ischemic processes can produce acute renal failure. There presently exists no experimental evidence that cyclosporine is directly toxic to renal tubular epithelial cells after either acute *in vitro* or *in vivo* exposure.[33,34] Thus, no experimental evidence supports a structural basis for the acute effects of cyclosporine on renal excretory function.

Instead, a number of experimental studies have suggested a functional hemodynamic basis for the acute and subacute forms of cyclosporine nephrotoxicity. Cyclosporine has been clearly demonstrated to produce dose-dependent increases in renal vasculature resistance resulting in declines in RBF and GFR, both acutely after intravenous administration and subacutely after several days of parenteral or oral administration.[33,35,36] This persistent modest decline in RBF is consistent with the clinical observations that cyclosporine produces a mild to moderate reduction in GFR which is acutely reversible after discontinuation or dose reduction, and which is, in most instances, nonprogressive.

It has been suggested that direct stimulation of renal nerves producing an α-adrenergic renal vasoconstriction plays an important role in the renal hemodynamic effect of cyclosporine.[35,37] While the precise

role for the renin–angiotensin and prostaglandin systems in this process has not been clarified, an influence of the renin–angiotensin system in this drug effect has been suggested by some studies,[38,39] although not by others.[35] There is also evidence that alterations in renal prostaglandin metabolism produce renal hemodynamic consequences, based on experimental observations that cyclosporine treatment reduces plasma prostacyclin stimulating activity.[40] A direct toxic effect on renal microvasculature has been suggested,[41] but not well established.[42]

The pathogenesis of the acute effects of cyclosporine on renal function immediately posttransplantation is probably related to cyclosporine's renal hemodynamic effects. This dramatic form of acute nephrotoxicity is most commonly seen under circumstances where underlying structural renal tubular cell injury has developed from other processes, most commonly ischemia. In this setting, further cyclosporine-induced declines in renal perfusion may potentiate injury arising from ischemic, toxic, or obstructive processes already occurring in the newly transplanted kidney or delay recovery of injured tubule cells. Consequently, this functional derangement in blood flow produced by cyclosporine could potentially aggravate a separate, but concurrent structural injury process. This thesis, if correct, may explain the clinical observations that acute cyclosporine nephrotoxicity after renal transplantation prolongs recovery from ischemic ATN and potentiates nephrotoxic acute renal failure, thereby resulting in a greater requirement for posttransplant dialysis treatments.

Although disagreement as to whether cyclosporine can produce chronic progressive renal injury and dysfunction continues, preliminary animal data suggest that cyclosporine has the potential to induce an interstitial process characterized by interstitial cell proliferation and fibrosis.[34] Further experiments are necessary to define the precise mechanism of this renal interstitial process by determining whether it is dose dependent and whether it is confined only to the kidney, and to define its relationship to the renal vascular alterations produced by cyclosporine.

1.2.3. Diagnosis

In most clinical conditions, cyclosporine nephrotoxicity results in a mild to modest decline in renal excretory function which is stable, nonprogressive, and reversible with decrease in drug dosage. This diagnosis of cyclosporine nephrotoxicity, therefore, is relatively easy, and renal function is improved with dose adjustment. In the clinical situation of renal transplantation, however, a downward dose adjustment or discontinuation of cyclosporine therapy may precipitate transplant rejection

and, perhaps, graft loss. Therefore, accurate diagnosis of cyclosporine nephrotoxicity and appropriate therapeutic response is essential in the clinical setting of renal transplantation.

Several methods have been suggested to aid in the diagnosis of cyclosporine nephrotoxicity, including measurements of blood, plasma, or serum drug levels, renal biopsy, renal interstitial hydrostatic pressure measurement, and fine-needle aspiration of renal tissue. Although helpful, none of these evaluation maneuvers have proven to be sufficiently specific for or highly predictive of cyclosporine nephrotoxicity.

Whole blood, plasma, or serum cyclosporine levels have been used to adjust dose and diagnose toxicity. Cyclosporine levels can be measured by both radioimmunoassay (RIA) and high-performance liquid chromatography (HPLC) techniques.[43] The RIA method is rapid, but measures both the parent compound and its metabolites. The HPLC method measures only the parent compound, but is much more cumbersome and time consuming than the RIA method. In either method, because cyclosporine is lipophilic, whole-blood levels are at least twice as high as plasma or serum levels owing to the partitioning of the drug into membranes of cellular elements.

The limitation of drug level measurements is the tremendous interpatient and intrapatient variability of cyclosporine absorption and metabolism. Wide day-to-day variations in trough levels are commonly seen in the early postoperative months in individual patients on the same daily dose. Trough plasma levels of cyclosporine ranging from 0 to 1500 ng/ml have been reported in the same patient with no change in clinical, renal, or dosing status.[25] Furthermore, measurements of average serum trough cyclosporine levels during 28 days of daily administration of this drug have demonstrated marked interpatient variabiliy, with values ranging from 40 to 1000 ng/ml.[44]

Interpretation of levels is further confounded by conflicting data on their significance. Although it has been reported that maintenance of plasma or serum cyclosporine trough levels below 250 ng/ml may avoid cyclosporine nephrotoxicity,[45] other data have suggested no correlation between drug levels and the development of cyclosporine-associated declines in renal excretory function.[46]

Histopathologic evaluation of renal biopsy specimens has also been suggested to aid in the differentiation of cyclosporine nephrotoxicity from rejection in the kidney transplant.[47,48] Diffuse interstitial fibrosis with little cellular infiltration or patchy focal areas of interstitial fibrosis surrounding atrophic tubules have been found more commonly in renal biopsy material from cyclosporine-treated patients than non-cyclosporine-treated individuals. Renal tubular cell alterations, including giant mitochondria, tubule cell vacuolization, and microcalcification, have also

been described with cyclosporine therapy. Of note, recent animal work suggests that vacuolization of renal cells may be related to the lipid vehicle used to administer the agent.

Cyclosporine-associated alterations occurring predominantly in the renal arterioles, as opposed to alterations that predominate in the arteries during transplant rejection, have been suggested to be a discriminating feature in cyclosporine nephrotoxicity, but occur in only a small fraction of patients with clinically diagnosed cyclosporine nephrotoxicity.[47] These morphologic alterations, however, are relatively nonspecific for cyclosporine nephrotoxicity, since arteriolopathy can be observed as a consequence of rejection as well. Furthermore, these findings do not correlate well with the degree of renal functional impairment. For these reasons, cyclosporine nephrotoxicity is extremely difficult to accurately differentiate from renal transplant rejection using renal biopsy material. In this regard, many transplant physicians and pathologists will presume cyclosporine nephrotoxicity in the absence of histologic changes consistent with acute rejection. This approach, however, cannot be used to evaluate the possibility of simultaneous transplant rejection and cyclosporine nephrotoxicity.

Elevation in intrarenal hydrostatic pressure[49] and the presence of cyclosporine deposits[50] or changes in the ratio of T-helper cells to T-suppressor cells[51] obtained by fine-needle aspiration of renal tissue have also been utilized in attempts to differentiate between transplant rejection and cyclosporine nephrotoxicity. Unfortunately, these techniques, like cyclosporine levels and renal biopsy material, do not provide sufficient specificity or sensitivity to reliably make this distinction, nor are they predictive of developing cyclosporine nephrotoxicity.

The diagnosis of cyclosporine nephrotoxicity, therefore, is based largely on clinical judgment and exclusion of other processes that may be responsible for a decline in renal function. Verification of this tentative diagnosis may be only empirically achieved if renal function improves within a day or 2 following a decrease in cyclosporine dosage.

1.2.4. Treatment

Since in most clinical conditions cyclosporine nephrotoxicity results in a mild to modest decline in renal excretory function, which is stable, nonprogressive, and reversible with a decrease in drug dosage, no treatment is required except dose adjustment. The risk that transplant rejection may result from this maneuver, however, indicates the importance of careful selection of the appropriate therapeutic response.

Experience indicates that the use of cyclosporine immediately following renal transplantation can potentiate the degree of, and time for

recovery from, ATN that may be present owing to surgical or mechanical difficulties such as extensive warm ischemia time or prolonged machine preservation. Because of this potentiation of delayed graft function, one approach has been to delay cyclosporine therapy until renal excretory function has fully recovered. This approach, however, eliminates the important beneficial effects of cyclosporine on the recognitive phase of the immune response and is probably not the best strategy to address acute cyclosporine nephrotoxicity in allografts with ATN. Many transplant centers have instead elected to treat these patients with antithymocyte globulin plus azathioprine during this early critical phase, followed by conversion from azathioprine to cyclosporine once renal function has improved. This approach has had excellent early results. If renal failure and oliguria persist for more than 3 weeks, however, conversion from cyclosporine to azathioprine therapy may be indicated, since recovery of renal function with continued cyclosporine therapy is unlikely.

On the other hand, an increased risk of graft rejection has been associated with this conversion of therapy.[52,53] Consequently, a compromise approach, consisting of a marked reduction in cyclosporine dosage and addition of azathioprine to convert from double (prednisone and cyclosporine) to triple (prednisone and lower doses of cyclosporine and azathioprine) therapy, may be the best alternative.[54,55]

In the early period following renal transplantation, a renal biopsy may assist in choosing a therapeutic strategy, since the finding of diffuse interstitial fibrosis with mild cellular infiltration may be more consistent with the diagnosis of cyclosporine nephrotoxicity than with acute transplant rejection.[47] As discussed, however, the utility of this procedure to differentiate between these two possibilities remains under considerable debate.

The most difficult decision point regarding alterations of cyclosporine dosing in the renal transplant patient occurs several weeks or months following allograft placement when renal excretory function begins to deteriorate after initial improvement has occurred. A clinical diagnosis of rejection as a cause of renal deterioration requires an increase in immunosuppressive therapy, while a diagnosis of cyclosporine nephrotoxicity necessitates a decrease in immunosuppression therapy with downward dose adjustment of cyclosporine. An error in diagnosis and therapeutic response at this stage will, therefore, increase the risk of rejection if a mistaken diagnosis of cyclosporine nephrotoxicity is made, or increase the risk of overimmunosuppression or progressive nephrotoxicity if a mistaken diagnosis of transplant rejection is reached.

At this critical juncture, further corroborative information can be obtained from the history and physical examination, renal biopsy material, serum drug levels, and small-needle aspiration. As previously dis-

cussed, while this additional information may indicate a high probability diagnosis, more often, no definitive diagnosis can be made. Hence, whether or not to undertake a potentially risky invasive procedure such as renal transplant biopsy is currently a highly debatable topic, since the likelihood of making an unequivocal diagnosis from morphologic evaluation is small.

Instead, under most circumstances, an empiric trial of cyclosporine dose reduction is probably the best approach to this problem. Since subacute cyclosporine nephrotoxicity is due predominantly to a functional renal hemodynamic effect of the agent, renal excretory function should show some improvement within 24 hr of dose reduction. If no improvement in renal function occurs after this empiric trial, rejection can be presumed to be the basis of the renal deterioration, and more aggressive antirejection therapy can still be initiated.

A small but substantial number of renal transplant patients will continue to have persistent elevations in serum creatinine concentration exceeding 3 mg/dl which does not improve after either cyclosporine dose reduction or increased steroid therapy. This group may well define patients with chronic cyclosporine nephrotoxicity.

Two approaches have been used to treat this clinical presentation, namely, complete conversion from cyclosporine to azathioprine, or conversion to triple therapy (prednisone plus lower doses of cyclosporine and azathioprine). Superior results appear to occur with use of triple therapy using low-dose cyclosporine (2–3 mg/kg), azathioprine, and steroids rather than complete conversion to azathioprine from cyclosporine.[52–55]

There are other strategies that should be considered to minimize the occurrence of cyclosporine nephrotoxicity. Doses much lower than those used during early clinical trials are uniformly recommended. Most investigators now agree that the initial doses of cyclosporine should be 14 mg/kg orally or less. The dose of cyclosporine should be aggressively tapered to achieve 6–8 mg/kg by 60 days and 5–6 mg/kg by 180 days if no significant rejection episodes have occurred. To avoid high peak serum levels, intravenous cyclosporine administration should be given as a slow infusion over 6 to 24 hr, rather than as a bolus injection.

Other drugs that either alter the metabolism of cyclosporine or alter renal hemodynamics should also be avoided. Since cyclosporine is metabolized by the hepatic cytochrome P-450 system and excreted in the bile, drugs that alter the activity of the hepatic P-450 system may have pronounced effects on the metabolism and clearance of cyclosporine and consequently may alter cyclosporine blood levels.[56] For instance, drugs that inhibit hepatic cytochrome P-450 activity, such as ketoconazole and erythromycin, increase cyclosporine plasma levels[43,56,57] and should

be avoided, if possible, during cyclosporine therapy or cyclosporine dose adjustments made if their use is necessary. Other drugs, including phenytoin and rifampin, increase the metabolic conversion of cyclosporine and decrease the plasma trough levels of cyclosporine.[43] Changes in cyclosporine levels resulting from these drug interactions undoubtedly influence the development and degree of cyclosporine nephrotoxicity and should be kept in mind when dose adjustments are being made.

Other drugs that are nephrotoxins or that influence renal hemodynamics have the potential to aggravate cyclosporine nephrotoxicity. As an example, amphotericin B promotes renal vasoconstriction and is commonly used in the bone marrow transplant population to treat fungal infections. The concomitant use of this antibiotic has been clearly shown to increase the incidence of cyclosporine nephrotoxicity,[58] undoubtedly because of the combined renal vascular effects of the antibiotic and cyclosporine. Dose adjustments may help to prevent this complication.

Drugs that inhibit prostaglandin synthesis, including aspirin and nonsteroidal antiinflammatory agents, also promote declines in renal blood flow in disease states in which vasodilatory prostaglandins are required to maintain RBF. Prostaglandin inhibition with these agents may potentiate the declines in RBF produced by cyclosporine[43] and should also be avoided if possible during cyclosporine treatment. Concomitant use of other nephrotoxic agents, especially aminoglycoside antibiotics,[43,59] should also be used with care while administering cyclosporine.

Cyclosporine is an exciting new immunosuppressive agent with well-defined, but ever-expanding clinical utility. Nephrotoxicity is the key limiting complication for its clinical use. Recent data have demonstrated that this agent exerts its acute and subacute nephrotoxic effects via a functional prerenal hemodynamic effect which is reversible with dose reduction. A chronic nephrotoxic effect resulting in renal interstitial fibrosis also appears to occur with the use of cyclosporine. The pathophysiology and ultimate modulation of this chronic complication is presently being investigated. Although much needs to be learned, substantial information about the nephrotoxicity of this new immunosuppressive agent has occurred, so that cyclosporine is already being used more effectively and with less nephrotoxic complications.

2. Postischemic Acute Renal Failure

It is now generally accepted that postischemic acute renal failure is due to injury limited to segments of the nephron which lead primarily to intratubular obstruction from cellular debris and backleak of glo-

merular filtrate across a damaged renal tubular epithelium. The understanding of the pathogenesis of postischemic acute renal failure is thus the understanding of the pathophysiology of ischemic cell injury. From the available data it now appears that the pathogenesis of ischemic cell injury is due to the simultaneous derangement of several critical metabolic processes that act in concert to produce a cascade of events that finally lead to plasma and subcellular membrane dysfunction incompatible with the maintenance of cell viability and integrity. As detailed in Fig. 2, a tentative scheme can be formulated from available data interrelating a variety of metabolic processes including depletion of high-energy phosphate compounds, cellular calcium derangements, and membrane phospholipid degradation and loss. Ischemia directly leads to declines in the rate of oxidative phosphorylation due to a lack of oxygen availabilty. A fall in cellular ATP levels develops. Ischemia also promotes redistribution of intracellular calcium pools and results in phospholipase activation and phospholipid degradation. When phospholipid degradation occurs concomitantly with a decline in levels of high-energy phosphate compounds, phospholipid synthesis cannot keep pace with phospholipid degration, and net membrane phospholipid loss occurs with accumulation of potentially toxic lipid by-products. An increase in plasma membrane calcium permeability develops, and the influx of calcium down its electrochemical gradient from extracellular to intracellular spaces occurs. This calcium is taken up and sequestered in

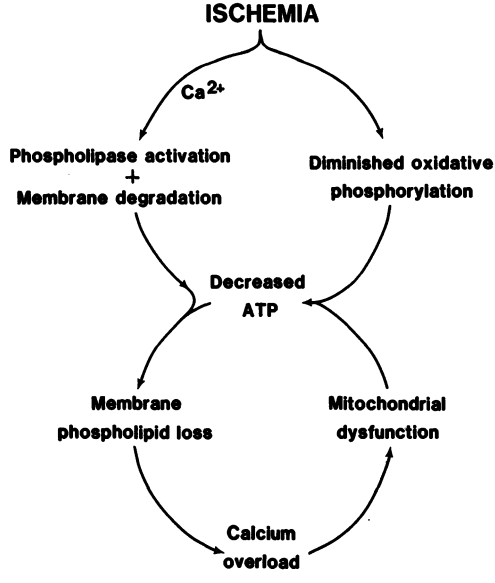

Fig. 2. Proposed pathogenesis of ischemic cell injury.

mitochondria and causes further alterations in mitochondrial structure and function, leading to further decline in cellular ATP content. Therefore, there eventuates an unremitting vicious cycle of progressive cellular membrane deterioration, cellular energy store depletion, and deranged cellular bioenergetics, until reparative and synthetic processes cannot keep pace with degradative events. The irreversible and lethal cell injury is the ultimate outcome. The following sections of this chapter will detail the experimental evidence of the role of these critical processes in the pathophysiology of ischemic cell injury. In addition, the role of free-radical production with resulting lipid peroxidation as a critical event in the pathogenesis of this disease process will be summarized.

2.1. Plasma Membrane Alterations and Renal Ischemia

The minimum criterion for cell viability is continued maintenance of the intracellular space as a distinct compartment relative to the extracellular fluid. This process is ultimately dependent on the structural and functional integrity of the permeability barrier provided by the plasma membrane. This integrity allows for retention of cytosolic macromolecules within the cell and restricts ionic permeability to degrees compatible with the capacity of energy-requiring plasma membrane pumps, such as Na,K-ATPase and Ca-ATPase, to fully compensate for normal ionic leakage which does occur. The role of the plasma membrane as a permeability barrier is probably its most critical function in the maintenance of cell viability. It has become evident that ischemia causes lethal cell injury by inducing critical membrane alterations via various intracellular mechanisms.

One of the earliest morphologic changes that occurs in renal ischemia is the loss of brush border membranes of the proximal tubules.[60–62] After 25 min of ischemia without reflow, proximal tubular cells and their intracellular organelles appeared considerably swollen morphologically. Brush border microvilli appeared club-shaped and bulbous, but maintained their general architecture. Immediately following resumption of renal blood flow, a striking morphologic transformation occurred in the brush border. The microvilli appeared to lose their stability, assumed misshapen configurations, and coalesced laterally at multiple points by a process of membrane fusion. This process rapidly led to incorporation of large areas of brush border membranes into the apical cytoplasm of the epithelium in the form of sacs, vesicles, and whorls. Consequently, almost the entire brush border showed a picture consistent with major loss of membranes.[60–62] Brush border membrane disintegration and formation of free-floating membrane blebs occurred to a moderate degree. In contrast, after 60 min of ischemia, membrane

blebs derived from brush border membrane were found in large numbers in tubule lumen and practically obstructed every nephron of the ischemic kidney.[63]

These data indicate that even mild reversible renal ischemia can cause extensive brush border membrane alterations. The extent of brush border membrane damage correlated with the duration of ischemia. Physiologic alterations implicating surface membrane dysfunction at the nephronal level include loss of selective permeability,[63] reductions in proximal tubular fluid reabsorption,[64] and sodium and glucose transport.[62] At the cellular level, ischemic plasma membrane alterations can cause increases in intracellular sodium[65] and calcium[66,68] and loss of intracellular potassium.[65]

The pathogenesis of plasma membrane damage during renal ischemia is complex and involves several interrelated processes. These intracellular events occur simultaneously and cause injury not only to the plasma membrane, but also to other subcellular organelles. These processes, as detailed in the following sections, include activation of phospholipases with resulting membrane phospholipid alterations,[67] elevated cytosolic free calcium, which can affect membrane permeability directly or indirectly,[68] free-radical-induced membrane lipid peroxidation,[69] and loss of high-energy phosphate stores.[70]

A recent study has examined more precisely the specific changes of brush border (BBM) and basolateral membranes (BLM)[71] during ischemia. Fifty minutes of ischemia without reflow caused a loss of BBM enzyme markers, leucine aminopeptidase, and alkaline phosphatase. This loss of enzyme markers reflected the alterations of BBM seen during ischemia. There was, however, a significant increase in the specific activity of Na,K-ATPase, a BLM marker enzyme, in the ischemic BBM fraction. In addition, Na,K-ATPase was localized cytochemically in abnormally whirled membrane fragments of apical BBM.[71] A loss of surface membrane polarity appears to be the cause of increases in ischemic BBM Na,K-ATPase, although contamination of the BBM fraction by BLM could not be entirely ruled out. The maintenance of surface membrane polarity is essential for tubular cells to accomplish net transcellular transport. Therefore, the loss of membrane polarity could cause important functional alterations at both cellular and nephronal levels. In addition, ischemia resulted in major changes in the BBM lipid composition. The cholesterol-to-phospholipid ratio decreased, while the individual phospholipid, sphingomyelin and phosphatidylserine, levels decreased,and phosphatidylcholine and phosphatidylinositol, as well as lysophosphatidylcholine, levels increased in ischemic BBM fractions.[71]

Although there was no change in the BLM marker enzyme Na,K-ATPase, there was, however, an alteration in BLM phospholipids during

ischemia. Significant increases in both phosphatidic acid and lysophosphatidylcholine were found.[71] The role of lysophosphatidylcholine and phosphatidic acid in increasing membrane transport of calcium has been described.[72,73] Therefore, ischemia results in abnormal accumulation of putative calcium ionophores in the BLM of proximal tubule cells. This process, in turn, could lead to increased tubule cell calcium permeability during ischemia.[66]

It has become clear that alterations in membrane structure and function represent the most critical factor leading to lethal cell injury in renal ischemia. The understanding of mechanisms responsible for membrane damage is, therefore, of great importance to develop methods to prevent cell injury and the clinical occurrence of ischemic acute renal failure.

2.2. Phospholipids in Ischemic Injury

Phospholipids provide the major structural framework for cell membranes[74] and also participate in the regulation of membrane enzyme activity, permeability, and hormone activation. It has been proposed that membrane phospholipid alterations induced by tissue ischemia account for metabolic changes critical in the evolution of cell injury and death.

2.2.1. Phospholipid Alterations During Ischemic Cell Injury

The effects of tissue ischemia on cellular phospholipid composition have been extensively studied in liver and heart. The review of those studies is relevant to renal ischemia. The nature of phospholipid changes during ischemia, however, varies, depending on experimental models, the nature of tissues, or animal species in use. Finkelstein and co-workers[75] have made significant contributions to our understanding of lipid metabolism in liver ischemia. Within 3 hr of liver ischemia, 30% of the cellular phospholipid was lost. All phospholipid species were equally affected, and there was no accumulation of lysophospholipids. There was no increase in the size of the free fatty acid pool, and the content of long-chain acyl CoA esters decreased by 50%. The acyl chain composition of the free fatty acid and neutral lipid pools changed, however, to resemble more closely that of the phospholipids. These data are consistent with a loss of fatty acyl chains from the phospholipids into the free fatty acid pool. Similar loss of phospholipids was found in primary cultures of adult rat hepatocytes made anoxic by evacuation of the CO_2–O_2 atmosphere with N_2.[76] The depletion of cellular lipids was paralleled by an accumulation of hydrophilic degradation products in the culture medium. Phosphorylethanolamine accounted for 50% of these products,

with equal amounts of glycerophosphorylethanolamine and ethanolamine accounting for the other 50%.

During ischemia subcellular membranes also undergo change in lipid composition. Interruption of the blood supply to rat liver produced a 55% loss of phospholipids from microsomal membranes.[77] Phosphatidylcholine and phosphatidylethanolamine were predominantly affected, without accumulation of either lysophosphatidylcholine or lysophosphatidylethanolamine. Alterations in liver mitochondrial membrane in response to ischemia have also been reported.[78] Similarly, studies on ischemic myocardial cell injury have shown depletion of membrane major structural phospholipids without[79,80] or with accumulation of lysophospholipids,[81] increased tissue levels of free fatty acids,[82] long-chain acyl-CoA, and acyl carnitine.[83]

Phospholipid metabolism has recently been studied in ischemic renal cell injury. Complete occlusion of the renal artery for variable time durations[75] significantly decreased phospholipid content of renal cortex only after 6–8 hr. The phospholipid loss increased with the duration of ischemia and reached 30% of the total phospholipid by 18 hr of ischemia. Most major classes of phospholipids were affected, and there was no accumulation of lysophospholipids. The number of acyl chains in total lipid extracts of ischemic renal cortex decreased parallel with the loss of phospholipids. The decrement in phospholipid acyl chains could be entirely accounted for in an accumulation of free fatty acids. These data suggest that the elevated free fatty acids were the product of the degradation of phospholipids. Matthys *et al.*[67] studied the change in lipids of the renal cortex and outer stripe of outer medulla in rats during ischemia and 2 hr after blood reflow. After 15 min of ischemia, there were marked elevations of free fatty acids and diacylglycerol, increasing further at 60 min of ischemia. These elevations were accompanied by alterations in phospholipids, including elevation of lysophosphatidylcholine at 15 min and phosphatidic acid at 15 and 60 min. Two hours after 15 min of ischemia, lysophosphatidylcholine returned to control levels and other phospholipids were normal, except phosphatidylinositol, which was decreased, and phosphatidic acid, which remained elevated. Free fatty acids and diacylglycerol approached or reached control values. Two hours after 60 min of ischemia, lysophosphatidylcholine, free fatty acids, diacylglycerol, and phosphatidic acid remained elevated; phosphatidylcholine and phosphatidylinositol remained decreased. Histologic injury was seen only in kidneys injured by 60 min of ischemia. Thus, irreversible ischemic damage correlated with persistent abnormalities of content of specific membrane phospholipids and accumulation of free fatty acids. Preliminary experiments on the effects of *in vitro* hypoxia on phospholipid metabolism of isolated proximal tubule seg-

ments in suspension showed that the composition of individual phospholipids, expressed as percent of total phospholipid content, did not change even in the presence of severe cell injury induced by 60 min of hypoxia. In contrast, free fatty acid levels increased after as early as 22 min of hypoxia. This level continued to rise with increasing duration of hypoxia and reached several times the control levels after 60 min of hypoxia. There was a good correlation between the degree of cell injury and free fatty acid levels.

2.2.2. Mechanisms Responsible for Phospholipid Alterations during Ischemic Injury

Ischemia-induced phospholipid depletion can be a result of either an accelerated rate of breakdown, including deacylation, or an inhibited rate of synthesis, including reacylation of lysophospholipids. In order to determine the role of increased rates of phospholipid degradation during liver ischemia, Chien *et al.*[77] prelabeled cellular phospholipids *in vivo* with [^{14}C]glycerol. Ischemia produced a rapid loss of specific radioactivity from the total phospholipids and from phosphatidylcholine and phosphatidylethanolamine compared to control. Similar results were found in anoxic rat hepatocytes in culture[76] and ischemic myocardium.[79] These results indicate that accelerated degradation is the major factor accounting for phospholipid depletion. At the same time there is decreased reacylation of lysophospholipids in ischemic liver cells,[75] as suggested by the decreased rate of incorporation of [^{3}H]arachidonic acid into phospholipids after this radioactive tracer was injected into the portal vein at the beginning of blood reperfusion. The reduction in the rate of esterification of radiolabel occurred even with as little as 20 min of ischemia and became more marked with ischemia of longer duration.

The accelerated phospholipid degradation is probably due to activation of membrane phospholipase activity. The phospholipase activity is suggested by accumulation of free fatty acids and lysophospholipids,[67] hydrolysis products of phospholipases A_1 and A_2 that specifically remove the fatty acid esters of the alcohol glycerol during ischemia. A relatively larger increase in polyunsaturated free fatty acids[67,84] than in saturated free fatty acids indicates the predominant action of phospholipase A_2, since this enzyme specifically removes the fatty acid attached to the second carbon of glycerol. This position is usually occupied by an unsaturated fatty acid. The role of phospholipase is further clarified by treating isolated rabbit renal proximal tubule segments with exogenous phospholipase A_2.[85] Phospholipase treatment of well-oxygenated tubules caused mild phosphatidylethanolamine depletion and moderate increases in lysophosphatidylcholine and lysophosphatidylethanolamine

levels, but did not result in alterations in tubule cell viability. Phospholipase treatment of mildly hypoxic tubules greatly potentiated cellular injury which occurred in response to hypoxia alone. This potentiation of cell injury was associated with marked phospholipid loss and increases in the levels of lysophospholipids and free fatty acids. Addition of ATP-$MgCl_2$[86] to hypoxic tubules treated with phospholipase A_2 lessened the phospholipid alterations and significantly reduced the degree of cell injury. These data suggest that accelerated phospholipid degradation can be produced by exogenous phospholipase. When this degradation is not balanced by an increased rate of phospholipid resynthesis because of a decrease in high-energy phosphate stores within cells during ischemia, phospholipid loss occurs, leading to severe cell injury. Further evidence of the role of phospholipase in ischemic injury was provided by Chien *et al.*,[77] who treated liver microsomes with exogenous phospholipase A_2. This treatment resulted in decreased phospholipids and membrane dysfunction such as inhibition of glucose-6-phosphatase, calcium pump activities, and increased calcium permeability. Moreover, pretreatment with phospholipase inhibitors, chlorpromazine,[77] or dilazep[87] protected against ischemic injury and prevented the loss of phospholipids.

Although there is good evidence for the role of phospholipase activation in ischemic injury, the mechanism responsible for the activation of phospholipases is not clear. A role for elevated cytosolic free calcium induced by ischemia[88] in the activation of phospholipase has been suggested. In this regard, Chien *et al.*[89] studied endogenous phospholipase in rat liver microsomes. Incubation with 5 mM $CaCl_2$ at 37°C caused progressive loss of phospholipids, with phosphatidylethanolamine being the predominant lipid that was degraded. Phosphatidylcholine levels declined at approximately half the rate of phosphatidylethanolamine. The loss of these two major phospholipids was accompanied by only a very slight accumulation of their respective lysophospholipids, suggesting the presence of lysophospholipase activity. These data suggest that endogenous phospholipase can be activated by calcium addition *in vitro*.

Free radicals have also been suggested to stimulate endogenous phospholipase activity. Au *et al.*[90] studied the effect of xanthine oxidase/hypoxanthine/ADP-Fe^{3+}, an exogenous free-radical-generating system, on isolated brain capillaries. Free radicals induced a significant loss of phosphatidylcholine and phosphatidylethanolamine and an increase in free fatty acids, in particular polyunsaturated fatty acids. Pretreatment with chloroquine or mepacrine, amphiphilic cationic inhibitors of phospholipase A_2, effectively inhibited the action of xanthine oxidase, preserving the phospholipid profile pattern. This report suggests that a free-radical-generating system has significant effects on membrane phos-

pholipid degradation, and that these effects can be prevented by phospholipase A_2 inhibitors. It is unclear, however, whether free radicals have direct stimulatory effects on membrane phospholipase or act indirectly by promoting release of free calcium ions from subcellular organelles[91] with subsequent phospholipase activation by increased cytosolic calcium levels. The evidence that free radicals can induce calcium-independent phospholipid degradation is demonstrated by incubating rat liver lysosomes with an exogenous free-radical-generating system (dihydroxyfumarate + Fe^{3+}-ADP) in a calcium-free medium at a pH adjusted to 6.0. Free radicals induced a rapid degradation of phosphatidylethanolamine and phosphatidylcholine and a significant rise in their related lysophospholipids, without any change in free fatty acids.[92] These data suggest that free radicals can accelerate membrane phospholipids by a mechanism independent from calcium. This effect may be related to the increased susceptibility of membrane-peroxidized phospholipids to the action of phospholipases.[93] Alternatively, the physical changes within the membrane bilayer, resulting from free-radical injury, may result in increased phospholipase activity.

2.2.3. Correlation between Phospholipid Alterations and Ischemic Cell Injury

Ischemic injury results in membrane structural phospholipid depletion and accumulation of phospholipid lipid by-products. The pathogenetic role of these lipid alterations in ischemia remains hypothetical at this time. Declines in phospholipid content may not be a critical event in the pathophysiology of ischemic cell injury.[94,95] Instead, the accumulation of a variety of products of abnormal lipid metabolism during ischemic cell injury, such as free fatty acids,[94,95] acylcarnitine, acyl-CoA, and lysophospholipids, may be more critical in the pathogenesis of cellular injury in ischemia. The main study of these agents has been in models of heart and liver injury. The general propensity for these lipid metabolites to induce cellular damage probably rests, in large part, on their amphiphilic nature, i.e., the presence of both hydrophilic and hydrophobic components within their molecular structure. This property allows their interaction with and incorporation into cellular membranes in a variety of ways, depending on the size, configuration, and quantity of amphiphile present. This interaction may markedly alter the structural and functional properties of those membranes.[83]

Unsaturated fatty acids and lysophosphatidylcholine or lysophosphatidylethanolamine demonstrated marked cytotoxicity when added to primary cultures of proximal tubule cells.[67,96] Fatty-acid-free bovine serum albumin protected, in part, against the injurious effects of exogenous

phospholipase A_2 treatment of hypoxic isolated rabbit proximal tubules.[94] This protective effect is probably related to the ability of albumin to bind free fatty acids generated by the action of phospholipase A_2. The decreased levels of free fatty acids would cause less cell injury. The mechanisms by which free fatty acids cause cellular damage are still uncertain. Free fatty acids have been shown to induce a calcium efflux from isolated liver and kidney mitochondria.[97] This calcium efflux was not inhibited by ruthenium red but was specifically inhibited by sodium and lithium. Free fatty acids and their metabolites, acyl-CoA and acylcarnitine, have also been reported to cause a calcium-dependent alteration in the permeability of the mitochondrial inner membrane, large amplitude swelling, inability to generate a membrane potential, loss of respiratory control with ADP, dinitrophenol, or valinomycin, and changes in ATPases of rat liver mitochondria,[98,99] as well as inhibition of sarcolemmal Na,K-ATPase activity.[100] In ischemic tissue it has been proposed that high levels of acyl-CoA esters may produce inhibition of mitochondrial adenine nucleotide translocase activity, contributing to the inability of oxidative phosphorylation to recover when reoxygenation occurs.[101] Moreover, acyl-CoA, acylcarnitine, and lysophosphatidylcholine were found to potentiate the free-radical-induced lipid peroxidative injury to sarcolemmal membranes.[102] Lysophospholipids induced profound electrophysiologic derangements in canine and sheep Purkinje fibers *in vitro* analogous to those seen in ischemic myocardium *in vivo*, implicating these lipid metabolites as potential progenitors of dysrhythmia during ischemia.[103,104] In liver, lysophospholipids caused the release of calcium from isolated mitochondria.[105] However, this effect of lysophospholipids is not associated with a significant impairment of the integrity of the mitochondria, as monitored by measurement of membrane potential and the rate of respiration. In short, these lipid metabolites clearly have detrimental effects on cellular membrane functions and integrity. The extent to which these processes may be active in *in vivo* models of ischemia, however, has not been clearly established.

2.3. Alterations of Cellular Calcium Metabolism and Ischemic Injury

Tissue calcium levels invariably increase when lethal cell injury develops in a tissue that is perfused or reperfused with extracellular fluid containing calcium.[106–108] This increase in tissue calcium levels is due predominantly to mitochondrial accumulation which occurs even when the cell is severely injured. The simultaneous occurrence of cell calcium overload and lethal cell injury, however, only establishes an association between these two events, but does not prove causality. The presence

of calcium overload in irreversibly injured tissue may only be the product of processes that produce membrane dysfunction, so that cellular calcium overload only occurs as a final event after the cells within the tissue have progressed beyond the stage of reversible injury.[106,107]

Interpretation of data that attempt to provide insight into the role of calcium in cell injury must, therefore, be accomplished with the view that damaged tissues often have cells within the area of injury with varying degrees of cell injury. In particular, the structural heterogeneity of the kidney adds a complexity to any analysis of the role of calcium in the pathophysiology of tubular cell injury. Susceptibility to ischemic injury varies greatly between segments of the nephron. S_3 segments of the proximal tubule exhibit substantially greater sensitivity than S_1 and S_2 segments to ischemic insults.[109] Accordingly, heterogeneous cell populations will exist ranging from lethally damaged cells to sublethally damaged and apparently normal cells. Although tissue calcium overload may occur owing to irreversible injury in cells that are already destined to become necrotic, this finding cannot be interpreted to suggest that altered cell calcium metabolism is the critical causal event in the injurious process. Furthermore, a maneuver that protects both tissue injury and tissue calcium overload does not necessarily suggest that calcium was a critical determinant in the transition from reversible to irreversible cell injury. Any maneuver that limits the degree of cell injury within a tissue consequently will limit the degree of tissue calcium overload, since the calcium accumulation by necrotic and lethally injured cells will also be reduced.[68]

To better implicate an important role for calcium in the development of cell injury, it is required to show that alterations in cell calcium metabolism precede the stage determining the transition from reversible cell injury to irreversible cell injury. But since the definition of transition from sublethal to lethal cell injury is difficult and the damaging action of calcium rapid, it is often difficult to show that derangements in cell calcium precede critical events determining irreversible cell injury. Owing to these problems, the proof for an important role for calcium in cell injury has been attempted by determining whether the development of lethal cell injury can be altered by modifying the effects or availability of calcium on various cellular processes and subcellular sites critical in the pathogenesis of cell injury.[106]

Major sites of deleterious calcium action in the cell include the plasma membrane, the mitochondria, the endoplasmic reticulum, and the cytoskeleton. Mechanisms by which calcium promotes injury include activation of free and membrane-bound phospholipases, alterations of membrane permeability properties, due to direct effects of calcium both on permeability and on activity of membrane phospholipases,[66] and ef-

fects on intracellular contractile and cytoskeletal structures. Of those critical sites for cell injury, mitochondria appear to play a major role in evolving cell injury for several reasons. A large fraction of intracellular calcium is normally sequestered within mitochondria; this sequestered mitochondrial calcium pool is, in large part, releasable to the cytosol to redistribute to critical sites within the cell.[106,110,111] The quantitation of mitochondrial and cytosolic free-calcium Ca_i^{2+} levels and changes in these levels during developing cell injury is thus critical in better understanding the role of calcium in cell injury.

The study of changes in cytosolic free-calcium levels has been hampered by technical difficulties until recently. Snowdowne *et al.*[88,112] used aequorin, a photoprotein, to measure Ca_i^{2+} in anoxic monkey kidney cells (LLC-PK_1) in culture. The lack of oxygen caused a rise in Ca_i^{2+} within a few minutes to a plateau approximately 25% above normal basal levels. Ca_i^{2+} remained constant at this plateau for almost 10 min. This initial rise in Ca_i^{2+} probably came from sequestered Ca_i^{2+} in mitochondria whose calcium transport was altered by lack of oxygen.[113] Support for this statement is suggested by the finding that the rise in Ca_i^{2+} above basal levels evoked by the uncoupler of mitochondrial oxidative phosphorylation, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone, was similar to that observed during the first 10 min of anoxia. In addition, this initial increase in Ca_i^{2+} was not caused by an increase in calcium influx into cells, since lowering perfusate $CaCl_2$ content from 1.3 to 0.1 mM or addition of lanthanum, which presumably blocks calcium entry into cells, did not abolish this initial rise in Ca_i^{2+}. In a second phase, Ca_i^{2+} rose again in a quasilinear fashion to a peak, 2.6-fold rise above control levels after 60 min of anoxia. This rise in Ca_i^{2+} was coupled to a comparable increase in cellular calcium efflux, which rose 2.5-fold above control levels after 60 min of anoxia. The rise in Ca_i^{2+} during the secondary phase is probably due to an increase in calcium influx, since cellular ^{45}Ca uptake was increased sevenfold during this period. Moreover, this rise in Ca_i^{2+} was markedly reduced or abolished by lanthanum as well by lowering the extracellular calcium concentration.

These data suggest that the initial rise in cytosolic free calcium levels due to the release of calcium sequestered in mitochondria during the first 10 min of anoxia is a critical step in initiating metabolic alterations in plasma membrane and other subcellular organelles. The resulting plasma membrane permeability alteration leads to further increases in Ca_i^{2+}. However, total cellular calcium did not increase, because of the simultaneous increase in calcium efflux as well as the markedly reduced calcium uptake by anoxic mitochondria.[113]

The mechanism by which calcium induces increased membrane permeability remains unclear. It has been suggested that the initial rise in

Ca_i^{2+} activates endogenous phospholipase A_2.[89,107] The activation of phospholipase causes degradation and loss of major structural phospholipids and the accumulation of potentially toxic lipid by-products, free fatty acids, and lysophospholipids,[67,75] which increase the plasma membrane permeability. Of note, since the initial rise in Ca_i^{2+} takes place in the early phase of anoxia, all the events leading to cell membrane permeability alterations may occur in less than 10 min. The time course study of phospholipid changes during ischemia has shown that accumulation of lysophospholipids,[67,95] free fatty acids,[95] is a relatively early event and precedes the loss of major phospholipids, which becomes apparent only after several hours of ischemia.[95] Therefore, if calcium-induced phospholipase activation is a major cause of membrane dysfunction, the increased membrane permeability will be more likely related to accumulation of phospholipid metabolites, free fatty acids, and/or lysophospholipids, rather than to the loss of structural phospholipids.

Upon reperfusion of ischemic renal tissue, both cellular and mitochondrial calcium overload develop.[114,115] After 45 min of bilateral renal pedicle clamping, the calcium content of mitochondria isolated from renal cortex was slightly elevated and progressively increased over the next 24 hr.[115] Although the magnitude of mitochondrial calcium content was less when isolation occurred in the presence of ruthenium red,[114] significant and progressive mitochondrial calcium overload developed during reperfusion and therefore reflected calcium uptake that occurred *in vivo*. The functional alterations related to increased mitochondrial calcium content are described in Section 2.4.

Microsomal function is also altered during ischemia.[116] After 90 min of bilateral renal pedicle clamping, ATP-dependent calcium uptake by microsomes isolated from renal cortex was significantly impaired immediately after the clamp release as well as after 1 hr of reflow, but not at 24 hr. Since the calcium uptake process in endoplasmic reticulum is energy dependent, low renal cortex tissue concentrations of ATP[113,117] could reduce the calcium uptake by the endoplasmic reticulum. In addition, high cytosolic free-calcium levels can activate endogenous phospholipases, which may then injure endoplasmic reticulum membranes.[89] Although the endoplasmic reticulum plays a critical role in regulating Ca^{2+} during normal cellular processes, the contribution of endoplasmic reticulum to the regulation of cytosolic calcium in ischemic tissue is not known. During injurious events with large increases in cellular Ca^{2+}, mitochondrial Ca^{2+} buffering appears more critical.

These experiments using both the *in vivo* approach and studies of organelles isolated after *in vivo* maneuvers are limited by an inability to clearly interpret results regarding the role of critical processes in evolving

cell injury. Consequently, more direct and controllable experimental designs utilizing *in vitro* systems have been developed to further probe the role of calcium in ischemic renal tubular cell injury. Studies utilizing *in vitro* suspensions enriched in isolated rabbit proximal tubule segments demonstrated that increases in tubule calcium content occurred between 15 and 30 min of hypoxia.[118] These elevated levels, however, returned close to normal levels during a 60-min reoxygenation recovery period after hypoxia, indicating that a phase of reversible net calcium entry into renal tubule cells occurs during hypoxic injury. Additional studies have been conducted, using primary cultures of microdissected rabbit proximal tubules,[119] which demonstrated that lethal cell injury developed in virtually all cells if posthypoxic recovery occurred in normal calcium-containing media. If the posthypoxic recovery occurred in media lacking in calcium, however, survival of approximately 30% of cells occurred. These results suggest that hypoxia-induced lethal cell injury can be attenuated by decreasing calcium availability during the posthypoxic recovery period.

Further support for a role of calcium in ischemic acute renal failure derives from the protective effects of calcium channel blockers in experimental models of this disorder. It has been reported that treatment with the calcium channel blocker verapamil during an ischemic insult to the kidney induced by norepinephrine administration lessened the resulting degree of acute renal failure without affecting renal perfusion.[120] This effect was accompanied by lesser rises in tissue and mitochondrial calcium levels at 24 hr. These data are consistent with a protective role of verapamil against renal tubule epithelial injury in this ischemic mode, but do not prove that it occurred by an antagonistic effect on cellular calcium uptake during or just following the ischemic insult. In fact, a study in the rat has demonstrated that verapamil protected against ischemic renal injury when the injury was induced by norephinephrine, but not when global ischemia was produced by renal artery clamping.[121] The protective effect of verapamil during norepinephrine infusion appeared to be due to the ability of the calcium channel blocker to ameliorate norepinephrine-induced renal vasoconstriction. Preliminary *in vitro* studies also do not completely answer the question of whether the protective effect of verapamil on ischemic acute renal failure *in vivo* is due to direct cellular or indirect hemodynamic alterations. In studies of hypoxic damage to *in vitro* suspensions of proximal tubule segments, verapamil had mild effects to preserve various metabolic parameters reflective of cell injury.[122] These protective effects were observed only at levels of the agent that are not achievable after *in vivo* administration. Studies utilizing primary cultures of proximal tubule cells, on the other hand, demon-

strated that protection against hypoxia-induced cell injury occurred at concentrations of verapamil that could be achieved after *in vivo* administration.[119]

In summary, in renal cell injury evidence exists for both intracellular calcium shifts during ischemia and abnormal net calcium influx into the cell during reperfusion. The redistribution of intracellular calcium pools during the early stages of cell injury appears to play a more critical role in development of ischemic cell injury. The precise role that Ca^{2+} plays in the complex pathophysiology of ischemic renal cell injury, and hence in postischemic acute renal failure, has only just begun to be understood.

2.4. Mitochondrial Alterations in Ischemic Cell Injury

Maintenance of normal cell function is dependent on the production and utilization of adenosine triphosphate (ATP). Renal tubular cells contain large numbers of mitochondria to provide sufficient quantities of ATP via oxidative metabolism to maintain transport and other energy-consuming processes. The functional capacity of mitochondria during complete ischemia is relatively unimportant for the tissue, since the availability of oxygen, the final necessary component of oxidative phosphorylation, is severely limited. Impaired mitochondrial function, however, becomes of great importance when oxygen supply is restored and the capacity for repair requires replenishment to the cell of abundant high-energy phosphates, a function dependent on intact mitochondrial oxidative phosphorylation. For this reason much attention has been focused on mitochondrial functional changes during and following ischemic processes.

The structural changes of mitochondria *in situ* during progressive ischemia have been well described[123–126] in different tissues. The initial alteration is a loss of normal intramitochondrial dense granulation followed by condensation of the mitochondrial matrix and a dilatation of the intercristae spaces. The next change is a modest degree of swelling of the mitochondria, but the ultrastructure otherwise remains intact. Up to this point, the mitochondrial morphologic changes correlate with reversible duration of ischemic injury. The transition to irreversible cell injury is characterized by an increase in the degree of mitochondrial swelling and by the loss of mitochondrial structural integrity, with fragmentation of cristae and the appearance within the mitochondrial matrix of flocculent densities. These flocculent densities are thought to consist of lipid and protein components of damaged mitochondrial membranes.[127] From this point on, if reperfusion does not occur, loss of mitochondrial membrane integrity progresses until dissolution. On the

other hand, if perfusion of the tissue occurs, granular dense bodies consisting of calcium phosphate develop within the mitochondrial matrix.

A number of functional changes in mitochondria isolated from various ischemic tissues after progressive periods of ischemia without reperfusion have been described. State 3 respiratory rates are substantially depressed in renal cortical mitochondria isolated after as little as 10–15 min of ischemia. The degree of inhibition is increased with longer duration of ischemia.[128] The effects of ischemia on state 4 respiratory rates have been smaller and inconsistent.[128] The efficiency of phosphorylation, measured as the P:O ratio,[129] is well maintained during the first 60 min of ischemia, during which time the major reductions in state 3 have occurred. Thereafter, the P:O ratio falls significantly. The ability of mitochondria to eject protons in response to pulsed oxygen increases during the first 60 min of ischemia, but falls afterwards.[128] Mitochondrial DNP-stimulated ATPase activity is rapidly lost within the first 15 min of ischemia.[130] Adenine nucleotide translocase activity is decreased in mitochondria isolated from rat liver ischemia for 3 hr.[126] At the same time, there is a decrease in state 3 and state 4 respiratory rates, as well as a progressive loss of respiratory control and a loss of the ability of dinitrophenol to stimulate O_2 uptake.[126] In addition, an increased permeability of the inner membrane is demonstrated by the shrinkage of swollen, ischemic mitochondria in isosmolar solutions of polyethylene glycol[98] after 3 hr of liver ischemia. This increase in permeability of the inner membrane is associated with a loss of capacity to generate a membrane potential, a loss of 80% of mitochondrial K^+ and Mg^{2+}, and a 10-fold increase of mitochondrial Na^+ content.[98]

Scanty information is available on the functional state of mitochondria during the period of reperfusion after ischemia. Studies under these conditions are of importance in deciding whether the mitochondrial alterations that developed during the ischemic period persist when the tissue is reoxygenated and thereby contribute to further tissue injury. Farber and co-workers[98,126] demonstrated that 3 hr of ischemic injury to the liver *in vivo* resulted in severe alterations of mitochondrial function and ultrastructure, as described in the previous paragraph. Reperfusion of ischemic liver resulted in widespread cell necrosis, dramatic increases in tissue and mitochondrial calcium content, and a loss of membrane phospholipids with persistence of severe mitochondrial alterations.[77] Pretreatment of animals with chlorpromazine, a phospholipase inhibitor, prevented the associated decline in tissue phospholipid content, increases in tissue calcium, and development of lethal hepatic cell injury.[77,131] The mitochondrial alterations of ischemic liver still occurred in animals pretreated with chlorpromazine; however, these alterations were reversible

after 2 hr of reperfusion.[98,126] These studies suggest that mitochondrial alterations are not causally related to the development of irreversible cell injury in ischemia. In other words, the inability to restore mitochondrial function is a consequence of the biochemical alterations accompanying reperfusion itself and not a consequence of the pattern of mitochondrial dysfunction prior to return of the blood supply. On the other hand, although mitochondrial alterations during ischemia may not directly cause irreversible cell injury, mitochondrial dysfunction may be the first step that leads to a cascade of critical events which eventually result in cell necrosis. In this respect the early release of calcium from anoxic mitochondria resulting in rise of cytosolic free-calcium levels[88] can affect numerous cellular metabolic functions, including plasma and subcellular membrane alterations and finally cell death.[107]

Recent work concerning renal mitochondrial dysfunction during reperfusion after renal ischemia has provided further insight into this area.[113,115] After 45 min of bilateral renal pedicle clamping, renal mitochondrial respiration, including state 3, uncoupled FCCP respiration rates, and acceptor control ratio, was severely depressed. The mitochondrial respiration improved significantly at 1 and 4 hr after reflow, although remaining below sham-operated controls. At 24 hr, when ischemic acute renal failure was established, mitochondrial respiration was again severely depressed. State 4 respiration did not significantly change during ischemia and reperfusion.[115] Mitochondrial calcium content was increased progressively at 1, 4, and 24 hr, and mitochondrial calcium accumulation demonstrated a significant correlation with the decreased state 3 respiration and the rising serum creatinine level.

Study of mitochondrial calcium transport following renal ischemia further illustrates the progression of mitochondrial damage during reperfusion.[113] After 50 min of bilateral renal pedicle clamping, energy-linked mitochondrial calcium uptake was unmeasurable, whereas mitochondrial calcium efflux was increased. Three hours of reperfusion was associated with a normalization of mitochondrial calcium uptake, release, and steady-state buffering. However, progressive deterioration with increasing reperfusion time subsequently occurred in these processes. During reperfusion both cellular and mitochondrial calcium contents also increased progressively.[115] These data are consistent with the thesis that mitochondrial calcium accumulation is due to a progressive increase in cytosolic calcium concentration. This mitochondrial calcium uptake is an active process in the early phase of reperfusion, but becomes a passive one during the later phase of postischemic reperfusion as the mitochondrial calcium transport gradually deteriorates.

Studies have demonstrated that mitochondria are able to accumulate and retain calcium up to certain levels without deleterious effects.[106] But above these levels, greater calcium uptake produces a spontaneous efflux

or release of mitochondrial calcium. This calcium efflux occurs via both ruthenium red-sensitive, i.e., via the electrophoretic uniport, and ruthenium red-insensitive pathways. This release is associated with loss of other intramitochondrial cations and adenine nucleotides and loss of the membrane potential across the inner mitochondrial membrane.[132,133] These mitochondrial permeability changes produced by calcium uptake appear to occur via nonphysiologic pathways.[132,133] Lesser amounts of calcium uptake are required to produce these permeability alterations if calcium is accumulated in the presence of a variety of compounds, including phosphate.[132,133] These mitochondrial permeability alterations can be lessened by either inhibiting the initial calcium uptake with ruthenium red or by incubating the mitochondria with Mg^{2+}, adenine nucleotides, fatty-acid-free albumin or agents that inhibit membrane phospholipase, such as dibucaine, promethazine,or trifluoperazine.[132–135]

Calcium uptake by mitochondria produces these mitochondrial membrane damage and permeability alterations, in large part, by activating mitochondrial phospholipase,[136,137] which results in the hydrolysis of mitochondrial phospholipids and accumulation of free fatty acids and lysophospholipids.[132,133] These by-products of phospholipid breakdown alter the structure and permeability of the mitochondrial membrane, resulting in functional deterioration. Since alteration in total phospholipid levels only occurs late in this process,[138] the early functional change has been attributed to the products of phospholipid metabolism, most importantly free fatty acids and lysophospholipids, and to loss of small amounts of phospholipid in limited but important areas. In favor of this proposal are the reports that demonstrate the direct detrimental effects of free fatty acids and lysophospholipids on mitochondrial function,[98,105] and the stimulation by free fatty acids of phospholipase activity.[131–133]

The role of free radicals in mitochondrial damage during postischemic reperfusion is unclear. It has been suggested that calcium and free radicals promote injury to mitochondria between site I and II of the electron transport chain[139] with complete uncoupling of oxidative phosphorylation. This detrimental effect is probably due to impairment of NADH CoQ reductase enzyme activity and can account for impaired ability of the tissue to generate adenosine triphosphate for restorative processes during postischemic reperfusion. Similar synergistic damaging effects of free radicals and calcium on rat brain mitochondria resulting in a decrease in state 3 have also been reported.[140]

In summary, mitochondria play a crucial role in numerous intracellular metabolic processes; among those, production of ATP via oxidative phosphorylation and control of intracellular calcium metabolism are important in the maintenance of cell function and integrity. Thus, mitochondrial alterations have an important contributory role in ischemic tubular cell injury.

2.5. Role of Depletion of High-Energy Phosphate Stores in Ischemic Injury

Several available studies are in good agreement concerning the changes in content of adenine nucleotides during renal ischemia. During the first 5 min of kidney ischemia, whole-kidney ATP dropped to 25% of control levels.[141] This fall in ATP levels was more severe than that seen in either liver[142] or heart[143] and may be accounted for by the low levels of glycolytic capacity in kidneys compared with these other organs. Concomitant with the fall in ATP levels during 5 min of ischemia was a six-fold rise in AMP levels.[141] Most of the decrease in cell ATP was accounted for by increased cell AMP in this early phase.[141–144] No accumulation of ADP was detected, probably because of continued activity of adenyl kinase.[144] Total adenine nucleotides fell by approximately 11% during the interval; lactate levels increased to 5 times those of controls.

Metabolic parameters have also been assessed for longer periods in order to encompass the stage of irreversible cellular injury.[141] After 15 min of renal ischemia, ATP levels were decreased to 16% of controls, and were not significantly decreased further after 2 hr of ischemia, a time that resulted in irreversible renal cell injury. These data indicate that the absolute concentration of ATP at the end of the ischemic period was not a reliable criterion of cell viability. ADP levels did not significantly change until 120 min of ischemia, when they decreased to 37% of control levels. At 15 min AMP levels were 4.3 times control, but thereafter decreased steadily, so that after 120 min they were back to control levels. Total adenine nucleotide levels decreased from 89% of control levels at 15 min to 28% of control levels at 120 min. Lactate levels were 5.9 times control at 15 min and increased to 12 times control at 120 min.[141]

Thus, ATP levels fall dramatically in the earliest phases of renal ischemia, well before the development of irreversible injury. As the duration of the ischemic insult increases, ATP levels fall further, but only mildly, with the greatest change being a decrease in the total adenine nucleotide pool secondary to steady decreases in AMP levels. Experiments with hypoxic proximal tubules have shown that substantial rises of medium AMP occurred under conditions where cell integrity was lost,[144] suggesting that AMP was released from damaged cells. However, most AMP formed during ischemia is further metabolized to precursor nucleotides.[117,145] Within 1 min after the onset of ischemia, a sixfold increase in tissue levels of adenosine occurred. Maximum levels of adenosine to approximately 8 times control level were achieved at 10 min of ischemia. Concomitant with the increase in adenosine are marked increases in inosine and hypoxanthine, which by 60 min of ischemia reach levels of 18 and 289 times normal, respectively. Fifteen minutes of reperfusion after a 60-min ischemic period essentially cleared all the ac-

cumulated excess inosine and adenosine from the kidney and markedly reduced the amount of hypoxanthine present, thereby leading to a profound decrease in total adenine nucleotide levels[117,145] owing to loss of these nucleotide precursors.

With reoxygenation, cell adenine nucleotide levels recovered toward those of time controls.[117,141,144] The degree of recovery generally correlated with the severity of the oxygen deprivation insult. After 15 min of ischemia and 24 hr of reoxygenation, tissue ATP and total adenine nucleotide levels returned to control levels. However, after 60 min of ischemia and 24 hr of reflow, ATP and total adenine nucleotide levels were still 46% and 50% of control levels, respectively.[141] There is a good correlation between the recovery of ATP and total adenine nucleotide levels. As cells are more severely damaged with increasing ischemic periods, greater quantities of adenine nucleotides are metabolized into nucleosides which are able to permeate the cell membrane and lost to the extracellular space. To restore the adenine nucleotide pools, three main pathways exist[146] (Fig. 3). Adenosine can be directly phosphorylated to AMP by adenosine kinase. This pathway is energetically economical. In hypoxanthine salvage, hypoxanthine can be phosphorylated

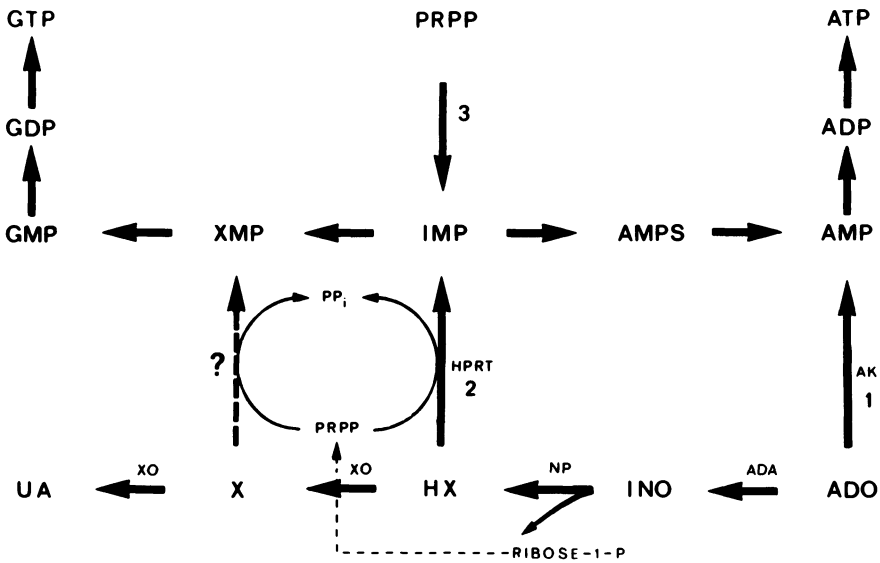

Fig. 3. Biosynthesis of ATP and GTP. Pathway 1, adenosine phosphorylation; pathway 2, hypoxanthine salvage; pathway 3, *de novo* synthesis. ADA, adenosine deaminase (EC 3.5.4.4); ADO, adenosine; AK, adenosine kinase (EC 2.7.1.20); AMPS, adenylosuccinate; HPRT, hypoxanthine phosphoribosyltransferase (EC 2.4.2.8); INO, inosine; NP, purine-nucleoside phosphorylase (EC 2.4.2.1); PP_i, pyrophosphate; PRPP, phosphoribosylpyrophosphate; UA, uric acid; XMP, xanthine-5'-monophosphate; XO, xanthine oxidase (EC 1.2.3.2.). (Reprinted from Harmsen *et al., Am. J. Physiol.* **246**:H37-H43, 1984, with permission.)

with 5-phosphoribosyl-1-pyrophosphate to form IMP and subsequently AMP. In *de novo* synthesis, IMP is also synthesized from small precursor molecules, including, among others, glycine and CO_2. The *de novo* synthesis rate of IMP in rat heart[146] is much slower compared to the production rate of adenine nucleotides from adenosine and hypoxanthine salvage mechanisms. Thus, the restoration of intracellular adenine nucleotide pools becomes more difficult as more nucleotide precursors are lost from the cells severely damaged by ischemia.

2.5.1. Relationship between Cellular ATP Depletion and Ischemic Cell Injury

A relationship between ATP depletion and lethal myocardial injury has been noted in different studies.[147–150] At ATP levels below 2 μmoles/g wet weight, an increasing number of cultured adult cardiocytes[149,150] or myocardial cells in anoxic myocardial slices became irreversibly damaged.[147,148] A close correlation was also found between decreases in mitochondrial adenine nucleotide contents and deterioration of oxidative phosphorylation capacity in liver[151] and state 3 mitochondrial respiration in myocardium.[152] Treatment of mitochondria with ATP was effective in maintaining mitochondrial function.[151]

The relationship between ATP depletion and lethal cell damage is unclear in renal ischemia. The ATP levels in rat kidney after 15 min of ischemia with no reflow were not significantly different from those after 120 min of ischemia.[141] However, when these ischemic periods were followed by 24 hr of reflow, 15 min of ischemia resulted in a reversible lesion with almost complete restoration of adenine nucleotide levels, whereas 120 min of ischemia resulted in death within 24 hr. A similar result was found in mouse kidney.[153] These data indicate that the absolute concentration of ATP at the end of the oxygen deprivation period was not a reliable criterion of cell viability. The pattern of decreases in total adenine nucleotide (TAN) levels, however, was significantly different from that of decreases in ATP alone, since the content of TAN after 120 min of ischemia was approximately 50% of that after 15 min of ischemia.[141] The loss of ATP precursors may prevent effective resynthesis of ATP during reflow and contribute to irreversible damage. Therefore, the loss of TAN and reduction of the cell's ability to resynthesize nucleotides during reoxygenation may correlate better with cell injury.

The pathogenesis of irreversibility in ischemic injury has not been clearly established. Numerous changes are occurring simultaneously during ischemia, and it is very difficult to assess independently the relationship, if any, between ATP depletion and cell death. In order to study the

effects of ATP depletion alone on cell viability, various studies have used metabolic inhibitors to deplete the ATP content of normoxic tissue. No significant loss of viability of hepatocytes was found even when ATP levels were lowered to 12% of control, although similar levels of ATP depletion induced by anoxia caused severe loss of viability.[154] In cultured neonatal rat myocardial cells, cell injury occurred only when ATP levels were depleted below 6% of control levels.[155] A similar result was found in renal LLC-PK_1 cells in culture,[156,157] where ATP levels were completely depleted by antimycin and deletion of medium glucose. This treatment resulted in the LLC-PK_1 cells becoming nonviable with phospholipid breakdown, accumulation of free fatty acids, and loss of mitochondrial potential. However, when ATP levels were maintained about 5% of control, the cells remained viable. Studies in the isolated perfused rat kidney exposed to hypoxia or various metabolic inhibitors showed a similar type of morphological response to ATP depletion in the first segments of the proximal tubule (S_1 and S_2) regardless of the agent used. These injuries consisted of clubbing of the microvilli and mitochondrial swelling. The magnitude of these changes was proportional to the degree of ATP depletion.[158]

2.5.2. Effects of Exogenous Adenine Nucleotides on Ischemic Injury

Treatment with exogenous adenine nucleotides has been reported to have protective effects in different models of experimental ischemic acute renal failure, including *in vivo* renal ischemia and intravenous ATP–$MgCl_2$ administration,[159–161] *in vivo* renal ischemia and intrarenal administration of ATP–$MgCl_2$ as a part of a flush solution,[162] and the isolated perfused kidney.[163] Protective effects on overall renal function parameters,[161] individual tubule function parameters,[159] and tubule morphology[159] have been demonstrated and have been associated with increases in postischemic ATP levels in the kidney.[164] Improvement of ischemia-induced renal mitochondrial dysfunction has been reported with exogenously administered adenine nucleotides.[165] However, the mechanisms by which exogenous nucleotides protect against ischemic acute renal failure are not well defined by these *in vivo* studies. Improvement in postischemic cell ATP contents may result from any measure favoring recovery from the ischemic injury regardless of a primary effect on cellular high-energy phosphate stores.

Allopurinol has also been shown to have protective effects on ischemic acute renal failure[69,166]; however, inconsistent results in which protective effects by allopurinol were absent have been reported for kidney,[144,167,168] liver,[169] and myocardium.[170] The mechanism by which this

agent protects against ischemic cell injury may be related to its inhibitory effect on the precursors which are important for the replenishment of cellular nucleotides during reperfusion.[167,169] Furthermore, allopurinol may also protect against cell injury by preventing the free-radical generation during xanthine oxidase-mediated degradation of hypoxanthine to xanthine.[69,170,171]

Treatment of hypoxic isolated proximal tubules with exogenous adenine nucleotides during hypoxia allows a more direct study of intracellular metabolism of adenine nucleotides. The intracellular levels of ATP and TAN did not change during hypoxia prior to reoxygenation by exogenous addition of ATP–$MgCl_2$, ADP–$MgCl_2$, or AMP–$MgCl_2$.[144,172] In contrast, during reoxygenation, tubules treated with exogenous ATP–$MgCl_2$ usually showed significantly greater rises of their cell ATP levels than untreated tubules.[144,172] The effect on cell ATP levels was seen mainly in milder hypoxic insult as opposed to more severe hypoxic insult.[144] These data show that exogenous nucleotides are effective precursors for the replenishment of cell ATP levels during the reperfusion period, but this effect depends on the presence of intact cellular metabolic processes to synthesize and maintain these higher intracellular ATP levels.

The mechanism by which exogenous nucleotides increase cellular ATP content is unclear. Exogenously added ATP–$MgCl_2$ during anoxia is rapidly metabolized extracellularly, as reflected by a large increase in medium AMP content, most likely by a 5′nucleotidase. During reoxygenation as cell ATP levels rose, the medium nucleotide levels markedly decreased.[144] These data suggest that AMP may be the major precursor that is transported across the tubule cell membrane during reoxygenation for resynthesis of ATP. Adenosine has also recently been reported to be an important precursor for ATP synthesis.[177]

A dose-dependent effect of exogenous ATP has also been reported. A recent study has shown that treatment of hypoxic proximal tubules with large amounts of exogenous nucleotides in divided doses during hypoxia prevented the alterations of functional parameters reflective of cell injury, even in tubules exposed to severe forms of hypoxic injury.[172]

In summary, ischemic renal injury is accompanied by substantial and relatively early falls in renal cortex ATP levels, and these declines are generally paralleled by decreases in total adenine nucleotide pool size, reflecting the activity of degradative enzyme systems to break down adenine nucleotides to precursor substances that are lost from the cell. However, when metabolic inhibitors are used to deplete cell ATP, only extremely low ATP levels (close to zero) cause irreversible cell damage. Thus, although a decline in high-energy phosphate pool size contributes to ischemic or hypoxic renal cell injury, it is rarely sufficient to produce irreversible cell injury.

2.6. Oxygen-Derived Free Radicals in Postischemic Cell Injury

Recent evidence suggests that oxygen-derived free radicals may be abundantly produced in ischemic tissues during reperfusion and that this may play an important role in postischemic cell injury. The biologic important free radicals include superoxide anion ($O^{\cdot-}$), free hydroxyl radical ($OH\cdot$), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$), a high-energy state of oxygen which is not a free radical *per se* but can interact with substances such as polyunsaturated fatty acids to initiate lipid peroxidation, a free-radical chain reaction process.[174]

The major source of superoxide in postischemic tissues appears to be the enzyme xanthine oxidase.[175] This enzyme is widely distributed among tissues; the intestine, lung, and liver are particularly rich sources in most species. The enzyme is synthesized as xanthine dehydrogenase (type D). This form appears to account for about 90% of the total activity in a healthy tissue.[175] The dehydrogenase cannot transfer electrons to molecular oxygen to form hydrogen peroxide or superoxide. The oxidase (type O) can use molecular oxygen, producing superoxide or hydrogen peroxide or both. The conversion of xanthine dehydrogenase to xanthine oxidase may occur *in vivo* in ischemic tissues.[175] The mechanism underlying this conversion is unclear, but may be related to the elevated cytosolic free-calcium concentrations[88] that occur during ischemia, thereby activating a protease capable of converting the dehydrogenase to the oxidase.[175] Concomitantly, the depletion of the cell's ATP results in an elevated concentration of AMP. The AMP is catabolized to adenosine, inosine, and finally hypoxanthine.[176] Hypoxanthine, as well as xanthine, serves as an oxidizable purine substrate for xanthine oxidase. Hence, during ischemia two important changes occur in tissue: a new enzyme activity (xanthine oxidase) appears, along with one of its two required substrates (hypoxanthine). The remaining substrate required for type O activity, molecular oxygen, is supplied during the reperfusion of the tissue. A rapid burst of superoxide radical and hydrogen peroxide thereby results. This sequence of events is diagrammed in Fig. 4.

Normal tissues protect themselves from free-radical-induced injury by a number of mechanisms. An important defense system is the enzyme superoxide dismutase.[177] Superoxide dismutase scavenges the toxic superoxide free radical by catalyzing the following reaction:

$$O_2\cdot^- + O_2\cdot^- + 2H^+ \rightleftharpoons O_2 + H_2O_2$$

The H_2O_2 is then decomposed by the actions of catalase:

$$2H_2O_2 \rightleftharpoons O_2 + 2H_2O$$

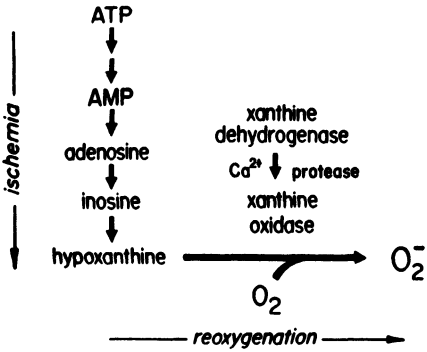

Fig. 4. Proposed mechanism for ischemia-induced production of superoxide and hydrogen peroxide. (Reprinted from McCord, *N. Engl. J. Med.* **312**:159–163, 1985, with permission.)

The importance of superoxide dismutase and catalase in the protection of cells from superoxide radical injury is well documented.[178] Other types of antioxidants have been demonstrated to function *in vivo:* water-soluble antioxidants, which include ascorbic acid and glutathione, and lipid-soluble antioxidants, which consist primarily of the tocopherols, especially vitamin E or D-tocopherols.[179] However, during ischemia supplies of endogenous scavengers may be depleted,[180,181] permitting cellular injury by free radicals during the reperfusion period.

Cellular damage due to ischemia has been attributed to a multiplicity of factors. Therefore, it is difficult to clarify the role of free radicals in ischemic cell injury. To assess independently the role of free radicals in causing cell membrane dysfunction, most studies have used oxidants such as menadione, t-butyl hydroperoxide, or free-radical-generating systems in the presence of transition metals as catalysts for initiating free-radical reactions involving oxygen in biologic materials *in vitro.* The studies have clearly shown that reactive free radicals are able to produce chemical modifications of and damage to proteins, lipids, and nucleotides.[179,182,183] In particular, lipid peroxidation produced by free-radical reactions in biologic membranes is a destructive process.[184] The unsaturated fatty acids are especially prone to this degradative process. The presence of the double bond in an unsaturated fatty acid weakens the carbon–hydrogen bond on the carbon atom adjacent to the unsaturated carbon–carbon bond. These allylic hydrogens are less tightly bound and are susceptible to abstraction by free radicals.[2] Since cellular membrane phospholipids, especially from mitochondria and endoplasmic reticulum, possess large amounts of polyunsaturated fatty acids, these membrane systems are highly susceptible to lipid peroxidative damage.[185] Dramatic alterations in membrane structure and function can be ultimate consequences of this process owing to disruptive effects of loss of membrane lipids, production of toxic lipid breakdown products, and peroxidative damages to nearby membrane proteins.[179,183,186,187] There has

also been suggestion that increased membrane phospholipase activity may participate in the selective elimination of specific fatty acyl moieties in phospholipids, particularly those which have been damaged by peroxidation reactions.[90,92,93] Furthermore, a potentiation of free-radical-induced lipid peroxidative injury has been demonstrated in sarcolemmal membrane by products of activated phospholipases on membrane phospholipids during ischemia, including palmitoyl–CoA, palmitoyl–carnitine, or lysophosphatidylcholine.[102]

The uncontrolled peroxidation of cellular membranes can thus lead to profound effects on membrane structure and function and may be sufficient to cause cell death.[183,186,188,189] Although lipid peroxidation can be an important pathway leading to cell injury, free radicals can cause oxidation of protein and nonprotein thiol groups leading to additional changes in cellular enzyme activity. Various oxidants (t-butyl hydroperoxide, menadione) have been shown to cause a disruption of calcium homeostasis due to a decline of plasma membrane Ca^{2+}-ATPase activity[190,191] and inhibition of ATP-dependent microsomal calcium sequestration,[192] resulting in a rise in cytosolic free-calcium content and loss of cell viability.[91] The injurious effects of these compounds have been linked to depletion of both soluble and protein-bound thiols and were prevented by the sulfhydryl protective agent dithioerythritol.[190,191,193]

The role of free radicals in ischemic cell injury has been extensively studied in intestine, myocardium, and other tissues. Because free radicals are extremely labile and rapidly removed by cellular scavengers, current available techniques are unable to measure directly cellular free-radical levels. Thus, experimental data concerning the role of free radicals in ischemic tissue injury emanate from studies of the possible consequences of a free-radical attack in the tissue, i.e., changes in naturally occurring tissue antioxidants and membrane phospholipids, the accumulation of peroxidation products, in particular malondialdehyde, or the protective effects of various free-radical scavengers on ischemic injury.

The intestinal mucosa is very sensitive to ischemic injury. In cats, 1 hr of local arterial hypotension (30 mm Hg of arterial pressure) followed by reperfusion greatly increases intestinal vascular permeability.[194] Pretreatment of animals with antihistamines, indomethacin, or methylprednisolone had no effect in preventing the ischemia-induced increase in capillary permeability, but intravenous administration of superoxide dismutase nearly abolished the change in permeability.[195] These results provided the first evidence that superoxide played a potential role in ischemic injury of the intestine. Subsequent studies found that the increase in vascular permeability could also be prevented by pretreatment of the animals with allopurinol, a xanthine oxidase inhibitor, or with

dimethyl sulfoxide, a scavenger of the hydroxyl radical.[196] These data suggest that the damage may be caused primarily by the superoxide-dependent generation of hydroxyl radicals.

If the period of ischemia is lengthened to 3 hr, the injury is characterized by development of mucosal lesions, such as extensive lifting of the epithelium from the villi, epithelial necrosis, disintegration of the lamina propria, hemorrhage, and ulceration. Pretreatment of the animals with superoxide dismutase markedly attenuated villus and crypt epithelial necrosis and essentially eliminated denudation of the villi.[197] Further evidence linking superoxide radical and mucosal damage has been obtained by intraluminal perfusion of animal bowel with a superoxide-generating system consisting of hypoxanthine and xanthine oxidase. Animals were injected intravenously with radiolabeled albumin, and its leakage into the luminal perfusate was monitored to assess the integrity of the mucosal membrane. Such exposure increased permeability of the membrane to a level comparable to that produced by 2 hr of partial arterial occlusion; the increase was limited by infusion of superoxide dismutase in the luminal perfusate.[198] All the studies of mucosal injury described above deal with partial ischemia and indicate a major role for superoxide or secondary radicals. There is evidence, however, that if arterial occlusion is complete, other mechanisms not dependent on free-radical generation become the dominant factors. Pretreatment with superoxide dismutase was unable to prevent the morphologic damage produced by 4 hr of total arterial occlusion in cats[199] or the increase in mucosal permeability produced by 1 hr of total arterial occlusion in dogs.[198] Similarly, there is recent evidence that free radicals may play an important part in ischemic liver injury since catalase and superoxide dismutase were able to provide partial protection against liver injury produced by 40 min of ischemia.[200]

Stewart *et al.* [201] studied hypothermic global ischemia in a canine model. A group of dogs undergoing hyperkalemic cardioplegia, with and without the addition of superoxide dismutase and mannitol, were compared with a group undergoing simple hypothermia. After 60 min of ischemia and 45 min of reperfusion, all parameters reflective of left ventricular function were significantly better in the enzyme-treated hearts. In addition, this group had higher calcium uptake rates by the sarcoplasmic reticulum.

Myers *et al.* [202] studied reversible regional ischemia in dogs that underwent a 15-min occlusion of the left anterior descending coronary artery followed by 2 hr of reperfusion. Pretreatment with superoxide dismutase (SOD) and catalase (CAT) significantly enhanced recovery of regional myocardial function, although SOD and CAT did not produce any hemodynamic changes that might account for this beneficial effect

or any differences with respect to collateral blood flow and size of the occluded vascular bed compared to the control group. In an earlier study[203] pretreatment (15 min before reperfusion) with SOD and CAT resulted in a significant decrease in infarct size in a canine preparation of 90-min occlusion of the circumflex coronary artery. However, no protection was afforded when SOD and CAT were given 40 min after reperfusion, suggesting that injury had occurred within the early phase of reperfusion. These data suggest that superoxide radical and secondary radicals play a role in postischemic injury on myocardium.

The xanthine oxidase inhibitor allopurinol has been used to inhibit superoxide anion generation from purine degradation during the reperfusion period. However, the protective effect of allopurinol against postischemic lesions has been inconsistent. Allopurinol did not decrease the infarct size of the canine heart after the circumflex coronary artery was occluded for 40 min.[170] Other studies have reported a significant protective effect of allopurinol in limiting myocardial infarct size.[204,205] The reasons for these conflicting effects of allopurinol in myocardial ischemia are unknown.

Recent studies have suggested that free radicals play a pathogenetic role in renal ischemic injury.[69,166,206] Paller and co-workers[69] studied the role of superoxide radical ($O_2\cdot^-$) and its reduction product (OH·) in mediating injury in rat kidneys after 60 min of ischemia. Pretreatment with $O_2\cdot^-$ scavenger, SOD, and OH· scavenger, dimethylthiourea (DMTU), provided partial protection against renal excretory dysfunction following renal ischemia and reperfusion. SOD prevented the reflow-induced increase in lipid peroxidation in renal cortical mitochondria but not in crude cortical homogenates, presumably because of the more heterogeneous nature of cortical tissue than of the mitochondrial fraction. Similarly, the xanthine oxidase inhibitor allopurinol, given before the onset of ischemia, also provided protection against postischemic acute renal failure. Neither SOD nor DMTU caused an increase in renal blood flow, urine flow rate, or solute excretion in normal rats. A similar protective effect against ischemic injury by SOD was found in canine kidneys[206] and swine cold-preserved ischemic kidneys.[166] An OH^- scavenger, dimethylsulfoxide, given at the termination of a 60-min period of ischemia in rat kidneys significantly lessened the degree of renal failure and mortality.[207] These data suggest that oxygen-free radicals produce lipid peroxidation during reperfusion injury and that these processes play some role in the pathogenesis of ischemic cell injury in the kidney.

Treatment with hypoxanthine oxidase inhibitor, allopurinol, in renal ischemia has yielded conflicting results, not unlike those on myocardial ischemia.[69,166,167,208–210] Negative protective effects with allopurinol have been found during *in vitro* study of isolated proximal tubule segments[211]

and in preserved cadaveric kidneys,[167] in contrast to positive results in *in vivo* experiments. These findings suggest the protective effects of allopurinol on *in vivo* models of renal ischemia may be due to effects on nephronal processes rather than directly related to its effect on free-radical generation.

Most studies of the role of free radicals in mediating renal injury after ischemia have been accomplished *in vivo,* in which several variable factors, humoral as well as hemodynamic, can influence the outcome of the studies. Preliminary work studying lipid peroxidation during recovery from hypoxic stress to isolated rabbit renal proximal tubule segments (PTS) have recently been published.[212] PTS was gassed with 95% N_2/5% CO_2 for 30 min followed by reoxygenation with 95% O_2/5% CO_2 for periods ranging from 15 min to 180 min. Lipid peroxidation measured as malondialdehyde (MDA) was not different from control after 30 min of hypoxia. However, after 15 min of reoxygenation MDA rose and remained 50% above the control level after 180 min of reoxygenation. Functional viability parameters measured as tubule potassium and ATP contents and tubule CCCLP-uncoupled respirations decreased after 30 min of hypoxia and then progressively returned to control levels after 60 min of reoxygenation while MDA levels remained elevated. These data suggest that lipid peroxidation due to free-radical activity occurs during reoxygenation. However, the pathogenetic role of lipid peroxidation in this model of renal ischemic injury remains uncertain in view of the lack of correlation between lipid peroxidation activity and tubule viability parameters during the reoxygenation period.

In summary, free radicals contribute to ischemic cell injury mainly by initiating lipid peroxidation which results in membrane dysfunction. Whether free-radical generation is a critical process in the pathogenesis of postischemic reperfusion injury is still not certain. The role of lipid peroxidation may, in fact, be more contributory to other processes, such as activation of phospholipase.

2.7. Protective Measures in Ischemic Cell Injury

2.7.1. Protective Effects of Acidosis on Hypoxic Injury

Tissues deprived of oxygen rapidly accumulate hydrogen ion leading to extra- and intracellular acidosis. In the globally ischemic rat heart, intracellular pH dropped from 7.05 to 6.2 within 13 min.[213] A fall in intracellular pH from 7.0 to 6.6 occurred in rabbit gastrocnemius muscle subjected to 4 hr of ischemia.[214] The intracellular pH after 60 min of ischemia in rat kidneys perfused with saline was 6.56 as measured by nuclear magnetic resonance.[215]

Although intracellular acidosis is a prominent feature of ischemia, it is not clear to what extent the acidosis contributes to the morphologic and functional abnormalities observed with ischemia. Some investigators have attributed the ischemic injury to the associated acidosis.[215] There is evidence, however, that acidosis may actually protect cells against anoxic injury. Nayler *et al.*[216] showed that respiratory acidosis improved the recovery of heart tissue function when exposed to pH 6.9 rather than 7.4 during hypoxia. This protective effect of acidosis did not appear to be related to decreased contractility, and therefore energy consumption, during the hypoxic period. Furthermore, it is not only in excitable tissue that acidosis has proven to be protective of hypoxic cell function. Bonventre and Cheung[217] demonstrated that hepatocytes were protected against anoxic injury at pH 6.9 as compared with cells incubated at pH 7.5 or 6.6. Similar protection by medium acidosis was found in renal cortical tubules.[217,218]

The mechanisms of the protective effect of acidosis on hypoxic injury are unclear. The cellular effects of acidosis are multiple. Acidosis may stabilize and thereby protect cellular membranes, making them more resistant to injurious effects of ischemia.[219] The protective effect of acid pH on hypoxic injury may also be related to an effect of acidosis on intracellular calcium metabolism. Oxygenated and hypoxic renal tubules incubated at low pH maintained significantly lower total intracellular Ca^{2+} levels than those incubated at normal pH levels.[218,220] The decreased cell Ca^{2+} content in cells incubated at low pH was associated with a decrease in the rate of cell Ca^{2+} uptake[220–224] compared to those incubated at normal pH levels. Furthermore, acidosis significantly depressed calcium flux between the cytosolic and mitochondrial pools.[220] Calcium accumulation in mitochondria extracted from hearts perfused at pH 6.9 was less than in mitochondria isolated from hearts perfused at pH 7.4 and 6.6.[216] Since cellular and subcellular Ca^{2+} accumulation plays a major role in hypoxic cell injury,[68] the effect of acidosis on cell calcium metabolism may protect tissue structure and function during oxygen deprivation. Of note, the dissociation constant (K_D) for the binding of Ca^{2+} to calmodulin is also dependent on pH. It has been reported that the K_D is about 10-fold higher (0.25 μM) at pH 6.5 than at pH 7.5 (0.02 μM).[225] Thus, changes in cell pH play an important role in regulating the numerous cellular metabolic processes controlled by Ca^{2+} and calmodulin.[225] In addition, studies measuring the activity of plasma membrane mitochondrial and microsomal phospholipases A have demonstrated that the pH optima for these enzymes are above 7.0, with marked inhibition of phospholipase activity when pH is decreased below 7.0.[89,226,227] Thus, reduction of the activity of membrane phospholipases by low pH effects on membrane Ca^{2+} and Ca^{2+}–calmodulin complexes,

as well as by direct effects of pH on enzymes themselves, may be an important mechanism by which low pH protects ischemic tissues.

2.7.2. Protective Effects of Calcium Channel Blockers and Adenine Nucleotides

Increased cytosolic and total cell calcium levels play a pathogenetic role in ischemic cell injury.[68,88] Calcium channel blockers have been shown to have significant protective effects on ischemic injury.[119,120,122] Treatment with exogenous adenine nucleotides, either systematically after an acute ischemic insult or intrarenally after ischemia, has been shown to ameliorate the resulting renal tubular cell injury and ischemic acute renal failure.[159–164] The protective role of calcium channel blockers and adenine nucleotides is discussed in more detail in other sections of this chapter.

3. Summary

From the available data, it appears that the pathogenesis of ischemic cell injury is due to the simultaneous derangement of several critical metabolic processes that act in concert to produce a cascade of events that finally lead to plasma and subcellular membrane dysfunction incompatible with the maintenance of cell viability and integrity. These metabolic processes include high-energy phosphate depletion, cellular calcium derangements, free-radical production, and membrane phospholipid degradation with accumulation of toxic lipid by-products. The understanding of mechanisms responsible for membrane damage is, therefore, of great importance to develop methods to prevent cell injury and the clinical occurrence of ischemic acute renal failure.

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10

The Kidney in Systemic Disease

Wadi N. Suki

1. Introduction

By virtue of the fact that they receive 25% of the blood pumped by the heart during any time interval, and that they process over 150 liters of ultrafiltrate of plasma everyday, the kidneys are often victims of a large number of extrarenal diseases. The injury that results may involve the microvasculature and the glomeruli, or the tubules and interstitium—each alone or in combination. The vastness of this subject precludes its coverage in one chapter; besides, such an attempt would overlap other chapters in this volume. The scope of this chapter will be limited instead to systemic disorders involving the renal microvasculature and the renal consequences of tumors.

2. Disorders of the Renal Microvasculature

This section deals primarily with the thrombotic microangiopathies (TMA) and scleroderma, and to a lesser extent diabetes mellitus.

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2.1. Thrombotic Microangiopathies

The term TMA refers to syndromes characterized by microvascular thrombosis and microangiopathic hemolytic anemia.[1] Two syndromes, hemolytic—uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP), and possibly a third syndrome, postpartum renal failure (PRF), are encompassed by this term. The triad of hemolytic anemia, thrombocytopenia, and acute renal failure succinctly describes the clinical features of HUS. Histopathologically the glomerular lesion most commonly consists of detachment of the endothelium from the basement membrane, with filling of the subendothelial space with plasma proteins including fibrinogen and, occasionally, fibrin, and of rarefaction of the mesangium and subsequent sclerosis of the matrix.[2] In some older lesions a new basement membrane is formed under the endothelium, and this, coupled with occasional mesangial interposition and proliferation, may be reminiscent of mesangiocapillary glomerulonephritis.[2] The small arteries and arterioles are usually involved, with a lesion of the endothelium similar to that affecting the glomerulus, and this is accompanied by intraluminal thrombosis resulting in cortical necrosis.[2]

Not unlike HUS, TTP is characterized clinically by relapsing microangiopathic hemolytic anemia, thrombocytopenia, fever, neurologic manifestations, and variable renal involvement, and histologically by diffuse intraluminal and subendothelial hyaline thrombi in different tissues.

The pathogenesis of HUS and TTP is not fully known, but several factors have been proposed[3]: (1) absence of fibrinolytic activity in diseased microvascular segments indicative of endothelial cell injury, (2) absence of a plasma factor which stimulates vascular prostacyclin production, (3) immune injury of the endothelial cells, (4) presence in plasma of platelet-agglutinating factor, and (5) presence in plasma during remission, and disappearance during relapse, of unusually large multimers of Factor VIII (von Willebrand factor), which presumably bind to and agglutinate the platelets. These hypotheses implicate the platelets, plasma, or vessel walls in a process capable of inducing adhesion and deposition of fibrinoplatelet aggregates and swelling of the endothelial cells, thereby narrowing the vessel lumen and inducing mechanical deformation of the erythrocytes and causing their lysis. While this hypothesis may explain the fragmentation of erythrocytes and the formation of schistocytes, it does not very well explain the micro- and macrocytosis, echinocytosis, and acanthocytosis often observed. Instead, a primary or secondary disorder of erythrocytes causing them to become inflexible, inelastic, and rigid may be at play.[4] These alterations impair the cells' ability to pass through the microvasculature, with resulting tissue hypoxia, acidosis, and cell fragmentation.[4] Whichever of these hypotheses

may be correct, a role for platelet activation received support from the finding of low intraplatelet serotonin levels, a reliable index of *in vivo* platelet activation, in children with HUS during the early stage of the disease.[5] All children who completely recovered renal function exhibited normalization of the platelet serotonin level, suggesting that this measurement may be of prognostic value.[5]

Of the many bacterial and viral infections associated with this syndrome, leptospirosis had not been heretofore reported to result in HUS. Although acute renal failure occurs frequently in leptospirosis, the lesion most commonly is that of tubular degeneration and interstitial inflammation.[6] Recently, however, a course compatible with HUS was described in a middle-aged man from Thailand with leptospirosis.[7] Unfortunately, no biopsy was allowed to confirm this unusual association.

Gastrointestinal symptoms occur commonly in the prodromal phase of HUS. Hepatic involvement with mild jaundice and enzymatic evidence of mild hepatocellular damage also occurs. Severe cholestatic jaundice, however, has been described in a young girl with this syndrome.[8] Because of the gastrointestinal symptoms, this patient was not receiving enteral feeding at the time cholestatic jaundice developed, and the jaundice resolved promptly following the resumption of enteral feeding. It is possible, therefore, that the cholestasis was not a manifestation of HUS, but the consequence of the interruption of enteral feeding.

Unlike in the adult, the prognosis of HUS in children is quite favorable. In a large series from France, 60% of the children had no functional sequelae, 13% had mild sequelae, and 8% had hypertension but normal renal function.[9] Only 7% of the children died, 5% developed severe chronic renal failure, and another 5% required maintenance dialysis. Arterial thrombotic microangiopathic lesions, cortical necrosis, and age above 3 years were associated with a poor prognosis.

Of particular interest for the patient who goes on to develop chronic renal failure and eventually requires renal transplantation is the possibility of recurrence of TMA in the transplanted organ. The development of TMA in renal allografts is usually a manifestation of antibody-mediated rejection and may occur from hours to as long as 20 months after transplantation, although most often within 10 days of transplantation.[10] Recovery of the hematologic abnormalities in these patients usually follows nephrectomy. There are now, however, reports of 4 cases of recurrent TMA in patients who had HUS (2 patients) or TTP (2 patients) as the original diagnosis.[10] All these recurrences have been in cadaveric allografts with onset from 12 to 240 days following transplantation. In these cases there were no associated signs of rejection such as fever and graft tenderness, the biopsy showed no evidence of rejection, thrombocytopenia and hemolytic anemia were present at the time allograft

dysfunction was noted, and plasma infusion, when used, was able to reverse the TMA. This is in contradistinction to cases of *de novo* TMA attributed to rejection wherein there is a delay between the clinical features of rejection, which are invariably present, and the onset of allograft dysfunction on the one hand, and the development of thrombocytopenia and hemolytic anemia on the other.

The recurrence of TMA in four cases should not, however, discourage the performance of transplantation in patients with this disease. Of 16 additional transplants carried out in patients with TMA (15 HUS, 1 TTP), 10 (6 cadaveric, 4 related) grafts have functioned for an average of 25 months without evidence of recurrence of the disease.[10] The remaining six grafts were lost to rejection and other unrelated causes. These findings attest to the safety of transplantation in HUS and TTP.

With respect to the medical therapy of TMA, the evidence continues to accumulate against drug therapy and in support of plasma infusion and/or replacement. Successful treatment of adult patients suffering from HUS with plasmapheresis using fresh frozen plasma for replacement has been reported in two separate reports.[11,12] Similar success with plasmapheresis[12,13] or with plasma infusion alone[13] has been reported in TTP. Although in most of these cases other therapy was employed as well, such as splenectomy, steroids, and antiplatelet agents, these therapies alone have heretofore failed to favorably affect the outcome of these disorders until the introduction of plasma infusion and replacement. Of great interest to nephrologists is the demonstration of the feasibility of performing plasmapheresis using a hollow-fiber plasma separator.[12] The use of such a device allows sequential plasmapheresis and hemodilaysis in patients whose kidneys have failed.

As intimated earlier, acute renal failure occurring a few days to as many as 10 weeks following what seems to have been a normal pregnancy and delivery is now considered akin to the TMAs because of its association with microangiopathic hemolytic anemia and thrombocytopenia, and the similar histopathologic features.[14] Clinically, PRF is characterized by renal insufficiency or anuria and, invariably, microscopic hematuria and proteinuria. The disease may persist for weeks or months and ultimately proves fatal in 50–60% of patients. Death in most patients results from hemorrhage or brain damage. The etiology of PRF is not known, but a role for elevated estrogen levels in the raised levels of coagulation factors and depressed fibrinolysis has been proposed in this disorder as in patients receiving oral contraceptives.

Because of the similarities between PRF, on the one hand, and HUS and TTP on the other, it is intriguing that plasma infusions and/or exchange have recently been reported to have resulted in complete recovery in four pregnant women presenting with TMA.[15] In two the

disorder was diagnosed at 31 and 34 weeks of gestation and, therefore, the diagnosis of PRF cannot apply. In the other two, however, the disorder was observed 9 and 12 hr postpartum, and while the pregnancy and delivery were normal in one, in the other patient headache, hypertension, and proteinuria developed 9 days before delivery and may have been the harbinger of events to come. Nevertheless, while none of these cases may have represented true PRF, a trial of plasma infusion and/or exchange is strongly indicated by this experience, and by the striking similarities between PRF and the other TMAs.

2.2. Scleroderma

Having dermal sclerosis in common, a heterogeneous group of disorders comprise scleroderma. These disorders include progressive systemic sclerosis (PSS), acrosclerosis, the CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telengiesctasia), and localized scleroderma. Localized scleroderma itself is subdivided into localized morphea, generalized morphea, and linear scleroderma (LS). The degree of visceral involvement differs in these disorders, and some, like CREST syndrome and LS, have been said to not usually be associated with visceral disease.

Linear sclerosis is characterized clinically by the presence of usually unilateral brown or hypopigmented sclerotic bound-down skin in linear distribution on the extremities, trunk, or head. In a study of 24 juveniles with LS an association was found between the presence of antinuclear antibodies (ANA) and rheumatoid factor (RF) and the presence of systemic disease.[16] Three of thirteen patients with ANA, and two of five patients with both ANA and RF, had systemic disease such as nephritis. In another report[17] a patient with LS, in association with progressive facial hemiatrophy and ipsilateral total hemiatrophy, developed Henoch–Schönlein nephritis which responded to steroid therapy. The same patient subsequently developed paroxysmal nocturnal hemoglobinuria. During both episodes the ANA was positive. These reports underscore the importance of vigilance in patients with LS when ANA and RF are present and demonstrate that immunologically mediated diseases may develop even in this localized variant of scleroderma.

In PSS the hide binding or tethering of the skin must be proximal to the metacarpophalangeal joints. Frequently, many of the components of the CREST syndrome are present in patients with PSS. Controversy exists as to whether PSS and CREST are different disorders, and whether CREST syndrome is of lesser severity. In a study of patients with PSS and CREST syndrome matched for age, sex, and disease duration,[18] there was greater skin, muscular, and pulmonary involvement in PSS,

but equal visceral involvement. In specific, renal involvement as determined by a reduced creatinine clearance and/or proteinuria was of equal frequency in the two groups of disorders. When renal involvement is defined as the abrupt onset of arterial hypertension often followed by development of rapidly progressive oliguric renal failure, "scleroderma renal crisis," or "scleroderma kidney," a large difference is noted in the relative incidence of PSS versus CREST syndrome. Whereas 18% of 259 patients with PSS developed scleroderma kidney, only 1% of 239 patients with CREST syndrome did.[19] In investigating the risk factors that might predict the development of renal involvement, it appeared that patients who show rapid evolution of the skin lesions early in their illness with the development of anemia, pericardial effusion, and congestive heart failure are at high risk for developing "scleroderma kidney."[19] Isolated mild hypertension did not serve to predict the later development of scleroderma renal crisis.

In addition to "scleroderma renal crisis," PSS patients may develop other immunologic renal disease. A patient with PSS was reported to develop Hashimoto's thyroiditis and nephrotic syndrome.[20] Renal histopathology revealed findings of membranous nephropathy with associated lesions of glomerular capillary tuft necrosis accompanied by crescents, and of arterial and arteriolar fibrinoid necrosis.

Although until recently invariably fatal, a number of reports have appeared reporting control of the hypertension and survival in patients with scleroderma renal crisis, especially with the use of angiotensin converting enzyme inhibitors. Members of the Department of Clinical Research at E. R. Squibb and Sons, Inc., report that 23 patients with scleroderma renal crisis have been treated with captopril.[21] Of these, 87% responded with a drop in blood pressure and 61% with a reduction in the serum creatinine. Only 26% of the patients died, and 30% (seven patients) ended on dialysis; in two of these captopril had been discontinued. This favorable response, however, is not shared by others. In a report from Switzerland[22] three patients with PSS, hypertension, and renal insufficiency responded to captopril with a sustained normalization of blood pressure, but renal function deteriorated inexorably culminating with dialysis and ultimately death of respiratory failure within 3–4 weeks after dialysis was started. Thus, while captopril may have improved the outlook for PSS patients who develop a renal crisis, a great deal more needs to be learned about the pathogenesis of this disorder and its treatment.

When scleroderma culminates in renal failure, dialysis and/or transplantation becomes necessary. Little has been written about peritoneal dialysis for these patients. The experience with the management of one

patient with CAPD over a period of 18 months is reported.[23] Two points are of considerable interest, one being the fact that with progression of the disease and increased skin binding, distensibility of the abdomen becomes limited, causing discomfort with the 2-liter exchanges. The second point has to do with seasonal variations in the vascular disturbances in the disease. Probably as a reflection of these variations, there was a 10-fold increase in B_{12} peritoneal clearance in the summer months, when compared with the winter months, while no change was noted in the clearance of urea nitrogen or of creatinine. These findings are of considerable interest since they demonstrate the feasibility of long-term peritoneal dialysis in PSS patients and point out important practical aspects of the dialysis in them.

2.3. Diabetes Mellitus

A great deal has been learned about the course of diabetic nephropathy in the last decade, and work in experimental models of diabetes has shed further light on the pathogenesis of the glomerular lesion in this disorder. The onset of clinically detectable proteinuria (0.5 g/24 hr) is an ominous milestone in the course of the disease, with survival limited to an average of 7 years beyond this point.[23] However, the proteinuria has a more interesting and revealing background than merely this grim prognostication. When albumin in the urine is measured by radioimmunoassay much smaller amounts can be measured than is possible by standard techniques employed in the routine urinalysis. Employing RIA, it can be shown that in normal individuals the albumin excretion rate (AER) is 1.0–12 μg/min.[23] Applying this technique to the study of diabetics has allowed a clearer definition of the course of diabetic nephropathy and the influence of different therapeutic interventions on it.[23] It appears that microproteinuria, although absent at rest early in the course of diabetes, can be provoked by moderately vigorous exercise, the magnitude of the increase rising with increasing duration of diabetes as resting microproteinuria sets in. Meticulous control of the diabetes, as manifested by normalization of the glycosylated hemoglobin level, can reverse or ameliorate both resting and exercise-induced microproteinuria.[23] At this stage of the disease, i.e., AER of 12–30 μg/min, the ratio of the clearance of IgG to that of albumin (selectivity index, SI) is intact, indicating that pore size and charge of the glomerular barrier is intact. The increase in excretion of albumin, IgG, and neutral dextram must represent an increase in the mean transglomerular filtration pressure ($\overline{\Delta P}$) and/or a raised ultrafiltration coefficient (K_f). Since K_f is a function of hydraulic permeability (L_p) and of the filtration surface area (A), it

is of interest that enlargement of the kidneys[24] and of the glomeruli is characteristic of diabetes. The SI does decline with time, however, reaching its nadir at an AER of 60–90 μg/min, indicating a loss of the fixed negative electrical charge on the glomerular membrane which normally constitutes a barrier to the passage of the polyanionic albumin.[23] The factors determining the progression from this point to the point of macroproteinuria, i.e., an excretion of greater than 500 mg of protein in 24 hr, are not known but may be related to metabolic control and to blood pressure. However, when macroproteinuria is reached and excess protein excretion can be detected by routine urinalysis, the glomerular filtration rate (GFR) begins to decline inexorably at a rate of 0.6–2.4 ml/min per month.[23] Blood pressure control, rather than strict glycemic control, appears to be the only factor that can modify the degree of macroproteinuria or slow the rate of fall of GFR. When the GFR has fallen to or below 10 ml/min, the selectivity to albumin is lost and the excretion of IgG relative to albumin increases, suggesting that membrane defects have appeared.

Not all renal disease in diabetics is the consequence of the diabetic glomerulonepathy *per se*. A variety of acquired glomerular diseases not related to the diabetes have been described in diabetics. However, an additional form of renal involvement that is indirectly related to the diabetes may be encountered in diabetics exhibiting resistance to insulin.[25] Two types of insulin resistance have been described, one related to a reduced number of insulin receptors (type A), and one related to the presence of antibodies that block the interaction of insulin with its receptor (type B). In a study of 14 patients with type B insulin resistance,[25] a high prevalence of leukopenia, ANA, hypergammaglobulinemia, and elevated sedimentation rate was encountered. Proteinuria was present in seven and was heavy in four patients. Renal biopsy in these four patients revealed various degrees of proliferative and membranous glomerulonephritis and of tubulointerstitial nephritis, findings that are similar to those in lupus nephritis. All these patients had anti-DNA antibodies, and their renal disease and proteinuria improved with steroid therapy.

3. Renal Consequences of Tumors

In addition to direct involvement of the kidneys with tumors, disturbances of blood composition and immunologic disorders resulting from the tumor often may involve the kidneys. A large spectrum of fluid and electrolyte disorders and disorders of renal function may result. In

the section to follow the discussion will center primarily on hematologic and on solid tumors.

3.1. Hematologic Tumors

Both in multiple myeloma (MM) and in lymphoma (LM), involvement of the kidneys is frequent. Impairment of renal function is encountered in 40–50% of patients with MM and sometimes may require dialysis. Renal failure in these patients carries a bad prognosis and may be acute or chronic. Most frequently the renal impairment is related to excretion of immunoglobulin light chains, but it may also be related to the development of hypercalcemia, hyperuricemia, amyloidosis, and other related conditions. The mechanism of renal injury resulting from the excretion of light chains is not fully understood and may be related in part to cellular toxicity resulting from ingestion of these proteins by the proximal tubular cells. However, precipitation of light chains in the acid tubular fluid has been invoked, a postulate which would require that the proteins involved have a low isoelectric point (PI) which would make them uncharged and least soluble at an acid pH. Other experimental evidence has suggested that light chains of high rather than low PI are nephrotoxic. This postulate is quite plausible since cationic light chains should be more filterable through the polyanionic glomerular membrane barrier, and they may react with the anionic Tamm–Horsfall mucoprotein (PI 3.5) to form tubular casts. To investigate this possibility, 23 patients with MM were investigated with measurement of light-chain excretion rate, light-chain PI, and creatinine clearance.[26] There was no significant correlation between light-chain excretion and creatinine clearance, but a highly significant negative correlation was found between the PI and the creatinine clearance. There was no difference between kappa and lambda subtypes. These findings lend further support to the postulate that light chains of high PI may be responsible for myeloma kidney. Precipitation of the light chains in the renal tubular lumen, or some variant of this mechanism, is not the only manner whereby a paraproteinemia may cause renal failure. A patient with MM and IgA kappa paraprotein has been described to develop macroscopic polyarteritis nodosa and renal cortical necrosis culminating in the patient's death.[27] Another cause of renal failure that is often overlooked is hypercalcemia. Of 42 patients with MM who had responded to initial treatment, 11 died and of these seven had hypercalcemia, associated with renal impairment in five.[28] Therefore, while measurement of the light-chain excretion in urine may be a useful means of follow-up of patients with MM, other disorders that may also impair renal function and hasten death must be kept in mind.

Other than hypercalcemia, electrolyte disorders in MM may be the result of proximal or of distal renal tubular acidosis. A new disorder may now be added to this spectrum with the description of a patient who had hyperkalemic hyporeninemic hypoaldosteronism with a non-anion gap acidosis, interestingly combined with unexplained respiratory alkalosis.[29] Although known to occur in amyloidosis, this disorder had not heretofore been reported in MM. No evidence of amyloid deposits was detectable on examination of the renal biopsy, but infiltration of the interstitium with large numbers of lymphoid and mature plasma cells was observed.

The spectrum of renal involvement in LM, as in MM, is very broad and may directly or indirectly result from the tumor. Although reported, acute renal failure is only rarely (0.5–2%) the result of renal parenchymal infiltration with neoplasm.[30] This is in spite of the fact that lymphomatous infiltration of the kidneys is quite frequent (35%). The most frequent cause of acute renal failure in malignant lymphoma is bilateral ureteral obstruction.[31] Ureteral obstruction, due to either direct involvement of the ureters with lymphoma or to compression by enlarged retroperitoneal lymph nodes, occurs in 4–8% of patients. Another cause of acute renal failure in LM is the acute tumor lysis syndrome. Although it is most often attributed to hyperuricemia, the report of a patient with Burkitt's lymphoma who developed the syndrome while serum uric acid remained normal because of allopurinol therapy raises a question about uric acid always being the culprit and raises the possibility that hyperphosphatemia and hyperphosphaturia may be operative.[32] The serum calcium in this patient fell as expected, but systemic blood pressure and central venous pressure were normal. Precipitation of calcium phosphate in the kidneys consequent to deliberate urinary alkalinization also may have been a factor. Whatever the mechanism, the patient responded favorably to treatment with large-volume hemofiltration.

The nephrotic syndrome occurring in association with lymphoma has been well documented. Of particular interest is the close association of lipoid nephrosis with Hodgkin's disease and its remission following successful treatment of the LM. A recent report points out, however, that the occurrence of lipoid nephrosis in patients cured of Hodgkin's disease is not necessarily a harbinger of recurrent lymphoma.[33] In two patients previously cured of Hodgkin's lymphoma lipoid nephrosis occurred, but after 20 and 36 months of follow-up no recurrence of the lymphoma was observed. In one patient the nephrosis remitted spontaneously, and it resolved in the other following treatment with prednisone and chlorambucil. Whether these interesting occurrences were the result of the observed abnormal T4 : T8 ratio or simply a coincidence cannot be determined.

3.2. Solid Tumors

As in the hematologic tumors, a wide array of afflictions of the kidney may be encountered in patients having solid neoplasms, and a large number of neoplasms have been associated with renal disorders. One tumor infrequently discussed as having renal manifestations is neurofibromatosis of von Recklinghausen, a disorder that affects some 80,000 Americans. Retroperitoneal neurofibromas arising from the pelvic autonomic nerve plexuses involve the bladder and ureters, causing upper-tract obstruction and presenting with symptoms of hematuria, urinary frequency and urgency, enuresis, pelvic, abdominal or genital pain, and abdominal or genital enlargement.[34] Urinary diversion may be required when obstruction occurs.[34]

Hypertension resulting from renal artery stenosis or from pheochromocytoma represents another form of renal involvement in neurofibromatosis. Stenosis occurs at the origin of the renal artery in 50% of cases, is bilateral in 40%, and occurs on the left side alone in 44% and on the right side in only 13%.[34,35] The association of renal artery stenosis with coarctation of the aorta in 23% of patients is worthy of note. Surgical treatment with revascularization or with nephrectomy, which was necessary in 40% of patients, cured or improved hypertension in 95% of patients, whereas medical therapy or balloon angioplasty was uniformly unsuccessful.[35] In contrast to renal artery stenosis, pheochromocytoma, which occurs in 1–2% of patients with neurofibromatosis, occurs more commonly on the left side.[34]

In addition to hypertension, pheochromocytoma is sometimes associated with massive proteinuria which abates with excision of the tumor.[36,37] Renal biopsy performed in one patient revealed the lesion of focal segmental glomerulosclerosis.[37] This is a fascinating association in view of current thinking regarding the pathogenesis of focal glomerulosclerosis. Intraglomerular hypertension, which has been invoked as the basis of glomerular injury,[38] and which could have resulted from efferent arteriolar constriction in pheochromocytoma, may have caused the proteinuria and eventually the glomerulosclerosis.

Two other tumors that cause hypertension are aldosteronoma and juxtaglomerular apparatus tumor. An association between aldosteronoma and renal artery stenosis has been described, but in a recent case the hyperreninemia resulting from the stenosis of the renal arteries has been invoked in causing the development of bilateral aldosteronomas.[39] The patient who had hypertension, hyperreninism, and bilateral renal artery stenosis initially had no evidence of adrenal tumor by angiography, but a year later a left adrenal tumor was detected by scintigraphy and was removed but without benefit. A few months later a right adrenal

tumor was detected by scintigraphy, and when that was removed, the blood pressure returned to normal. The suggestion that prolonged elevation of the plasma renin could result in a "secondary primary," "tertiary," or "autonomous secondary" aldosteronism is provocative.[39]

Differing only in the raised peripheral and renal vein renin, juxtaglomerular apparatus tumor resembles in its clinical manifestations primary aldosteronism.[40] Hypertension, hyperaldosteronism, hypokalemia, and renal potassium wasting characterize both disorders, and only lateralization of renal vein renin and the demonstration of an intrarenal tumor by angiography and computerized tomography can distinguish between them.[40]

Of the metabolic disorders caused by tumors, an interesting entity is oncogenic hypophosphatemic osteomalacia, caused by mesenchymal neoplasms and resulting from a tumor-induced renal defect in phosphate absorption.[40] A variant of this disorder has been described in which the proximal tubular transport defect was more generalized (Fanconi syndrome) instead of being limited to phosphate transport.[41] In this patient the renal defect abated after resection of a nonossifying fibroma of the tibia. The exact mechanism of this renal defect and the nature of the tumor factor that causes it remains unknown at the present time.

The association between neoplasia and the nephrotic syndrome is now well documented, and neoplasms have been found in approximately 10% of nephrotic patients. The renal histologic lesion most commonly present has been membranous nephropathy, but mesangiocapillary glomerulopathy[2] and lipoid nephrosis[33] have also been described. Often there is a close temporal association between the remission or relapse of the tumor, on the one hand, and the remission or relapse of the nephrotic syndrome. This relationship is illustrated by the case of a patient with metastatic adenocarcinoma, subsequently determined to be of gastric origin, in whom the irradiation of the tumor metastasis to bone twice resulted in remission of the nephrotic syndrome caused by a membranoproliferative renal lesion.[42] The closeness of the association between neoplasia and nephrosis notwithstanding, a question remains regarding the mechanism of the renal disease and the reasons why it is not observed with greater frequency. In an effort to address this issue, a study by immunofluorescence microscopy of renal tissue obtained at autopsy from patients with solid tumors was carried out and the findings were compared to those in patients without carcinoma.[43] Immunoglobulin deposits were found in 17% of the neoplasm group but in only 5.4% of the other group, a significant difference. Deposits were seen most often in patients with digestive tumors, the deposits were usually mesangial, and in 64% of patients were IgG and/or IgM and/or C3. Interestingly, IgA deposits were present in 36%. There is no information whether

proteinuria was present antemortum, but considering the low frequency of nephrosis in neoplasia (as opposed to neoplasia in nephrosis), it is not clear why only very few patients develop the full-blown picture of nephrotic syndrome when the presence of immunoglobulins in the kidneys is so frequent.

Not usually thought of as a complication of malignancy is crescentic glomerulonephritis. However, in a recent report malignancy was found in 7 of 80 patients with crescentic glomerulonephritis but in only 1 of 80 patients with minimal-change or focal segmental glomerulosclerosis.[44] All 7 patients had type III crescentic lesions, i.e., no demonstrable immunoglobulins in the glomerulus. Even more interesting is the fact that the prevalence of malignancy in patients over the age of 40 years with crescentic glomerulonephritis was 20%. The authors suggest that a high tumor antigen load could have caused glomerulonephritis, or alternatively that the monocytes which had been sensitized to the tumor antigen cause glomerulonephritis by reacting with antigen deposited in the glomerular capillaries. These are fascinating suggestions, and while the exact mechanism remains to be elucidated, it seems now certain that a tumor lurking somewhere must be looked for in all patients over the age of 40 who develop type III crescentic glomerulonephritis.

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11

Congenital Renal Disorders and Kidney Tumors

Autosomal Dominant Polycystic Kidney Disease and Renal Cell Carcinoma

Manuel Martínez-Maldonado, Julio E. Benabe, and Luis Báez-Díaz

1. Introduction

This chapter reviews the literature until May 1986. The first part covers the anatomic and functional correlates of autosomal dominant polycystic kidney disease (ADPKD) and aspects of renal cell carcinoma that will be of interest to nephrologists. Several other tumors that produce symptomatology that is likely to result in nephrologic consultation, such as those seen with acquired cystic disease of dialysis and hemangiopericytoma, are also discussed.

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2. Autosomal Dominant Polycystic Kidney Disease

Adult polycystic disease has been recognized as an inherited disorder of autosomal dominant transmission with high penetrance. Originally described in the seventeenth century by Plater, a Swiss anatomist, the disease was further analyzed by Lejars in 1888.[1,2] Several series have reviewed the most salient clinical features of this disease,[3–7] which is the most important and common of the congenital renal disorders in the adult. The prevalence in several reported series has varied between 1:500 and 1:1000.[8–11] In a recent study from Olmsted County, Minnesota, the incidence of cases diagnosed during life was 1.38 per 100,000 person-years, whereas the annual incidence of cases diagnosed at autopsy was 2.75 per 100,000 person-years.[12] Thus, approximately 50% of the patients with ADPKD are clinically diagnosed during their lifetime. It has been estimated that patients with ADPKD account for 5–10% of all patients admitted for chronic dialysis in the United States.[10,13–18] It ranks third among the most common causes of chronic renal failure.[15,16]

The disease has almost the same frequency in females and males, with a slight preponderance in the latter.[12] It seems to be a more frequent cause of end-stage renal disease among Caucasians than blacks.[19,20] The diagnosis is usually established after the second decade; however, cysts have been found in the fetus and newborns of patients with ADPKD.[21–23] A recent study of a large population of nonazotemic patients with AKPKD found that 42% of those with suspected disease and 20% with the disease were less than 20 years old at the time of diagnosis; only 10% of subjects with full-blown ADPKD were over 50 years old.[24] In general, it appears that the younger the patient at the time of diagnosis, the longer the survival and the longer it will take for renal failure to supervene.[12,25] Garcia-Iglesias and co-workers[12] found that patient survival was significantly better in patients diagnosed before age 35 years than in those diagnosed at an older age. Early recognition of the diagnosis may permit proper treatment and/or prevention of the deleterious effect that pyelonephritis and hypertension may have on renal function.[26,45] This may be a factor that influences survival in the disease. Franz and Reubi[27] showed that renal function does not decrease at a constant rate between birth and end-stage renal failure. Renal function remains well preserved for many years but decreases rapidly at a later stage. The authors proposed that impairment of renal function is directly proportional to the growth of the cysts. However, if, as has been assumed, the radii of the cysts increase at a constant rate,[26,27] the initial growth results in displacement without reduction of functioning parenchyma. This allows kidney growth without detectable impairment of glomerular filtration rate (GFR). Nevertheless, at a later stage, compression of functional parenchyma

may no longer be avoided and leads to progressive atrophy. Since the renal capsule is relatively rigid, it could further limit kidney growth and accelerate parenchymal atrophy.[23] Clearly, secondary infections and hypertension, inasmuch as they produce cyst distention, would contribute to renal impairment. However, Franz and Reubi[27] and other investigators[5,45] could not find a faster decline in GFR in patients with recurrent infection in spite of the fact that longevity could be adversely affected.[5]

2.1. Pathogenesis

The basic biochemical defect that leads to the formation of cysts in ADPKD remains unknown. The initial hypothesis that ADPKD was a congenital disorder resulting from failure of the nephrons and collecting ducts to align properly has not been sustained by morphologic studies. Although abnormal branching of collecting tubules has been described by several investigators,[28,29] microdissection studies by Baert[30] did not confirm abnormal branching or attachment of collecting tubules. In the latter study cystic dilatation was found in proximal and distal tubules, loops of Henle, and collecting tubules intermingled with entirely normal nephrons and collecting tubules.[30] The distribution of the cystic lesions over the entire nephron and collecting ducts is random. This favors the hypothesis that loss of tubular wall support, induced through an effect in either the cell interconnection or the basement membrane, could cause cyst formation. Milutinovic and co-workers[31] examined the kidneys of 14 asymptomatic subjects at risk of developing polycystic kidney disease. These investigators could only find dilated distal and collecting tubules in 5 of the 14 subjects when their renal biopsies were examined by light microscopy. In three of the five subjects ADPKD was documented 3 years later. Electron microscopy revealed no differences in the fine structural details between specimens with and without tubular dilatation, except for splitting of the lamina densa of the glomerular capillary basement membrane. The latter change was found in two of the three patients with documented ADPKD.

Cuppage and co-workers[13] utilized electron microscopy to evaluate the morphology of 20 cysts from five patients with ADPKD. They found that basement membranes were highly variable in appearance. Some basement membranes had a normal thickness, whereas others were thickened or extensively laminated. Nevertheless, neither the basement membrane appearance nor its defects could be correlated with either cyst origin or formation. Darmady and co-workers[32] postulated that an inherited metabolic defect led to the formation of a circulating toxic substance which caused the morphologic changes responsible for cyst for-

mation. These investigators based their hypothesis on the experimental production of cysts in rats fed diphenylamine. They argued that examination of necropsy material from patients with ADPKD showed morphologic changes similar to those seen in the experimentally induced lesions in rats. Nevertheless, this has not been corroborated by others.

Several other antioxidants, such as diphenylthiazole and nordihydroguaiaretic acid, have been shown to cause renal cysts in normal animals.[33,34] This has lent support to the view that an alteration in the metabolism of some chemical substance stimulates the production of cysts. Evan and Gardner[35] studied rats that developed cystic kidneys after being fed 2% nordihydroguaiaretic acid (NDGA). These investigators monitored intratubular hydrostatic pressures while perfusing single surface nephrons in rats fed NDGA and normal control rat kidneys. They found a significant increase in intratubular hydrostatic pressures in cystic, but not in nondilated or normal nephrons. When the kidneys for NDGA-treated rats were examined, they found hyperplasia of collecting tubule segments with an increased number of cells comprising the periphery of the collecting tubules. There was also an increase in the number of nuclei around the circumference with the appearance of polyplike structures along tubule walls and definite or slight dilatation of collecting tubule lumens. Associated with these changes, thymidine uptake was increased. The authors concluded that partial obstruction caused by polypoid hyperplasia led to the formation of cysts in NDGA-treated animals.

To examine the presence, extent, and distribution of cellular hyperplasia in human ADPKD, Evan and co-workers[36] performed morphologic studies in four patients with the disease who had undergone nephrectomies. All showed areas of epithelial polypoid hyperplasia located in both the cortex and the medulla. The polyps were often located at the neck of the cysts and occluded over 80% of the tubule lumens. These findings confirmed the proposal that epithelial proliferation projecting like polyps into tubule lumens at or near the sites of change in luminal diameter could cause obstruction and formation of cysts. Nevertheless, Carone *et al.*[33] and Huseman *et al.*[37] found normal transmural pressure gradients in chemical-induced and in spontaneous human ADPKD. These investigators proposed and supported the hypothesis that the basic defect in ADPKD is altered compliance of tubular basement membranes. Since tubular basement membranes confer the elastic properties of tubules,[38] a defect of the basement membranes could lead to cyst formation even at normal intratubular pressures. Although the available literature provides support for both the hyperplasia–obstruction hypothesis and the tubule–basement membrane hypothesis, several kinetic considerations of cyst formation remain unanswered by either of

these hypotheses. Welling and Welling have made a critical appraisal of the kinetics of cyst formation[39] and concluded that stretching of the tubules because of increased transepithelial pressure or compliance would lead to thinning of the epithelial lining and basement membranes. Such a lesion has not been found in morphologic studies, as discussed above. Furthermore, if cysts fill up by accumulation of glomerular filtrate, the fluid-absorptive rate of the tubular epithelium must decrease as the cysts grow in order for them to reach the large sizes characteristic of ADPKD. This analysis has led Welling and Welling[39] to propose that ADPKD may be a disorder in which hyperplasia leads to radial proliferation of tubule epithelial cells initiated by endogenous modifiers in a genetically susceptible subject. Clearly, the cause of ADPKD is probably a combination of hyperplasia with obstruction and a tubular basement membrane defect.

Although several studies have addressed the issue of pathogenesis in ADPKD, little is known about the genetic defect responsible for this disease.

Recently, Watson *et al.*[40] obtained blood samples from 150 members of 12 ADPKD families and found a tight linkage between the disease and phosphoglycollate phosphatase (PGP) isoenzyme whose locus is in chromosome 16. In addition, Reeders and co-workers[41] found a highly polymorphic DNA marker linked to ADPKD and closely linked to the γ-globulin locus on the short arm of chromosome 16, close to the PGP locus. Measurement of the isoenzyme, therefore, may be an accurate predictor of the presence of ADPKD.

2.2. Cyst Function in ADPKD

The studies of Lambert[42] and of Bricker and Patton[43] helped establish that cysts from ADPKD are connected to functional renal units. In their studies these investigators showed that inulin injected intravenously could be detected in the cyst fluid. Furthermore, Lambert also measured creatinine and urea from cyst fluid samples and found that a concentration gradient in the cysts was present for both substances. He concluded that cystic nephrons in the adult retain functional activity and play a part in the formation of urine. Bricker and Patton also noted that the concentration ratio of creatinine in cyst fluid to that of plasma approximated unity in the majority of superficial cysts and exceeded unity in all of the deep cysts. The authors speculated that superficial cysts lie anatomically in the region of the proximal tubules and that deep cysts are more related to distal tubules. Thus, deep (i.e., distal tubule) cysts are able to sustain water reabsorption. Subsequently, Gardner[44] analyzed the chemical composition fluid in 12 cysts from a patient with ADPKD

and confirmed that the concentration of inulin, creatinine, and urea is higher in cyst fluid than in plasma, indicating that they are connected dynamically to patent and functional nephrons. Gardner also reported that the concentration of sodium varied directly with that of calcium and inversely with those of potassium, hydrogen, ammonium, and magnesium. This indicated that cysts function as either proximal or distal tubules.

Evidence that cysts function as proximal or distal tubules has been accrued by other investigators. Huseman and co-workers[37] studied the solute composition, volume, and hydrostatic pressure in cysts from eight patients with ADPKD. In all patients the pattern of solute concentrations in cyst fluid fell into two principal groups. Proximal cysts had sodium, potassium, chloride, hydrogen, creatinine, and urea values almost equal to their respective sera, whereas distal cysts had lower sodium and chloride concentrations and higher potassium, hydrogen ion, creatinine, and urea concentrations than their respective sera. Furthermore, Cuppage *et al.*[13] showed that proximal cysts are lined by epithelial cells with open or short closed apical junctions that are permeable to lanthanum. In contrast, distal cysts were lined by epithelial cells with long closed apical junctions impermeable to lanthanum. These findings are consistent with "leaky" proximal tubule cysts and "tight" distal tubule cysts. They provide strong evidence that cysts in ADPKD are enlarged segments of tubules that maintain the qualitative solute transport function of the respective segments from which they arise. Recently, Perrone[46] studied the transport characteristics of cyst epithelium from human ADPKD *in vitro* utilizing the Ussing chamber techniques. He demonstrated that cysts with fluid of low sodium concentration consistent with distal nephron origin had elevated potential difference (PD), high short-circuit current (Isc), and low conductance. These cysts had PD and Isc sensitive to amiloride and consistent with active transport. Cysts that did not maintain a sodium gradient were found to fall into at least two categories: cysts with functions similar to other leaky epithelia, such as the proximal nephron, and damaged or nonfunctional cysts.

These studies strongly support the notion that cysts are active tubular segments with rapid fluid turnover[47] which maintain the reabsorptive and secretory functions of the tubule segments from which they originate. Nevertheless, their function may not be completely preserved, as suggested by Welling and Welling[39] and Perrone's study.[46] In fact, clinical studies in patients with ADPKD show a number of functional defects. Martínez-Maldonado and co-workers[48] examined the ability to regulate sodium excretion in 13 patients with ADPKD (seven without azotemia and six with azotemia). They found that patients without azotemia attained balance on a high-sodium diet (100 meq/day) and were

able to reduce urinary sodium excretion on a low sodium (10 meq/day) diet. The group of azotemic patients, however, were unable to lower urine sodium concentration below 34 meq/day, a value not different from that seen in patients with renal disease of other etiologies. In the same study it was shown that a relationship could be found between sodium and calcium excretion regardless of the diet, but not between sodium and magnesium or phosphate excretion in the nonazotemic group. In the azotemic group, by contrast, the excretion of calcium, magnesium, and phosphate bore a significant relationship to that of sodium, suggesting that depression of ion reabsorption in advanced renal insufficiency was the result of a common mechanism. Thus, even though patients with ADPKD can maintain sodium balance, tubular function is not completely normal throughout their lifespan. In fact, even before any signs of renal insufficiency or gross pathologic defects are detectable, Martínez-Maldonado *et al.*[49] demonstrated a defect in maximal concentrating capacity (U_{max}). Similar findings were obtained in rats treated with diphenylamine.[50] Since the capacity to maximally concentrate the urine ($T^c_{H_2O}$) and maximally dilute the urine (C_{H_2O}) were normal in these studies, despite a marked reduction in U_{max}, the authors concluded that alterations in collecting duct function or medullary architecture were responsible for the concentration defect. The normal C_{H_2O} in human as well as in the experimental model argued against a gross defect in sodium reabsorption in the thick ascending limb or the distal tubule. Thus, it has been proposed that the defect is a functional change in the responsiveness to ADH exacerbated by altered medullary architecture.[25,90] The concentration defect has been confirmed in studies by D'Angelo *et al.*[51] and Preuss *et al.*[52] In the study by Preuss and co-workers, they also found an incapacity to lower urine pH in response to an acute acid challenge and decreased renal ammonium excretion during acid loading, even when corrected for GFR. Thus, tubular function, although generally well maintained in ADPKD, is not entirely normal.

2.3. Clinical Features and Associated Disorders in ADPKD

The most frequent clinical manifestations in ADPKD are summarized in Table I. Pain in the back or the lumbar area is the most common initial complaint. It is usually a dull ache or heaviness in the lumbar region which may occasionally be severe and accompanied by peritoneal irritation if an infected cyst ruptures. Obstruction caused by renal stone may cause colicky pain. In the study by Gabow and co-workers[24] as well as in other series,[3–7,53] hematuria was the second most common presenting symptom. In the former study the prevalence of hematuria was influenced by renal enlargement and hypertension, suggesting that vas-

Table I. Prevalence of Signs and Symptoms in ADPKD[a]

Proteinuria	70–80%
Flank and back pain	60%
Hematuria	30–40%
Headache	20%
Nocturia	8%
Dysuria	8%
Nausea	5–7%

[a] Approximate percentages.[4–7,24,53]

cular tears occur more commonly in vessels stretched by renal enlargement and subjected to higher hydrostatic pressure. Headache is another frequent symptom, occurring in 20% of patients with ADPKD. Its prevalence is not statistically related to hypertension and may suggest cerebrovascular abnormalities.[24] Less common, nonspecific symptoms in patients with ADPKD include nocturia, dysuria, nausea, and vomiting. Nocturia has been explained by the concentration defect commonly seen in PKD, while dysuria may indicate the presence of urinary tract infections. Nausea and vomiting have been attributed to uremia; nevertheless, in the study by Gabow *et al.*[24] 5% of the nonazotemic patients had these symptoms. Thus some other pathogenic mechanism must account for them. Among the signs and associated conditions in patients with ADPKD, proteinuria occurs in 70–80% of the cases (Table I), although massive protein loss leading to nephrotic syndrome is rare.[25]

One of the most common associated findings in ADPKD is diverticulosis, which may be present in over 80% of the patients with chronic renal failure and ADPKD (Table II). Scheff *et al.*[54] found an incidence of 83% among patients with chronic renal failure due to ADPKD and

Table II. Associated Disorders in ADPKD[a]

Diverticulosis	83%
Hypertension	60–75%
Renal infections	50–75%
Liver cysts	29%
Cardiovascular abnormalities	18%
Cerebral aneurysms	10–30%
Nephrolithiasis	10–20%
Pancreatic cysts	10%

[a] Approximate percentages.[4–7,20,24,53,54]

only 32% among those with other causes of chronic renal failure. Diverticulitis and perforation were also more frequent among patients with ADPKD and chronic renal failure. Another common finding among patients with ADPKD is hypertension, which is present in 60–75% of cases (Table II). The mechanism for hypertension has been studied by several laboratories. Nash[55] studied seven patients with ADPKD and glomerular filtration rates greater than 70 ml/min. He found evidence of volume expansion and sodium-dependent hypertension. The renin–angiotensin system was not consistently suppressed, and two of the seven patients had significantly increased plasma renin activity. Thus, hypertension was the result of sodium retention, volume expansion, and, in some patients, an incompletely suppressed renin–angiotensin system.

D'Angelo and co-workers[51] found a blunted natriuresis in response to volume expansion in ADPKD patients with normal GFR. These investigators proposed that incomplete arterial vasodilation resulting from the anatomic lesions of polycystic disease was responsible for the inadequate natriuresis. More recently, Valvo and co-workers[56] studied 32 patients with ADPKD, 16 with normal and 16 with decreased renal function. They found significantly higher plasma volume, cardiac output, and total peripheral resistance in patients with hypertension. Furthermore, hypertension did not correlate with plasma renin activity or GFR. A high degree of correlation was found between mean arterial pressure, plasma volume, and cardiac output. These data support the previous findings that hypertension is predominantly volume dependent and further define the hemodynamic state of ADPKD as characterized by increased cardiac output and total peripheral resistance independent of loss of GFR. In addition, Anderson *et al.*[57] found that the infusion of saralasin did not cause a vasodepressor response in patients with ADPKD consistent with the absence of a pathogenic role by the renin–angiotensin system in this disease. Reubi[58] has recently reported a series of 57 untreated patients with an average mean blood pressure (MBP) of 118 mm Hg. Most of the patients had mild to moderate hypertension, and severe hypertension was uncommon. Reubi could not find any significant correlation between MBP and GFR or renal plasma flow. In fact, he found that MBP could be elevated in some patients with normal renal function and normal in others with renal failure. Reubi also showed that there was no significant correlation between MBP and age. Yet he found a significant tendency for MBP to increase up to the age of 50 years and to decrease thereafter.[58] The explanation for these findings is unknown.

In addition to renal cysts, patients with ADPKD may have liver, cerebral, and pancreatic cysts, as indicated in Table II. Liver cysts may occur in as many as 30% of patients. Milutinovic *et al.*[59] found liver cysts in 46 of 158 patients (29%) who had ADPKD for over 10 years. He

showed that the prevalence of liver cysts increased with advancing age and with decrease of GFR. Nevertheless, liver cysts rarely caused liver function impairment or portal hypertension. The authors concluded that the presence of hyperbilirubinemia, liver failure, or portal hypertension in a patient with ADPKD should suggest a coexistent process such as liver cyst infection or malignancy.[59,60]

Clinically the most important extrarenal lesions associated with ADPKD are aneurysms of the cerebral arteries. About 10–30% of the patients may harbor this potentially lethal complication[61–63] and some series have reported a higher incidence.[64–65] The indications for cerebral angiography as a routine diagnostic tool for cerebral aneurysms were recently evaluated by Levey *et al.*[66] and Levey.[67] Their analysis showed that arteriography should not be carried out routinely because its benefits only exceed 1 year when the prevalence of aneurysm exceeds 30%, the surgical complications rate is 1% or less, and the patient is under 25 years of age. Their analysis also revealed that prevalence is the key variable in determining the benefit of arteriography. The higher the prevalence, the greater the benefit of arteriography at any age.[67] Their findings, however, apply only to patients not known to have cerebral aneurysms. Patients with symptoms suggestive of unruptured aneurysms should undergo investigation, and if the aneurysm is greater than 1 cm, surgery is indicated.[67–71] In addition, symptoms caused by compression of cranial nerve structures, other central nervous system structures, or distal thromboembolic phenomena have been shown to correlate with eventual rupture, particularly when aneurysmal size is greater than 7 mm.[71]

Cardiovascular abnormalities other than cerebral aneurysm and hypertension can be found in 18% of all hospitalized patients with ADPKD. Leier *et al.*[72] found 11 patients who had cardiovascular anomalies in a series of 62 patients with ADPKD. Seven patients had primary dilatation of the aortic root and annulus with aortic regurgitation. Mitral regurgitation was found in three patients, two with redundant mitral leaflets and ruptured chordae tendinae, and the third with mitral valve prolapse. One patient had coarctation of the aorta. The authors speculated that the best explanation for their findings in these patients with ADPKD was an inherited abnormality of collagen. Their hypothesis was supported by the histologic evidence of disruption and loss of collagen as well as the myxomatous changes of the aortic and mitral valves.[72] Abdominal aortic aneurysms have also been reported in ADPKD.[73,74] Finally, Gabow *et al.*[24] found a significant increase in the frequency of systolic murmurs unrelated to hypertension in nonazotemic patients with ADPKD.

Renal infection is another important complication associated with ADPKD. It has been estimated that from 50% to 75% of all patients with ADPKD have at least one symptomatic urinary tract infection during the course of their illness.[75,76] Cyst infection by the ascending route from the lower urinary tract is more common than by hematogenous spread. Nevertheless, Kime and co-workers[77] showed that experimental animals with diphenylamine-induced cystic disease developed *Escherichia coli* infections and pyelonephritis after intravenous injection of bacteria and external kidney massage when compared to control animals. Thus, the renal architectural alterations in ADPKD, as has been shown for obstructive uropathy, may increase susceptibility to infections. The effect of infections on the progression of renal disease is still unclear, as has been previously discussed. An interesting suggestion is that bacterial infections may play a role in the development of cysts. Werder and co-workers,[78] using an experimental model of ADPKD in mice, showed that when the animals grew in a germ-free environment, the incidence of cystic disease decreased from 70% to less than 1%. Similar findings were reported by Gardner and Evan,[79] using the NDGA-treated rat model. These investigators showed that the evolution of experimental cystic disease was accelerated by moving the animals from a germ-free to a conventional environment.

The diagnosis of pyelonephritis in ADPKD is based on the same signs and symptoms as those of subjects without cysts. Fever, renal pain, leukocytosis, pyuria, and positive urine or blood cultures virtually assure the diagnosis. Nevertheless, when a single cyst is infected, bacteriuria may be undetectable and urine cultures may be negative since bacteria from one infected cyst may be diluted in the relatively large urine volume of these patients. Computerized tomography (CT) may help in the diagnosis of cyst infection.[80] Thickening and irregularity of the cyst wall, increase in the attenuation value of the cyst content, and localized thickening of the renal fascia are found on CT. These changes however, are not specific for infected cysts since tumors may present similar changes.[81] An important complication of cyst infection is the development of a perinephric abscess. Sweet and Keane[82] found symptomatic urinary tract infections in 8 of 24 patients with ADPKD. The clinical course was complicated by the development of perinephric abscesses in five patients, three of whom died. They reported accumulation of gallium-67 in the areas of perinephric abscess in two of the five patients. Once the diagnosis is established, treatment with the appropriate antibiotic may be complicated by several problems. First, the infecting organism may be difficult to identify, as already mentioned, and second, antibiotic penetration and trapping may be different, depending on the functional nature of the

infected cyst. Schwab *et al.*[83] showed that intracystic pH determined the extent to which basic lipophilic antibiotics with relatively alkaline pKa, like clindamycin, achieved higher intracystic concentrations than lipophobic antibiotics, like gentamicin. Muther and Bennett[84] obtained fluid from 79 cysts in six adult patients and found that gentamycin, tobramycin, cephapirin, and ticarcillin were either undetectable or present in subtherapeutic concentrations. Subsequently, Bennett has shown that erythromycin, vancomycin, metronidazole, and clindamycin attain therapeutic concentrations after short-term administration even when severe renal failure is present.[76] Schwab has also shown that chloramphenicol may be effective in treatment of patients with infected cysts that had not responded to initial antibiotic treatment.[85] Thus, one can predict that lipophilic antibiotics with relatively high pKa's can be expected to accumulate in distal cysts.[85] Failure of antibiotic therapy is frequently the result of kidney stones or obstruction which must be removed or relieved for optimal response.

Obstruction usually results from extrinsic compression of calyceal infundibula, pelvis, or ureter by large cysts or from luminal obstruction caused by calculi or blood clots. Sonography is not useful in evaluating obstruction in ADPKD since calyces and renal pelvis are often compressed and are difficult to differentiate from adjacent cysts.[86] CT without contrast may be the most effective method to exclude obstruction by showing normal-sized renal pelvis outlined by renal fat.[87] The presence of blood clots of calculi producing obstruction may be detected and differentiated by CT.

Renal stones are another well-recognized complication in ADPKD. The incidence of stone formation in patients with ADPKD has been estimated to be from 10% to 20%.[7,8,10,75,88] The stones usually contain calcium; however, there are reports that the stones may be composed mostly of urate.[75] Recently, Martínez-Maldonado[90] reported eight patients with ADPKD who had kidney stones. He found that calcium excretion was normal in all of them, arguing against a possible role for hypercalciuria as an important factor in the genesis of stone disease. The explanation for stone formation in patients with ADPKD remains to be determined. It is likely that a cyst acidification defect, infection, stasis, or the absence of an inhibitor of stone formation in cyst fluid could contribute to the association of nephrolithiasis in ADPKD.[90]

Finally, renal cell carcinoma has been reported in over 30 patients with ADPKD. It has been calculated that renal cell carcinoma is bilateral in 20% of patients with ADPKD, whereas it is bilateral in only 5% of patients without ADPKD.[89] This finding has suggested that in ADPKD the influence of an unknown stimulus may lead to malignant changes of the hyperplastic epithelium in the cyst wall.[86]

3. Renal Cell Carcinoma

Primary renal tumors represent 2% of all malignant tumors diagnosed in the United States; the estimated annual incidence for 1986 is 20,000 new cases.[91] Adenocarcinoma of the renal parenchyma (hypernephroma) accounts for 85% of primary renal cancer in the adult population. Transitional and squamous cell carcinoma of the renal pelvis comprise the other 15% of malignancies considered as primary renal cancer in the adult.[92] The 5-year overall survival for primary renal cancer is 50%, and approximately 9000 deaths each year are from renal cancer.

The mean age at the time of diagnosis is 60 years, but the age-adjusted incidence rate shows a progressive increase from 9.6 per 100,000 population at age 50 years to 28.5 at age 80 years.[93]

There is a 2-to-1 male-to-female predominance in renal adenocarcinoma, but no difference is found in cancer of the renal pelvis.[93] The previous notion that blacks have a lower incidence of renal cancer than the general population has been discarded since it was mostly attributable to lack of access of the black patient to adequate diagnosis and reporting facilities. The incidence of renal cancer is higher in urban than in rural communities, as demonstrated in population studies in Connecticut and New York.[94,95] There appears to be no relationship, however, between socioeconomic status and renal cancer incidence.

Several environmental and nutritional factors have been associated with the development of renal cell cancer (Table III). For most of them the association has been a loose one. The use of tobacco and tobacco products is the only risk factor to bear a strong relationship. Prospective and case-control studies have shown significant correlation between tobacco use and the development of renal cancer. Bennington *et al.* estimated the risk of developing renal cancer in a smoking as compared to a nonsmoking population. The risk was 5.4 times greater for male tobacco users as compared to nonsmokers. The risk was highest for cigar and pipe smoking (12.9 and 10.3), followed by cigarette smoking (5.1).[96]

Table III. Environmental and Nutritional Factors Implicated in Kidney Cancer

Tobacco use	Halogenated hydrocarbons
Coffee	Animal protein consumption
Alcohol consumption	Radiation
Vitamin deficiencies	Nitrosamines
Lead	Analgesic abuse
Asbestos	Potassium bromate

In a subsequent study by these same investigators a similar association was found between tobacco use and renal adenomas.[97] This is of interest since some histopathologists consider renal adenomas to be small renal adenocarcinomas.

The association between tobacco and kidney cancer is also apparent in a study of cancer mortality among active Mormons of the states of Utah and California.[98] Members of the Mormon Church observe the "word of wisdom," which advises against the use of tobacco, coffee, tea, alcohol, and other addictive drugs. Cancer mortality among male members of the Mormon Church is close to 50% less than that of a comparable U.S. white male population. The decrease in cancer mortality was most striking in the tobacco-associated malignancies, including renal cell carcinoma. Tobacco use cannot solely account for the decreased incidence of renal cancer. Comparisons made between active Mormons and U.S. white males who never smoked still showed a significantly decreased incidence of renal cancer in male Mormons.

Nitrosamines and *N*-nitroso compounds can be found in the volatile fraction of tobacco and may be etiologic agents in the development of renal cell carcinoma. These compounds are remarkable for their ability to induce epithelial tumors in experimental animals.[99] Systemic administration of dimethylnitrosamines (DMN) induces renal adenomas and adenocarcinomas in rats. A single dose of DMN induces renal tumors in 50% of the animals exposed.[100] DMN appears to function by activation of a specific oncogene (K-RAS) on cells of the proximal convoluted tubules, leading to tumor initiation and promotion.[101]

3.1. Acquired Cystic Disease and Cancer

Dunnill *et al.* initially reported the development of acquired cystic disease of the kidneys in patients on chronic hemodialysis.[102] Of 30 patients who had autopsy examination, 14 were found to have cystic degeneration of the renal parenchyma. In 6 of the 14, an incidental renal cell cancer was found. In a prospective study by Ishikawa *et al.*, 96 patients with chronic glomerulonephritis who were on dialysis had serial CT of their kidneys performed.[103] Acquired cystic disease was detected in the contracted kidneys of 43% of patients with less than 3 years of dialysis and 79% of patients with 3 years or more. In four cases (one adenoma and three adenocarcinomas), tumor was detected by CT and documented by nephrectomy. The mechanism of tumor development is unknown, but cystic degeneration of the renal epithelia in these patients can show cytologic features of anaplasia.[104] Acquired cystic disease has been considered a premalignant lesion. Patients with adult polycystic kidney disease also have an increased incidence of renal cell cancer.[105]

Bilateral tumors are found in 20% of cases of ADPKD, as compared with a 5% bilateral involvement in the rest of the patients with renal cell cancer.[89]

3.2. Hereditary Renal Cell Cancer

The most commonly recognized instances of hereditary renal cell cancer are those associated with von Hippel–Lindau disease.[106] Close to 60% of patients with this hereditable phakomatosis will develop renal cell carcinoma; in most the involvement will be bilateral and multifocal.

Tuberous sclerosis is another phakomatosis associated with renal tumors.[107,108] Renal angiomyolipomas are found in 40–80% of patients with this syndrome. This is a slow-growing and benign tumor, although at times it may attain large size. Pain and spontaneous retroperitoneal hemorrhage may occur.[109] Not all renal tumors associated with this phakomatosis are benign.[108]

Hadju and Foote[110] reported 14 cases of renal cell carcinoma and nine cases of cortical adenomas in patients with tuberous sclerosis. This latter association is important as radiologic differentiation between renal cell tumors and angiomyolipoma cells is at times difficult, if not impossible.

Familial renal cell cancer has been described in patients without an underlying phakomatosis or polycystic kidney. Cohen *et al.*[111] was the first to describe a family with hereditable renal cell cancer and a specific constitutional chromosomal abnormality. Ten members of this family went on to develop renal cell cancer. Chromosomal studies on peripheral lymphocytes of affected members revealed a balanced reciprocal translocation between chromosomes 3 and 8 (t3;8) (P21; q24). It has recently been shown that the MYC oncogene is located in chromosome 8 and might become activated in this chromosomal rearrangement.[101] A nonconstitutional chromosomal rearrangement, (3p; 11p), has recently been described in tumor cells of a patient with familial renal cell cancer.[112] It has been suggested that a cancer gene might be present in the short arm of chromosome 3 as this is the chromosome involved in both cases of familial cancer associated with a specific chromosomal effect.

3.3. Animal Tumor Models

A variety of clinical carcinogens, including nitrosamines, nitrosoureas, cadmium, lead acetate, and aflatoxin, can induce renal cell cancer in susceptible experimental animals.[99,113,114,116] These tumors are often multifocal and bilateral.

One of the most commonly used animal models is that of estrogen-

induced tumors.[115] Renal tubular cells have estrogen and progesterone receptors.[117] Castrated hamsters chronically treated with diethylstilbestrol (DES) develop renal cell tumors within 5 months. Treatment with progestational agents or discontinuation of DES administration induces tumor regression in this model. Other hormonal manipulations, including treatment with androgenic steroids, modify the behavior of this tumor. These hormonal-dependent tumors have served as the basis for the use of progesterone and androgens in human renal neoplasia.[117]

3.4. Histopathology of Renal Cell Cancer

The term hypernephroma is commonly used to designate renal cell carcinoma. This misnomer arises from Grawitz's original description of the resemblance of these tumors to adrenal rest tissue.[118] Today it is firmly established that renal cell carcinoma originates from renal tubular cells.[119] Antibodies directed against microvilli antigens of proximal tubular cells cross-react with antigens on the surface of renal cell carcinoma.[120] Electron microscopy studies show features suggestive of its tubular cell origin. Villous transformation of cell membranes, unfolding of plasma membranes, and formation of intercellular spaces are all suggestive of the tubular nature of these cells.[119,121]

On light microscopy, three cell types are identified: (1) clear cell, a polygonal cell with clear cytoplasm as a result of large amounts of triglycerides and phospholipids; (2) granular, dark round cell with abundant mitochondria and highly developed Golgi apparatus; (3) spindle-shape cells resembling those of a mesenchymal tumor.

Most tumors are of the clear cell variant. In 15% of cases, granular, spindle, or a mixture of these may predominate. Histologic grade is based on cytologic differentiations and pattern of organization. Well-differentiated neoplasms are usually composed of papillary or tubular structures with very little cellular and nuclear pleomorphism. Poorly differentiated or high-grade tumors are characterized by increasing cellular anaplasia, high mitotic activity, and extensive areas of necrosis and hemorrhage.

One of the most controversial areas among surgical pathologists is the distinction between renal adenoma and adenocarcinomas.[122,123] The former identifies a benign growth and the latter a highly malignant and aggressive tumor. Both tumors arise from proximal tubular cells. There are no histologic, histochemical, or electron microscopic features that can help to distinguish adenomatous growth from a true carcinoma.[124] Some investigators suggest that a lesion less than 3 cm in size, with no evidence of capsular or parenchymal invasion, is better designated as

adenoma. We have seen, as have others, tumor recurrences and metastases from lesions of 2–3 cm in size.[125] Size by itself is not a good enough criterion for malignancy. Some renal adenomas will behave as small adenocarcinomas, and the true nature of those tumors, whether benign or malignant, should rest on the evaluation of local and regional invasion and metastasis rather than tumor size alone. In some cases cytologic and nuclear grading can be of help in identifying small tumors with aggressive behavior and poor prognosis.

Oncocytomas, oxyphilic adenomas, and mitochondromas are descriptive terms used to describe a variant of renal adenoma.[126–128] These tumors are composed of transformed epithelial cells with an eosinophilic cytoplasm and abundant mitochondria. Oncocytomas can arise in other organs, including thyroid, pancreas, lung, and salivary glands. Oncocytomas of renal origin are thought to originate from distal epithelial tubular cells. They can attain large size and demonstrate evidence of local invasion, but no case of distant metastases has been reported. Nephrectomy is curative in the great majority of cases. In rare cases of local recurrence surgical resection is indicated.

3.5. Rare Primary Tumors of the Kidney

Most of the rare primary tumors of the adult kidney are of mesenchymal (sarcoma) origin. They account for less than 3% of all primary renal tumors. Frequently, sarcomas of the retroperitoneal space can invade the kidneys by direct extension. In these instances, the site of origin can be difficult to identify and might be erroneously attributed to renal structures.

3.5.1. Angiomyolipomas

Renal angiomyolipomas are benign mesenchymal tumors. They are most commonly associated with tuberous sclerosis,[107–109] although in 50% of cases, no history of a hereditary phakomatosis can be elicited. The tumor is most commonly seen in women, with a female-to-male ratio of 5:1. In some cases the initial presentation is flank pain and spontaneous retroperitoneal hemorrhage; hypertension is present in 6% of cases. Cure can occur with removal of the tumor. On pathologic examination, the tumor is composed of mature adipocytes, smooth muscle, and aberrant vessels. Most histopathologists consider this tumor to be a "choristoma," a congenital growth arising in tissue displaced from its normal position during embryogenesis. The natural history of this tumor is characterized by slow growth, compression and destruction of

renal parenchyma, and, rarely, invasion of the perirenal space. Surgery is curative in most cases, and due to its benign nature a conservative approach is recommended.

3.5.2. Hemangiopericytomas

Hemangiopericytomas are sarcomatous growth of pericyte cells of vascular smooth muscle. There are few reported cases of primary renal hemangiopericytomas. Benign and malignant forms of this tumor exist. Differentiation between the two rests on identification of tumor grade, mitotic activity, cellularity, necrosis, and the degree of invasiveness and metastasis.

One variant of benign hemangiopericytoma is the so-called juxtaglomerular cell tumor. Less than 20 cases are reported in the medical literature.[129] The average age at diagnosis is 24 years. There is no difference in incidence among males and females. All patients have had severe systemic hypertension. Hyperreninemia, hyperaldosteronism, and hypokalemia are present in most cases. Because of the small size of some of these tumors (less than 1 cm), renal vein renin levels must be used to localize the lesion. The histopathology of the tumor reveals spindle or polyhedral cells on a background of collagenous stroma. Renin storage granules can be identified using immunofluorescent antirenin antibodies. Electron microscopy reveals rhomboid crystals characteristic of prerenin within the cytoplasm of tumor cells. Renin activity of tumor tissues can be found up to 2000 times that of adjacent normal renal cortex. Most tumors do not metastasize, and if detected early, systemic hypertension can be cured by nephrectomy. Not all renin-producing tumors are juxtaglomerular cell tumors. Renin production and hypertension has been documented in patients with clear cell carcinoma of the kidney, Wilms' tumor, and renal leiomyomas. Other mesenchymal tumors of different histogenesis can arise from renal structures. These include leiomyomas, liposarcomas, chondrosarcomas, mesenchymomas, and others.[130–133] The histopathology and tumor biology are similar to those arising in extrarenal tissues.

3.6. Clinical Signs and Symptoms

Most patients will present with signs and symptoms attributable to local and regional spread of the primary tumor. Hematuria, palpable abdominal mass, and pain are the most common initial manifestation, although they are present concomitantly in less than 10% of cases. (See Table IV.) Symptoms and signs related to distant metastasis occur in less

Table IV. Presenting Signs and Symptoms of Renal Cell Cancer

	Patel[134] (n = 166)	Skinner[136] (n = 309)	Haertig[138] (n = 311)	Gibbons[137] (n = 110)	Ochsner[135] (n = 103)	All[a] (n)
Triad (%)	5%	9%	—	—	11%	8% (578)
Hematuria	35%	59%	46%	37%	40%	43% (999)
Abdominal mass	30%	45%	30%	21%	48%	35% (999)
Pain	34%	41%	24%	21%	39%	32% (999)
Weight loss	15%	28%	27%	30%	37%	27% (999)
Fever	11%	7%	16%	—	11%	11% (889)
Incidental	8%	7%	8%	—	5%	7% (889)
Erythrocytosis	6%	3%	—	—	3%	4% (578)
Anemia	16%	21%	—	—	—	18% (475)
Hypercalcemia	—	3%	—	—	—	3% (309)
Hypertension	33%	—	—	—	22%	27% (269)
Acute varicocele	—	2%	2%	—	—	2% (620)
Metastasis	25%	10%	—	—	—	17% (475)

[a]Average percentage of patients from all five series; some characteristics were not available for analysis in some individual series. Triad refers to the concomitant occurrence of hematuria, abdominal mass, and pain.

than 25% of cases. Eventually 65% of cases of renal cell carcinoma will develop evidence of metastatic disease. The most common sites of metastatic involvement are lungs (50%), lymph nodes (35%), bones (35%), liver (30%), and brain (10%). But metastases can occur practically to any site and lead to various clinical syndromes that can tax the clinician's diagnostic accumen.[139–141]

The clinical picture can at times be confusing since close to 50% of patients will develop systemic or paraneoplastic manifestations from the primary renal tumor or its metastases. (See Table V.) In many instances hormones and hormonelike activity have been isolated from patients' blood samples or tumor cells in cultures.[144,146,147] Erythropoietin endogenous pyrogens, gonadotropins, prolactin, ACTH, PTH, and renin are implicated in the development of some of the paraneoplastic syndromes seen with renal cell carcinoma.[142–147]

3.7. Diagnosis and Staging of Renal Cell Cancer

A variety of invasive and noninvasive renal imaging techniques are available to the clinician for the detection and evaluation of space-occupying lesions of the kidney.[148–158] A plain abdominal film can detect abnormalities in the renal contour and demonstrate abnormal calcifi-

Table V. Systemic Manifestation of Renal Cell Cancer

Constitutional
Fever, weight loss, cachexia, night sweats
Hematologic
Polycythemia, anemia, dysfibrinoginemia, leukemoid reaction, monoclonal and polyclonal gammopathies
Endocrinologic
Cushing's syndrome, galactorrhea, gynecomastia, hypercalcemia
Gastrointestinal
Enteropathies, constipation, hepatic dysfunction
Neuromuscular
Polyneuromyopathies, polymyositis
Renal
Hyperreninemia, systemic hypertension, nephrotic syndrome, salt-losing nephropathy
Other
Amyloidosis, arthritis, vasculitides, congestive cardiomyopathy

cations. Centrally located calcific deposits are typical of renal malignancies, but 20% of mass lesions with peripheral calcifications will harbor a malignant tumor.

Intravenous pyelography and nephrotomogram can detect up to 80% of renal space-occupying lesions. Abnormalities include (1) renal enlargement, (2) distortion in renal calyces and pelvis, and (3) delineation of cystic lesions.

Ultrasonography will detect close to 90% of renal masses, but its best use comes in the distinction of cystic from solid lesions.[154–156] Fine-needle aspiration of suspicious cyst lesions is needed to further evaluate the nature of this lesion. Fluid analysis must include protein content, LDH levels, and cytology.

Computerized tomography and angiography are the most sensitive and specific imaging techniques for the diagnosis and staging of renal cell carcinoma. Renal angiography will detect a hypervascular mass in 85% of cases.[152] Neovascularization, arteriovenous fistulas, and microaneurysm will confirm the diagnosis of renal cell carcinoma. Angiographic studies are most accurate in demonstrating renal vein and vena cava invasion. Flush aortogram is needed for identification of parasitic tumor vessels.

Computerized tomography can detect renal masses with a greater than 90% accuracy. It has supplanted angiography for the staging of renal cancer. With the use of an intravenous bolus of contrast material and a dynamic series of computed tomograms, accurate staging of the tumor can be made. Dynamic computerized tomograms can accurately identify tumor extension to the perirenal space, renal vein, and vena

cava, and regional lymph nodes.[151] Magnetic resonance imaging promises to be a valuable tool for the diagnosis and staging of renal cancer. Recent preliminary studies report comparable results with computerized tomography in staging renal cancer.[160,161]

Age, sex, histopathology, tumor grade, and stage are some of the prognostic variables in patients with renal cell cancer. By far the most important prognostic variable is anatomic extension of the tumor at the time of presentation. Clinical cancer staging can be done with the information gathered by chest x ray, liver scan, bone scan, intravenous pyelography, computerized tomography, and/or renal angiography. A pathologic staging can also be done with the results obtained at nephrectomy. Pathologic staging is more accurate in predicting disease-free survival.

3.8. Natural History of Renal Cell Carcinoma

Renal cell carcinoma disseminates by (1) direct extension, (2) vascular invasion, and (3) lymphatic invasion. The tumor originates in the renal cortex and is usually surrounded by a pseudocapsule. Intrarenal growth of the tumor leads to invasion and compression of the renal calyces and pelvis. Outward growth results in invasion of the renal capsule, the perinephric space, and, eventually, penetration into Gerota's fascia. This can be followed by direct invasion of abdominal viscera.

Lymphatic invasion is initially documented by metastatic involvement of renal hilar and retroperitoneal nodes. There is good clinical correlation between invasion of retroperitoneal nodes and supradiaphragmatic nodal metastasis. Invasion of the renal veins is present in 20% of cases.[134,159] Progressive vascular tumor growth can lead to supradiaphragmatic extension and tumor thrombus that can reach as far as the right atrium and superior vena cava.[160,161]

Distant metastasis can be documented in 25% of patients at initial presentation. But 50–60% of a patient's distant metastases will appear during the course of the disease. Even in patients surviving more than 10 years after curative nephrectomy, late-appearing metastases occur in 10–15% of cases.[141,162]

Bilateral simultaneous tumors occur in less than 5% of cases.[159] The overall survival of these patients approaches those of patients with metastatic disease on presentation. This suggests that contralateral renal tumors represent metastasis to that organ rather than another primary tumor.

Instances of spontaneous tumor regression are documented in the literature.[163] Also, regression of pulmonary metastases has been reported to occur following resection of the primary renal mass. In most

cases of spontaneous or nephrectomy-induced regression, tumor regrowth is the rule.

3.9. Treatment of Renal Cell Carcinoma

Early aggressive surgery appears to be the most important factor for the improved survival of patients with renal carcinoma. During the past 18 years, simple nephrectomy has been supplanted by radical nephrectomy with or without lymphadenectomy. Overall survival figures have improved from 30% to 60% 5-year survival with the more radical approach. Surgery involves removal of the entire kidney, adrenal, perinephric fat, and Gerota's fascia, with or without regional lymphadenectomy.

Invasion of the vena cava by tumor thrombus occurs in 5–10% of cases. In vascular surgery techniques have been refined to the extent that radical nephrectomy and venocavotomy with complete removal of tumor thrombus is now feasible. An 80%, 2-year survival has been reported following venocavotomy and thrombectomy in patients with an infrahepatic vena cava invasion.[164] Surgery has even been attempted in patients with suprahepatic tumor thrombus extension; however, the results have not been encouraging.

New renal imaging techniques, such as CT scan, digital angiography, and nuclear magnetic resonance, allow the establishment of the diagnosis of renal carcinoma at an early stage. Recent reports suggest that in patients with low-stage, low-grade tumors less extensive surgery (*in situ* partial nephrectomy, bench surgery, and autotransplantation) results in survival figures comparable to those obtained with a radical approach.[165]

Attempts have been made by combining other therapeutic modalities to improve resectability and overall survival. One approach has been preoperative embolization of large tumors.[166] Embolization of tumor vessels has been done utilizing microspheres, blood clots, gelatin, ethanol, and steel coils. These preoperative interventions are not without complication; most patients experience fever, pain, nausea, vomiting, and sudden hypertension. Embolization procedures plus nephrectomy have not been shown to be clearly superior to nephrectomy alone. But in patients with large, inoperable symptomatic tumors, palliation of pain, hematuria, and, rarely, temporary regression of metastasis have been observed following embolization of the renal tumor vasculature.

From 25 to 60% of patients with renal cell cancer will present or eventually develop distant metastases. In selected cases surgical removal of a metastatic growth can result in prolongation of life and palliation of symptoms. Candidates for such metastatectomy are patients with solitary metastases, particularly to lungs or brain. Patients with a long dis-

ease-free interval (time between resection of primary tumor and the appearance of metastases) will derive the greatest benefit from aggressive removal of metastases.

3.9.1. Radiotherapy in Renal Cell Cancer

Renal cell carcinoma is a relatively radioresistant tumor. Most clinical trials that have employed preoperative or postoperative radiation to the renal bed have resulted in minimal improvement in overall patient survival. In some trials, however, a decrease in local-regional recurrence has been noted after postoperative radiation to the renal bed.

Radiotherapy can still offer good palliation of local symptoms in patients with inoperable tumors.[167] Radiation of bone and brain when metastases to these areas are symptomatic is also indicated.

3.9.2. Chemotherapy

Chemotherapeutic agents have shown marginal benefits in patients with metastatic renal carcinoma. The best results at the present time are obtained with vinblastine (vinca alkaloid). Nevertheless, in numerous clinical trials using vinblastine, response rates have been less than 20%.[168] Most of the documented tumor regressions are partial and short lived. The use of combinations of drugs with different mechanisms of action and toxicities has not improved the number of responses seen with vinblastine as a single agent.

3.9.3. Hormonal Therapy

The fact that prolonged estrogen administration can induce renal adenocarcinomas in hamsters and the identification of hormone receptors in human cancer cells have prompted a variety of hormonal manipulations in patients with metastatic renal cancer. The use of progestins and androgenic and antiestrogen compounds has shown objective tumor regression of metastases in less than 15% of cases.[117]

Use of the combination of chemotherapeutic drugs and hormone treatment, however, has failed to improve the results obtained with either agent alone.

3.9.4. Immunotherapy

Spontaneous and nephrectomy-induced regressions of renal cell metastases has suggested that stimulation of the immunologic system might result in control of metastases and tumor growth. Together with the lack of effective systemic cytotoxic agents, multiple clinical trials using

different immune-modulating agents have been reported in the literature.[169] Unfortunately, many studies have used small numbers of patients and others have been proven to be of no benefit at all.

Among the nonspecific immunotherapeutic agents, interferons are currently undergoing clinical trials. Initial results using natural or recombinant interferons show regression of tumor metastasis in 10–30% of cases.[170,171] Responses are most notable in lung and lymph node metastases. Major drawbacks from these studies include (1) short duration of responses, (2) systemic drug toxicities, and (3) small number of patients. *In vitro* synergism between interferons and chemotherapeutic agents have recently been demonstrated. Therefore, it is likely that new trials using a combination of both agents will be conducted in the near future.

The most recent novel approach to cancer therapy involves the use of activated killer cells.[172] Autologous tumor killer cells (LAK) can be generated in the laboratory by harvesting lymphocytes from patients with cancer and stimulating them with lymphokines (interleukin). Rosenberg[173] has initiated clinical trials with LAK cells in patients with different malignancies. Two patients with metastatic renal cell carcinoma included in this trial experienced documented tumor regression.[173] It is too early to comment further on the impact of this new modality of treatment.

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12

The Uremic Syndrome

Garabed Eknoyan

1. Introduction

In 1827, Richard Bright described the clinical and morbid symptom complex that results from chronic renal failure. The term "uremia" was subsequently introduced in 1849, to reflect the failure of the diseased kidney to excrete waste products and the resultant poisoning of the blood due to "urine in the blood," the literal meaning of "uremia." Although the term "uremia" is now integrated into the English language, the definition given in Webster's *Third New International Dictionary* is "accumulation in the blood of constituents normally eliminated in the urine producing a toxic condition. . . ," which still reflects the origin of the term as introduced over a century ago. In light of the current understanding of the additional roles of the kidney as a regulatory and endocrine organ, however, failure of renal function entails more than is implied in the literal meaning of uremia. In fact, as the term should be defined today, renal failure means that the kidney no longer can perform all three of its primary functions: excretion of waste products, regulation of body volume and composition, and secretion of hormones. The symptom complex that results either directly or indirectly as a consequence of renal failure is more properly referred to as a syndrome: the uremic syndrome, which is characterized by some degree of derangement of

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most, if not all, of the organ systems of the body. It is for this reason that the title of this chapter has been changed in this edition of *Contemporary Nephrology* and the format followed slightly modified to emphasize organ involvement.

2. Uremic Toxins

The fact that dialysis reverses some of the abnormalities of the uremic syndrome indicates the possible removal of accumulated noxious substances, a concept strengthened by the definition of uremia as a "toxic condition"; but definitely stimulated by the quest for a scientific explanation for the uremic syndrome, a search for uremic toxins has long been underway. None of the agents incriminated over the years has withstood critical examination except for parathyroid hormone (PTH). The evidence for PTH as a principal agent responsible for the manifestations of the uremic syndrome is certainly convincing and is considered under the sections of this chapter addressing the different organ systems. It is clear, however, that the role of PTH is generally permissive and additive to whatever else accounts for the uremic syndrome. Thus, the relentless quest for other specific uremic toxins continues.

Despite intensive efforts, utilizing sophisticated and elaborate technology, by several laboratories to identify the middle molecular toxin(s), since the concept of the "middle molecule" was first suggested in 1965, no identifiable middle molecular toxin has been isolated.[1] Furthermore, although chromatographic techniques have identified a number of abnormal peaks in uremic serum, some of which have been coded and dignified by being labeled "the" uremic toxin,[2] there has been no consistent and clear relation of any of them with objective tests of the derangements that occur in the uremic syndrome.[1,3,4]

Attention has also been focused on trace elements.[5–7] Among these aluminum intoxication has received the most attention, and certainly results in a distinct and well-characterized syndrome.[8] Aluminum intoxication, however, is a consequence of the treatment of the uremic syndrome rather than its cause. Zinc deficiency has also been incriminated.[9] The evidence for supplemental zinc replacement in correcting the taste, endocrine, and immune dysfunctions of uremia is considered under the appropriate sections of this chapter.

A host of peptides, aliphatic and aromatic acids, and amines have also been isolated from uremic serum by different investigators.[10–14] The reservations and limitations expressed for the "middle molecule" as a uremic toxin also apply to these proteins.

3. Progression of Renal Dysfunction

Dietary restrictions have long been at the core of treating the patient with renal failure. Much of the original intent was aimed at facilitating the role of the kidney with reduced renal function, at maintaining homeostasis (by restricting sodium, potassium, phosphate, and fluids), at providing symptomatic relief of uremic symptoms (by restricting protein), and at correcting nutritional deficiencies (by providing vitamin supplements). However, based on old evidence that nutritional factors may also be responsible for the progression of renal failure and prompted by recent experimental evidence that dietary protein restriction may retard the progressive deterioration of renal function of aging or diseased kidney of rats, attention has focused on the use of dietary manipulation to slow the rate at which renal function is lost during the early and late stages of chronic renal failure. It is still not evident by which mechanism protein restriction affects progression of renal failure. That intraglomerular hemodynamic changes parallel the improvement or deterioration of renal function is well established from a number of elegant experimental studies. In the experimental model, if intake of protein is restricted, glomerular capillary flow and filtration pressure, which are elevated in renal failure, return to normal and, thereafter, parallel the reduction of progressive glomerular injury. The mechanisms that account for this adaptive change remain to be identified. The fact remains that dietary therapy has emerged as effective in slowing the progress of renal failure and can postpone the time when the patient with renal failure will require maintenance dialysis.[15–19]

3.1. Protein Restriction

Long-term prospective, cooperative studies to carefully examine the role of protein restriction in retarding the progression of renal disease are underway, and their results eagerly awaited. In the meantime, considerable convincing evidence for a protective effect of protein restriction continues to accrue. The cumulative number of patients reported from different centers and from different countries is certainly impressive, although several of the studies have been retrospective.[20–23]

In a retrospective study, from Italy, of 78 patients with renal disease of diverse etiology, the effect of dietary protein and phosphorus restriction (about 0.6 g/kg body weight of protein, 700 mg of phosphorus) was compared to that of 22 patients on a regular *ad lib* diet.[20] Regression analysis of the reciprocal serum creatinine as a function of time revealed a significantly slower rate of deterioration in the patients on the protein-

restricted diet. In about half the patients on the dietary regimen there was no decrease of renal function during the period of follow-up. The best results were attained in those with chronic pyelonephritis and the worst in those with proteinuria. While on the protein-restricted diet, the actuarial survival probability at 72 months, assuming "renal death" at a serum creatinine of 10 mg/dl, was 45% in patients with chronic glomerulonephritis, 44% in those with polycystic kidney disease, and 67% in those with chronic pyelonephritis. In a prospective randomized study, from the Netherlands, of 149 patients with various renal diseases, dietary protein restriction (0.4–0.6 g/kg body weight) significantly retarded the development of end-stage renal failure.[21] Regression analysis of the reciprocals of serum creatinine against time showed that the average rate of decrease in reciprocal creatinine was 3–5 times slower in the protein-restricted group than in the control group. In a subsequent report of a larger group of 199 patients at various stages of renal failure, from the same center, renal function was shown to be significantly better preserved in the 105 patients on protein restriction (0.4–0.6 g/kg body weight) when compared to the 94 patients on an *ad lib* diet.[23] The median serum creatinine increased in the control group, but remained stable in the treated group, indicating arrest of progression in some patients.

In a study, from England, of 47 patients with moderate to severe renal failure, restriction of dietary protein (0.6 g/kg) decreased the rate of deterioration of renal function in 87% of the patients.[22] Those patients with the most rapidly declining renal function showed the greatest response.

The suggestion has been made that the dietary fiber, by inhibiting colonic bacterial ammonia generation and increasing fecal nitrogen excretion, might decrease hepatic urea synthesis and, thereby, reduce blood urea nitrogen (BUN). In a study of a 6 to 8 week course of hemicelluloses, BUN was reduced by 10–20% while fecal nitrogen was increased by 30%.[24]

Concern has been expressed that a negative nitrogen balance might result from strict (25–30 g/day) protein restriction. This can be circumvented by supplementing the diet with essential amino acids or a mixture of essential keto acid analogs and amino acids. In a study of 17 patients with chronic renal failure placed on a low-phosphorus diet containing 20–30 g of mixed-quality protein, supplemented by amino acids and their keto analogs, the rise in creatinine levels was slowed or arrested when the regimen was initiated before the patient's creatinine levels had reached 8 mg/dl.[25] Ten of the seventeen patients (59%) had a slower rise of serum creatinine during follow-up (average 20 months) than predicted from their pretreatment rate of progression. In six of the seven patients whose treatment was instituted before the serum creati-

nine was 8 mg/dl, the serum creatinine remained at or below the level at the start of treatment.

The response to protein restriction is variable. The nature of the underlying nephropathy appears to be an important factor in the results obtained. In a study examining this issue, when a low-protein diet (0.5 g/kg body weight, 700 mg phosphate) was prescribed for 6 months, renal function was noted to improve significantly in nine patients with tubulointerstitial nephropathy, while it was only marginally effective in 12 patients with chronic glomerulonephritis and had no beneficial effect in nine patients with hypertensive nephrosclerosis.[26] In patients with glomerulonephritis the response of their proteinuria seemed to discriminate the type of response of renal function obtained. Those patients whose proteinuria persisted showed no improvement of renal function, while those whose proteinuria decreased showed improved renal function.

Thus, it would seem that protein restriction is effective, particularly in those with early renal failure (S_{Cr} 8 <mg/dl); appears to exert its greatest beneficial effect in those with rapid deterioration of renal function; and that the variable effect noted may depend on the underlying cause of renal failure, those with tubulointerstitial nephropathies showing the best results and those with proteinuria the worst results.

3.2. Phosphate Restriction

Phosphate restriction is an inevitable accompaniment of protein restriction, and evidence exists for a relationship between phosphate intake and the rate of decline in renal function.[15–19,27] Additional experimental evidence for an effect of phosphate has been advanced from a study of the rate of progression of renal failure in two homogeneous groups of chronic renal failure patients with early renal insufficiency.[28] In both groups the diet supplied 35 kcal/kg per day and was equally restricted in protein to approximately 0.6 g/kg body weight; however, in one group the phosphate intake was 6.5 mg/kg body weight and in the other group 12 mg/kg body weight. In both groups the rate of decline of creatinine clearance improved on institution of protein restriction, but in the low-phosphate group the improvement was significantly better. The serum phosphate, phosphaturia, and serum PTH levels increased in the high-phosphate group, but remained near normal in the phosphate-restricted group.

3.3. Uremic Symptoms

Protein restriction exerts a beneficial effect on uremic symptoms. An objective evaluation of this was made in a study of six patients on

dialysis whose dialysis time was reduced so as to result in the abnormally low resting transmembrane potential (E_m) of skeletal muscle cells characteristic of the uremic state and generally reversible with adequate dialysis.[29] Instituting a protein-restricted diet supplemented with essential amino acids resulted in correction of the abnormally low E_m.

Another, and more important, abnormality that appears to be corrected by protein restriction is the stunted growth of uremic children. In a study of 14 children (average 9.9 years) treated for 21 months with a low-protein (0.4–2g/kg body weight), high-energy diet (55–130 kcal/kg body weight) supplemented with essential amino acids, expected improvement of clinical parameters occurred. In addition, it was noted that following the institution of protein restriction, the earlier decrease of growth rate was interrupted and an almost linear growth with the standard growth scores was attained in 10 of the children.[30]

On the other hand, the abnormalities of lipid transport that characterize uremic patients do not seem to reverse with protein restriction.[31]

3.4. Acquired Cystic Disease

A detrimental result of the progressive changes that develop in the diseased kidney, when the life of patients with end-stage renal disease is prolonged by maintenance dialysis or transplantation, are cystic lesions which have the potential of becoming malignant.[32–35] The diagnosis of acquired cystic disease of the kidney can be established by either ultrasound or computerized tomography. The lesions may be suspected when an unexpected rise in hematocrit occurs or the patient develops hematuria. Autopsy studies reveal tumors in cases that were never suspected of the lesion, although occasional deaths due to metastatic renal cell carcinoma have been reported.[34,36]

4. The Skin

Uremic frost and poor wound healing, the two principal uremic abnormalities of the skin, have become a thing of the past since the advent of dialysis. Instead they have been replaced by an increasing number of cutaneous lesions that afflict most patients with renal failure on dialysis.[37–41] Some cutaneous abnormality was present in 79% of patients on hemodialysis and in 76% of those on peritoneal dialysis.[37] The most characteristic feature was a marked cutaneous dryness with evidence of premature aging of the skin and signs of disturbed keratinization, characterized by xerosis with fine scales and lamellar desqua-

mation which assumed an ichthyosislike appearance in three patients. The most bothersome lesion and the scourge of some patients, however, was pruritus, which was more common in those with xerosis. Pruritus is a significant problem in as many as 60–70% of patients on dialysis[38] and 25% of those not on dialysis.[41] A linkage between pruritus and some parathyroid function derangement has long been suggested on the basis of disappearance of pruritus after subtotal parathyroidectomy and the demonstration of high calcium concentration in the skin of patients with end-stage renal disease.[39]

Other disturbances must also account for uremic pruritus since it is not present in all patients with secondary hyperparathyroidism, and there is no difference in the level of circulating parathyroid hormone between those who itch and those who do not.[38] In about a third of dialyzed patients the pruritus can be severe enough to necessitate therapeutic intervention. In 17 patients with severe pruritus, treated thrice weekly with total-body exposure to ultraviolet irradiation (UVB), pruritus resolved in all.[38] However, in 55% of the cases pruritus recurred in 6 weeks. Skin biopsies obtained before and after UVB phototherapy revealed elevated contents of calcium, magnesium, and phosphorus. The resolution of pruritus following UVB treatment was associated with a significant reduction of only the skin phosphorus to values comparable to those of nonpruritic uremics or normal volunteers. This was taken to indicate that uremic pruritus may be due to increased divalent ion concentration resulting in the microprecipitation of calcium or magnesium phosphate in the skin. In another study showing the effectiveness of UVB treatment in 7 of 10 patients with severe pruritus, the putative role of vitamin A, another culprit incriminated in pruritus, was examined.[40] Before treatment, the retinol concentrations in serum and epidermis were significantly higher in pruritic patients. Treatment with UVB reduced epidermal retinol. There were no changes in epidermal 3-dehydroretinol, carotene, or serum retinol. Evidence has also been advanced that the cause of pruritus is an increase of dermal mast cells and a release of histamine.[41] It should be noted in this regard that hyperparathyroidism accelerates mast cell proliferation in organs such as bone marrow, spleen, and intestines. In this study,[41] however, there was no relationship between mast cell proliferation and serum parathyroid hormone levels. The authors concluded that pruritus may be the result of extracorporeal circulation in hemodialyzed patients. A study from Russia reports the successful treatment of severe pruritus by intermittent plasmapheresis.[42]

Not all itching of dialysis patients is due to the uremic syndrome, particularly when the staff begins to itch also, as the authors of a report

entitled "Pseudouremic Pruritus" found when 35% of the staff began itching, and a more careful search led to the finding that the itch was due to a nosocomial scabies epidemic.[43]

Epidermal perforation, as a histologic feature of skin lesions, is very rare among the general population but may be present in 6–10% of patients with chronic renal failure, with a significant predominance in blacks and diabetics.[44] These lesions are keratotic papules and nodules on the trunks and extremities which show perforation on biopsy. They are thought to represent examples of transepithelial elimination, a mechanism by which the skin rids itself of abnormal dermal substances by extruding them through epidermal channels. They have also been described in association with pseudoxanthoma elasticum.[45] Follicular pustules which evolve into perforating folliculities in prurigo nodularis also occur.[46]

Inflammatory lesions of the eccrine sweat glands are rare in the general population but have been described in dialyzed patients.[47] Another unusual entity is widespread epidermolysis mediated by a toxin elaborated by *Staphylococcus aureus* which has been described in a 10-year-old boy with *S. aureus* infection of the dialysis fistula.[48] This is a rare disease that affects only infants and children younger than 5 years.

Bullous dermatosis, which closely resembles porphyria cutanea tarda, but with normal porphyrin levels, also occurs and has been referred to as "pseudo-porphyia" or "idiopathic bullous dermatosis of dialysis."[49] A study of 50 chronic dialysis patients without skin lesions revealed significantly elevated plasma porphyrins, twofold that of normal controls. In five dialysis patients with bullous skin disease, the serum prophyrin values were extremely elevated and exceeded those of subjects with prophyria cutanea tarda and normal renal function.[50]

Hyperpigmentation, particularly in sun-exposed regions, is a characteristic feature of renal failure patients and may be due to increased melanogenesis. Hypopigmentation of hair and freckles occurred in a patient receiving chloroquine for malaria prophylaxis.[51] The accumulation of chloroquine, because of reduced renal excretion, was implicated.

5. The Muscles and Joints

5.1. Muscles

Next to skin complaints, arthralgias, and muscle cramps, weakness and pain are the most common problems confronting the end-stage renal disease patient. The myopathy affects primarily the proximal muscles

of the extremities, in sharp contrast to the distribution of peripheral neuropathy, which characteristically affects the distal musculature and leaves sensory perception and reflexes intact.[52,53] Muscle atrophy, restricted almost entirely to type II (glycolytic) fibers with only minimal type I fiber loss, is the characteristic histologic lesion that expresses itself as clinically detectable muscle atrophy. The exact pathogenesis of what has come to be termed "uremic myopathy" remains undefined. Although there is convincing evidence for hyperparathyroidism as a principal cause of the myopathy, suggestive evidence continues to mount for increased muscle catabolism due either to nutritional deficiency or to metabolic disturbances in lipid-derived energy and electrolyte homeostasis.[53] Uremic rats sustain increased muscle protein wasting which is due to protein degradation as evidenced from perfusion studies of muscles from uremic rats, which when compared to normal muscle reveal lower muscle concentration of most amino acid pools, decreased muscle protein synthesis, and greater net release of phenylalanine, tyrosine, alanine, nonessential amino acids, and total amino acids.[54–57] Of special and practical interest is the observation that in uremic rats exercise training ameliorates the elevated muscle protein degradation to control levels, as documented from measurements of the rates of release of phenylalanine and tyrosine and those of incorporation of radiolabeled phenylalanine into muscle protein.[58] Of practical and clinical import is the observation made on patients with chronic renal failure in whom skeletal muscle function is directly related to the nutritional status of the patient.[59] In fact, skeletal muscle function testing provided a functional measure of the nutritional status of the end-stage renal disease patients studied. Thus, more attention to exercise and nutritional status might prove useful in alleviating and preventing the symptoms of some patients.

Disturbances in muscle intracellular electrolytes also occur. Typically, muscle biopsies from end-stage renal disease patients reveal an increase in intracellular water and reduced intracellular concentration of potassium in relation to various reference bases of cell mass,[60] whereas those of chloride and sodium are increased.[61] The abnormalities in potassium appear to be corrected by long-term hemodialysis, whereas those of chloride, sodium, and water persist, suggesting that the former are mainly related to cell function disturbances due to the uremic state whereas the latter are an expression of expanded extracellular fluid volume.[61]

5.2. Joints and Supporting Structures

The carpal tunnel syndrome or median nerve entrapment, once considered a rarity, is being recognized as a more common problem than heretofore recognized which affects as many as 12–15% of patients.[62,63]

The mechanisms implicated are peripheral neuropathy, tenosynovitis, hypervascularization of the connective tissue on the side of the dialysis access, and deposition of amyloid in the connective tissue. The lesion is more common and starts on the angioaccess site, but ultimately becomes bilateral. It presents earlier (1 year) after the start of dialysis in those with diabetes, analgesic abuse nephropathy, and peripheral neuropathy, whereas it is slower to develop in nondiabetics and those with no neuropathy.[63,64] Treatment consists of surgical decompression, with immediate and dramatic relief of pain and a gradual partial relief of the motor and sensory deficit.[62–65] In unoperated cases there is a progressive loss of motor and sensory function within 1 to 4 years after onset of symptoms[63] which may progress to incapacitating digital ulcerations.[66]

A rare clinical syndrome that continues to be noted in patients is spontaneous tendon rupture. The rupture is more common in younger active patients whose treatment is delayed. Histologically, it is characterized by nonspecific degenerative changes and calcification of the tendons involved.[67,68]

Arthralgia, a common complaint, can be incapacitating. Erosion of the articular surfaces due to secondary hyperparathyroidism is one cause of joint symptomatology. Radiographic assessment of 59 patients with end-stage renal disease revealed severe symptomatic erosive osteoarthropathy in 12, an incidence of 21%.[69] Clinical episodes of arthralgias were most common in the hand and knee joints. Symptomatic patients had a longer mean duration of dialysis and serum alkaline phosphatase levels.[69,70] Although multifactorial in origin, arthralgias are generally related to secondary hyperparathyroidism. The possibility of aluminum toxicity has been implicated. Aluminum crosses the synovial barrier and accumulates in the joint structures. The aluminum concentrations in synovial tissue from uremic patients were 2.7–10 times the control level, in synovial fluid they were 2.5–8 times control values, and in cartilage 2.6 times the control concentration.[71] An unusual form of arthropathy due to amyloid deposits in joint structures has also been reported.[72] This is a slowly progressive arthropathy that involves the larger joints (knees, shoulders, ankles). Amyloid deposits were present in the synovial membrane and fluid. Of interest is the fact that four of the seven patients reported had an accompanying carpal tunnel syndrome, which is known to be caused by amyloid deposits, possibly related to the dialyzer membrane.[73] Analysis of the isolated amyloid fibrils from such patients revealed a new form of amyloid fibril protein which is of low molecular weight and is homologous to β_2 microglobulin.[73]

Finally, neuropathic arthropathy or Charcot's joint has been noted in three end-stage renal disease patients.[74] Given the frequency of neu-

ropathy in end-stage renal disease, it is unusual that this syndrome has not been noted previously.

6. The Gastrointestinal System

Symptoms related to the gastrointestinal tract are among the most common complaints of the end-stage renal disease patients. Although dialysis has resolved some of the more serious derangements previously encountered (uremic colitis and hemorrhage), a host of new ones have appeared and affect the vast majority of dialyzed patients. An autopsy of 78 cases revealed some gastrointestinal abnormality in all but two patients, with more than one organ abnormality in each patient.[75]

6.1. Oral Cavity

The oral physiology is disturbed in end-stage renal disease, and abnormalities of the oral cavity are common.[76] Hypogeusia and a progressive deterioration of taste acuity that is responsive to zinc supplementation continues to be documented.[77,78] A significant inverse relationship between creatinine clearances (ranging from 5 to 75 ml/min) and the recognition taste thresholds for sour and sweet can be demonstrated.[77] The zinc deficiency and taste abnormalities persist up to 1 year after transplantation.[78,79] This delay in recovery seems to be due to an increased urinary zinc excretion following transplantation, which corrects itself after a year, concomitant with the amelioration in tast acuity.

Changes also occur in the hard tissues of the oral cavity.[76,80] The decrease in caries in patients with end-stage renal disease results from the protective effect of the alkaline pH of the plaques that these patients form, with the elevated salivary urea nitrogen concentration being the major variable that accounts for plaque pH.[81] An idiopathic reduction of the dental pulp chamber which has been described[76] seems to be related to and aggravated by steroids used to treat progressive renal disease and transplantation.[82]

6.2. Esophagus

Esophageal abnormalities were present in over 40% of cases examined at autopsy, esophagitis being the most common (36%) lesion.[75] In fact, an erosive esophagitis accounts for 17% of cases of upper gastrointestinal (GI) bleeding of end-stage renal disease patients.[83] Abnor-

mal esophageal motility, characterized by depressed or absent peristalsis and sustained multiphasic contractions with swallowing, was noted in 7% of patient from one dialysis center.[84] This could contribute to the vomiting of some patients and may be consequent to the neuropathy and axonal degeneration that is the underlying pathology of uremic neuropathy.

6.3. Stomach and Duodenum

In an autopsy series of 78 patients erosive gastritis was present in 37.1%, atrophic gastritis in 39%, duodenitis in 25.6%, gastric ulcers in 10.3%, and duodenal ulcer in 7.7%.[75] This is different from what was encountered clinically and on endoscopy of 249 patients, where biopsy of sampled gastric tissues revealed gastritis in 29.6%, atrophic gastritis in 18.2%, and duodenal ulcers in 11.2%.[85] In any case, the prevalence of gastroduodenal ulcers does not appear to be higher than that in the general population in patients with end-stage renal disease before transplantation.[85,86] Angiodysplasia of the stomach and duodenum appears to be the most common cause of upper GI bleeding in end-stage renal disease patients, accounting for 24% of the cases of bleeding, a frequency significantly higher than the 5% encountered in the general population.[83] Recurrent bleeding is also more frequent in patients with renal failure (25% versus 11%), with angiodysplasia being the most frequent source of recurrent bleeding in end-stage renal disease patients (53%), whereas peptic ulcer was the more likely cause in those without renal failure.[83] Multiple gastroduodenal angioplastic lesions are present in 63% of uremic patients.[87] Colonic angiodysplasia can be detected in 50% of them.[87]

The incidence of multiple duodenal polyps is also significantly higher among end-stage renal disease patients than in the general population.[88,89] The polyps result from circumscribed nodular hyperplasia of Brunner's gland. Patients with polyps do not differ from other end-stage renal disease patients in variables such as gastric acid secretion, serum gastrin level, or length of dialysis. In those with polyps, the mean pH of resting gastric juice was lower,[88] and the mean serum concentration of pepsinogen II was higher.[89]

It is now well established that patients with end-stage renal disease secrete less acid and have elevated levels of gastrin. When the uremic state is ameliorated by dialysis, gastric acid secretion is restored to normal in the majority of patients, whereas hypergastrinemia persists. In patients (15–20%) who continue to have gastric hypoacidity on dialysis, the gastric hypoacidity persists after normalization of renal function after transplantation.[90] The hypergastrinemia of chronic renal failure is generally

attributed to the reduced clearance of gastrin and its increased production.[91] Morphometric analysis of biopsy tissue reveals that the density of antral gastrin cells is significantly increased in patients with end-stage renal disease,[92,93] providing a morphologic basis for the elevated circulating gastrin levels.

Gastric emptying of solids and liquids is normal in patients with end-stage renal disease who are dialyzed.[94]

6.4. Intestines

Changes in intestinal absorption occur in patients with renal failure. In rats made uremic by subtotal nephrectomy, the intestinal absorption of riboflavin is decreased,[95] whereas that of vitamin A is normal.[96] The increased plasma and red-blood-cells concentration of riboflavin reported in end-stage renal disease, therefore, cannot be attributed to increased absorption and must be due to increased ingestion.

Acute ischemic lesions of the intestines must be considered in the differential diagnosis of cases presenting with nonspecific abdominal symptoms and leukocytosis, in adults following dialysis-associated hypotension,[97] and in children on chronic intermittent peritoneal dialysis.[98]

Contrary to studies in rats with experimental renal failure which have shown that colonic mucosa Na,K-ATPase and fecal potassium excretion is increased,[99] Na,K-ATPase activity in human rectal mucosa is not different from normal in patients with end-stage renal disease and shows no relationship to their serum potassium level.[100]

6.5. Liver

An increased incidence of malignancies has been noted in uremic subjects. Cholangiocellular carcinoma occurred in two patients with end-stage renal disease secondary to polycystic kidney disease who were on dialysis.[101] In one case, *in situ* transformation of a liver cyst epithelium into cholangiocellular carcinoma could be identified.

In two patients with cirrhosis of the liver who developed end-stage renal disease, long-term management of the ascites and renal failure was obtained by combined use of a peritoneovenous shunt and maintenance hemodialysis.[102]

6.6. Pancreas

In patients with end-stage renal disease, the pancreatic release of polypeptides is normal but their renal clearance is reduced, with a consequent 10-fold elevation of measurable immunoreactive pancreatic

polypeptide levels.[103–105] In normal controls, pancreatic polypeptide suppresses somatostatin release from the gut and pancreas. However, in patients with end-stage renal disease and high circulating pancreatic polypeptides, the circulating levels of somatostatin are normal, in part because the renal clearance of somatostatin is also reduced when the functional renal mass is decreased.[106,107] The elevation of circulating levels of another pancreatic enzyme, amylase, which is common in patients with end-stage renal disease, remains a vexing problem in the diagnosis of pancreatitis despite the application of sophisticated technologic methods.[108,109]

Trypsinlike activity and immunoreactive cationic trypsinogen content are also increased in the pancreas, but the plasma levels remain normal apparently because the discharge and synthesis of trypsinogen and chymotrypsinogen induced by cholecystokinin is reduced in renal failure.[110] This experimentally demonstrated lowered responsiveness of the pancreatic acini may account for the clinical observation of pancreatic hypofunction in severely wasted end-stage renal disease patients on hemodialysis who respond to supplementations of pancreatic enzymes.[111]

In experimental chronic renal failure produced by 7/8 nephrectomy in rats, pancreatic weight, DNA, RNA, and protein content were increased.[11]

7. The Pulmonary System

Although pulmonary involvement is common in patients with end-stage renal disease, pulmonary symptoms are not a prominent complaint of most of these patients. Disturbances in several parameters of measured pulmonary function have been described in uremic patients and are confounded by changes introduced by dialytic therapy.[112] The most common acute problem is pulmonary edema, and as a rule, increased lung water as a cause of abnormal lung function tests is difficult to exclude.[112,113] Volume overload and left ventricular failure are the principal cause of these abnormalities, although lower protein oncotic pressure due to hypoproteinemia and altered pulmonary microvascular permeability are additional important contributory factors.[114] In a canine model of renal failure where left atrial pressure was altered by inflation of a balloon catheter placed in the left atrium, protein osmotic pressure was lower in renal failure and pulmonary edema occurred at lower left atrial pressures in dogs with renal failure than in controls. In addition, the ratio of extravascular lung water to pulmonary blood volume was greater in the uremic dogs at each level of left atrial pressure examined,

suggesting increased vascular permeability in those with renal dysfunction.[114]

Structural changes consisting of thickening, lamination, and fragmentation of alveolocapillary basement membrane and degenerative changes of the capillary endothelium can be identified upon ultrastructural examination of the uremic lung.[115] Another factor that could account for the alterations in lung function is pulmonary calcification, which can be detected in as many as 20% of long-term dialysis patients examined at postmortem.[116] On light microscopy, calcification may be observed either as a finely granular, linear deposit along the alveolar septa, or as a coarse, widespread deposit within the lung parenchyma. Histochemical examination of these deposits revealed evidence of calcium and phosphate, as well as magnesium, leading to the implication of altered magnesium metabolism as a contributing factor to visceral calcification.[116] Abnormal deposits of aluminum have also been detected in the pulmonary precipitates.[115] Since in the majority of patients with pulmonary calcification the chest radiographs will not detect calcium, the presenting symptoms and abnormal pulmonary function tests may be confused with pulmonary embolism[117] or pulmonary edema.[118] In such cases, technitium-99m diphosphonate scintigraphy can be useful.[118]

Sleep apnea, primarily obstructive in type, can affect a significant number of male patients on hemodialysis. In a study of five patients with polysomnography performed both on and off testosterone, no evidence could be obtained for the previously suggested role of testosterone in causing the sleep apnea.[119]

The mechanism of hypoxemia that occurs during the course of hemodialysis has been the center of considerable investigation.[112,119] Persuasive evidence has accrued implicating dialyzer-associated complement activation as the cause of leukocyte aggregation, with the consequent intrapulmonary sequestration of the activated leukocytes accounting for the pulmonary ventilation–perfusion defect noted during the early stages of hemodialysis.[120] The activator sites on the membrane are saturable, and the repeated use of the same dialyzer results in progressively less complement activation.[121,122] The variation in the symptomatology noted in different patients appears to be related to the magnitude of complement activation, which is unique to each individual, a feature that lends itself to *in vitro* measurement by measuring the complement activation in response to zymosan.[123] The hypoxemia due to leukocyte aggregation at the start of dialysis accounts for only 20% of the fall in oxygen tension that occurs during the course of dialysis. A more severe hypoxemia develops after 30 min into dialysis and is independent of dialyzer membrane composition. Rather, it is determined by the nature of the base

used to prepare the dialysis fluid. In acetate bath, carbon dioxide lost across the dialyzer membrane causes reflex hypoventilation. In bicarbonate bath, no carbon dioxide transfer occurs and no hypoventilation develops.[124–126]

Peritoneal dialysis does not seem to exert a detrimental effect on pulmonary function, during either the filling or emptying of the abdomen with dialysate.[127–129]

8. The Cardiovascular System

8.1. Heart

Impaired cardiac performance develops relatively early in the course of renal failure. By the time end-stage renal disease occurs, cardiac symptoms become a common problem that becomes incapacitating in some patients affecting their quality of life and ultimately prognosis. In a study of 12 asymptomatic patients at various stages of renal failure, who were studied by cardiac catheterization at rest and during supine exercise, left ventricular stroke work did not increase normally with exercise when the serum creatinine was greater than 50 μmoles/liter (5.6 mg/dl). Left ventricular end diastolic pressure was abnormally raised at rest in most of them and during exercise in all of them.[130] Although confounded by coexistence of overload, hypertension, and anemia as renal failure progresses to end-stage renal disease, the cardiac findings that characterize these patients are enlargement of the left ventricular cavity, reduction in myocardial contractility, thickening of the left ventricular posterior wall,[131,132] and the presence of a congestive cardiomyopathy in some.[133]

In a study of 321 patients on chronic hemodialysis, interventricular septal hypertrophy (51.3%) and left ventricular posterior wall hypertrophy (47.1%) on echocardiography and left ventricular hypertrophy on ECG were the most frequent abnormalities noted.[134] The cardiovascular response to exercise is blunted in these individuals, despite adequate activation of the sympathoadrenal system as assessed from plasma catecholamine levels.[135] This defect is more severe in patients with insulin-dependent diabetes.[136] Diminished exercise tolerance is strongly associated with cardiac abnormalities. In a study of the exercise tolerance (bicycle ergometer) of 54 patients on dialysis, the performance of only 17 patients (about 1/3) was within normal limits.[137] Left ventricular ejection fraction, assessed by gated blood pool scanning in 37 patients, was abnormal in nine who also had abnormal exercise tolerance. M-mode echocardiogram obtained on 45 of the patients was abnormal in 43 of them, with evidence of left ventricular hypertrophy in 56% of them.

There is a significant linear relationship between the severity of cardiac abnormalities and the worsening of exercise tolerance. The proportion of patients becoming unemployed within 1 year from starting dialysis increased from nil in those with the best exercise test results to 60% in those with the worst results.[137] Aerobic endurance exercise training of these patients appears to exert a beneficial effect by increasing the physical work capacity, improving the lipid profile, ameliorating the glucose intolerance, and lowering the dose of antihypertensive medications.[138]

Asymmetric septal hypertrophy (ASH), detected by M-mode and two-dimensional echocardiography, was present in one-third of normotensive end-stage renal disease patients on dialysis who had no signs of cardiac disease.[139,140] Although during dialysis cardiac index is reduced in these patients and intradialytic hypotensive episodes have been attributed to ASH, the presence of ASH does not seem to impair the percentage of fractional shortening, ventricular circumferential shortening, or ejection fraction, probably because of a compensatory performance of the posterior wall of the left ventricle of these patients.[139] However, after hemodialysis, standing causes a significant increase in the plasma norepinephrine levels of patients with ASH as compared to those without ASH and controls. Also, in those without ASH, there is a significant drop in blood pressure and increase in heart rate, whereas both values remain unchanged in those with ASH.[141] This sympathetic overactivity, in response to postural or dialysis-induced hypotension, may contribute to the development of asymmetric hypertrophy of the septal wall of these patients. Of note in this regard is the observation made on seven hemodialysis patients with ASH whose septal hypertrophy improved significantly following institution of peritoneal dialysis.[140] In another study, 10 patients on hemodialysis and 10 on continuous ambulatory peritoneal dialysis, all of whom had prior evidence of left ventricular hypertrophy, were evaluated 22 months after institution of dialysis by M-mode echocardiography. In the hemodialysis group, left ventricular hypertrophy persisted or increased, while in the CAPD group it was reversed.[142] These observations of a preferential beneficial effect during peritoneal dialysis certainly deserve further scrutiny and documentation.

Following correction of the volume overload and uremic derangements by hemodialysis, there is, generally, a decrease in left ventricular end diastolic volume (LVEDV) and systolic volume, a decrease or no change in stroke volume and cardiac output, an increase in the velocity of ventricular circumferential shortening fraction (VCF), and an increase or no change in the left ventricular ejection fraction.[132] In a careful study of patients who underwent three different dialytic procedures, regular hemodialysis with weight loss, ultrafiltration only and regular hemodialysis without weight loss, it was clearly shown that the improve-

ment in left ventricular (LV) contractility, as assessed by VCF, could be dissociated from any alteration in cardiac filling volume.[143] Convincing evidence has also been advanced that an increase in ionized calcium, which occurs during regular dialysis, is a key factor in the improvement of left ventricular contractility noted after dialysis.[144] Changes in serum potassium may also contribute to this non-volume-dependent improvement, as suggested from a study of 16 patients using three different isovolemic bicarbonate-dialysis procedures.[145] During the first procedure, when ionized calcium and potassium were decreased, LV performance remained unchanged. During the second procedure, when ionized calcium increased but potassium decreased, VCF improved, but declined gradually within 3–12 hr of dialysis. During the third procedure, when ionized calcium increased and potassium remained unchanged, VCF remained unchanged.[145] A drop in potassium was necessary for the increment in ionized calcium to exert its beneficial effect. It appears that the ratio of ionized calcium to potassium may be an important determinant of dialysis-related improvement in LV performance.

Parathyroid hormone has been implicated as a potential depressant of myocardial function.[146] In a study of patients on hemodialysis, the plasma parathyroid hormone level was lowered in 12 patients by the administration of 1α-hydroxycholecalciferol for 6 weeks, and in a second group of 20 patients by increasing the plasma magnesium. In both groups the lowering of parathyroid hormone resulted in significant improvement in VCF. In a third group of patients, who underwent parathyroidectomy for severe secondary hyperparathyroidism, VCF also increased.[147] However, in another study of seven patients who had no cardiac hypertrophy but underwent parathyroidectomy, only a transient, modest change in cardiac performance was noted, with return of the cardiac performance to preparathyroidectomy level within 3–6 months of the procedure.[148] This is not an unexpected result, since in the final analysis even the improvement noted during hemodialysis depends on the preexisting cardiac status. In a study of 31 dialysis patients, hemodialysis improved VCF in those with reduced values prior to dialysis, but produced no significant change in those with normal predialysis in VCF. The improvement was blunted in those with left ventricular hypertrophy and was noted to be significant only in those with normal predialysis LVEDV.[149]

Because of their compromised ventricular function many patients with end-stage renal disease are on digitalis. The measurement and subsequent interpretation of plasma digoxin levels in these patients is fraught with problems. An endogenous substance with digoxinlike immunoreactivity (DLI) has been noted in as many as 60% of patients with

renal failure not on digoxin [150] and in premature children.[151] In uremic patients on digoxin, differences as high as 2.9 ng/ml have been noted when measured by different immunoassay methods.[152] Neither the endogenous DLI nor the variability of digoxin levels obtained by different immunoassay methods seems to relate to the levels of renal function or the form of dialysis therapy. Nevertheless, the levels of measurable digoxin in uremic patients not on digoxin are in part methodologic, being subject to the assay method used, and are generally of a small magnitude, rarely exceeding 0.23 ng/ml.[153] However, the potential addition of these otherwise clinically insignificant values to true digoxin content in patients on digoxin can result in the wrong therapeutic decision to underdose. A combination of high-performance liquid chromatography and radioimmunoassay of the eluted fractions seems to resolve the methodologic problem.[154] Attempts to isolate the endogenous digoxinlike substance(s) from the plasma of uremic and essential hypertensive patients and its characterization as a Na,K-ATPase inhibitor continue, but its purification and isolation remain elusive.[155,156]

Next to myocardial hypertrophy, arrhythmias are the most common abnormality encountered in end-stage renal disease. In a review of 321 patients, although premature contractions were detected by ECG in only 4.7% of patients, ventricular premature contractions were noted in 45.7% and a significant arrhythmia in 27.2% of 92 patients examined with 24-hr Holter monitoring.[134] Furthermore, the PQ interval was noted to be progressively prolonged in relation to the duration of dialysis. A similarly high incidence of cardiac arrhythmias (40%) was noted by others, in patients while on hemodialysis but not on nondialysis days.[157] This does not seem to be a uniform finding.[158,159] The unduly high values noted in the first two reports[134,157] remain unexplained but could be due to the presence of preexisting coronary artery disease.[160] Actually, the presence of coronary artery disease is generally underestimated in these patients. In a study of 33 patients on hemodialysis, 55% of the patients had an abnormal thallium-201 scintigram, whereas typical anginal symptoms were present in only 33% of them.[161] Eleven patients died within 1 year of the scintigraphic study. Not unexpectedly, the risk of developing fatal cardiovascular events was higher in those with abnormal scintigrams (7/18) than in those with normal scintigrams (1/15).

The creatine kinase levels are elevated in patients with chronic renal failure, but should cause no diagnostic confusion in the evaluation of myocardial infarction in these patients. In a study of 88 patients on dialysis, the MB creatine kinase activity was within the normal range (less than 13 IU/liter) in 92%, and only modestly elevated (highest 20 IU/liter) in 8% of patients. In acute myocardial infarction the levels of MB creatine kinase are substantially higher (153 ± 4 IU/liter) than the

values noted in end-stage renal disease patients.[162] No significant differences were found between the MB creatine kinase values prior to or after hemodialysis.

Valvular abnormalities are noted in end-stage renal disease patients. When they are examined, mitral annular calcification, with consequent functional and ausculatory abnormalities, appears to be more common than heretofore recognized. In a study of 168 patients who were on dialysis, 16 had mitral annular calcification (MAC). This is more common than is encountered in nonuremic individuals, in whom the incidence of MAC has varied from 2.8 to 6.3% in large echocardiographic studies. Also, the mean age of uremic patients with MAC is younger (55.7 years) than that of nonuremics (73 years).[163] Pulmonic valve insufficiency is not uncommon in hemodialysis patients and is the cause of the diastolic decrescendo blowing murmur best heard with the patient supine. It is generally corrected or improved by dialysis and reflects remediable pulmonary hypertension.[164]

8.2. Pericardium

Although the availability of dialytic therapy has reduced the incidence of clinically noted pericarditis in end-stage renal disease, the availability of ultrasound has added a new perspective to the pericardial changes seen in these patients. In a prospective study of 50 uremic patients studied before dialysis, pericardial effusion was detected by M-mode echocardiogram in 18, whereas only three of them had clinical evidence of pericarditis. Following dialysis, the effusion improved or subsided in six, remained unchanged in six, and worsened in two. No patient developed new pericardial effusion during dialysis. Changes in effusion size, noted during follow-up on dialysis, were related to volume overload.[165]

Intensive dialysis remains the first line of therapy of uremic pericarditis. In a retrospective review of 97 patients with uremic pericarditis, 67 responded to intensive dialysis and 30 did not. By univariate analysis, nine factors correlated with failure to respond: fever over 102°, rales, arterial pressure under 100 mm Hg, jugular venous distention, peritoneal dialysis treatment only, leukocytosis of greater than 15,000/mm^3, left shift of WBC count, large pericardial effusion, and the presence of both anterior and posterior effusion.[166] By discriminant analysis the authors developed a seven-variable function to predict the possibilities of response to intensive dialysis.

Although the absolute majority of uremic pericardial effusions are sterile, infective pericarditis must always be considered in these immu-

nosuppressed individuals who are constantly exposed to invasive procedures.[167–169]

8.3. Hyperlipidemia

Disturbances of lipid metabolism are implicated in the increased risk for cardiovascular atheromatous disease of uremic patients. Hemodialysis patients have higher serum triglycerides (TG) and lower HDL cholesterol concentrations than their sex-matched controls, while the total and LDL cholesterol levels are normal; i.e., they have a type IV hyperlipidemia.[170,171] Both hepatic and plasma lipoprotein lipase activities are low in hemodialysis patients. There is an inverse relation between lipoprotein lipase activity and serum triglyceride concentration, and a positive correlation between lipoprotein lipase activity and HDL concentration, suggesting that the impaired catabolism of triglyceride-rich lipoproteins is responsible for the low HDL cholesterol concentrations.[170] The plasma lecithin:cholesterol acetyltransferase (LCAT) is also significantly lower in patients on hemodialysis, but the distribution of LCAT activity is not different from controls, 90% being associated with the HDL and VHDL lipoprotein fractions.[171] This lower LCAT activity, in the face of higher plasma TG and lower HDL, may contribute to the impaired lipolysis of uremic patients and suggests that it is decreased catabolism rather than increased production that accounts for the hyperlipoproteinemia that is present in 30–60% of uremic patients.[171] Similar results have been noted in rats with experimental uremia in which the serum triglyceride level was lower than control in the fed state but higher in the fasting state.[172] In uremic rats the serum insulin level was significantly decreased and the epididymal lipoprotein lipase activity was significantly reduced. This was construed as evidence against hepatic overproduction of triglycerides and suggests a lipoprotein lipase-mediated defect of peripheral lipid catabolism, possibly related to the insulin deficiency state.[170,172]

Another abnormality is that of a significantly increased apo I and apo/HDL cholesterol ratio, indicating the presence of qualitative changes in the HDL subfractions which could contribute to increased atherogenesis.[173,174] Alterations in apo-LDL have also been noted in chronically uremic patients. LDL isolated from 18 uremic patients was degraded less and had diminished ability to stimulate cholesteryl ester formation in cultured fibroblasts when compared to LDL from 13 normolipemic controls.[175] LDL carbamylated *in vitro* showed interactive properties with fibroblasts similar to those of uremic LDL. Carbamylation of lysine residues of apo B *in vivo* could account for the abnormal uremic LDL.[175]

In any case, the decrease in uptake by fibroblasts of uremic LDL may increase the residence time of these particles within the subendothelial region of the vasculature and contribute to increased atherogeneity. A defect in cholesterol transport from HDL to VLDL and LDL has also been described in uremic patients on long-term hemodialysis.[176] It is not clear, however, whether this would contribute to accelerated atherosclerosis.

Currently, there is no good evidence that specific correction of the type IV hyperlipidemia reduces the incidence of ischemic cardiac events in the presence or absence of renal disease. However, the available experimental evidence does suggest a detrimental effect on the vasculature of the abnormalities noted in uremics. It seems, therefore, prudent to attempt lowering lipids in uremia. There are several modalities which are safe and effective but require the patient's initiative and cooperation. These are achievement of ideal weight, adherence to a graded aerobic exercise schedule, and avoidance of simple carbohydrates and ethanol.[138,177] Other measures that have been suggested, on the basis of rather equivocal evidence, are changes in the dialysate buffer composition (acetate versus bicarbonate) and glucose content, increased frequency of dialysis, and the dialytic modality (hemodialysis versus peritoneal dialysis). Consideration should be given to using alternatives to drugs commonly prescribed to these patients that cause higher TG levels, such as beta blockers and androgens.[177] The use of specific drugs to lower lipids in end-stage renal disease is probably best avoided given the toxicity of most agents currently available.[177,178] Carnitine deficiency has been incriminated in the pathogenesis of the altered lipid metabolism. In experimental renal failure of rats, carnitine supplementation had no effect on plasma lipid parameters.[179] In fact, in clinical trials of L-carnitine supplementation, a detrimental effect on the lipid levels and an increased platelet aggregation were noted.[180]

Of interest in the propensity of uremic subjects to cardiovascular complications are the markedly elevated nicotine levels noted in hemodialysis patients compared to control subjects before smoking, during smoking, and at 4.5 hr after smoking.[181] It is certainly advisable to discourage this group, more than any other, to abandon smoking.

8.4. Vasculature

The vasculature of patients with chronic renal failure is subjected to a variety of hemodynamic and metabolic insults that ultimately result in an accelerated vascular degenerative process which accounts for the major and early cardiovascular morbidity and mortality of these patients. Hypertension, present in the vast majority (>75%) of patients with renal

failure, is considered a principal contributor to this premature vascular degeneration.[182] A variety of factors that increase peripheral vascular resistance or cause volume overload contribute to the pathogenesis of hypertension.[182,183] Although volume control exerts a favorable response in the majority of patients, there is a subgroup of patients whose hypertension is due to a hyperreactive renin–angiotensin system (RAS). Actually, the plasma renin activity (PRA) has been known to rise with progressive reduction of renal function, although there is considerably interindividual variation in the levels of PRA attained.[182,183] The plasma renin and renin substrate concentrations, however, are not altered in renal failure patients,[184] and the increments in PRA must be due to the subsequent chain of events in the RAS. The enzymatic activity of renin is greater in the plasma of uremic patients on dialysis than in the plasma of normal control subjects.

The plasma renin reactivity, or the rate of conversion of angiotensin I production after addition of renin to plasma, has been shown to be increased in patients with renal failure compared with that of normal plasma,[184] suggesting the presence of a deficiency of a normally occurring renin inhibitor in uremic plasma. Two plasma fractions obtained by chromatography of normal plasma have been shown to inhibit the enzymatic activity of both renal renin and highly purified mouse submaxillary renin.[185] The inhibitor appears to be a lipid associated with protein and was absent in the plasma of 10 uremic patients on dialysis.[185] A significant increase in the serum angiotensin-converting enzyme activity of these patients has also been noted.[186,187] The magnitude of the changes noted, however, was not related to the severity of the renal disease but tended to be higher in diabetic and proteinuric patients and those with liver disease, and was augmented by hemodialysis-induced hypotension.[186–188] Although these results are contrary to those obtained by others,[189] they certainly provide a biochemical basis for the use of converting enzyme inhibitors in the management of these patients.[190,191] Both of the currently marketed converting enzyme inhibitors are excreted by the kidneys, and it is important that they be used in reduced dosage in patients with reduced renal function, in order to avoid serious side effects.[192]

Another contributory factor to the hypertension of renal failure patients is the sympathoadrenal system. The concentration of circulating catecholamines is usually increased early in the course of renal failure.[193–195] The calculated appearance rate of norepinephrine (NE) is higher in uremic patients, suggesting hyperactivity of the sympathetic nervous system. Furthermore, when exogenous NE is infused, the appearance and metabolic clearance rate of endogenous NE is reduced in normal controls but not uremic patients, suggesting that the negative

feedback of circulating NE levels on the activity of the sympathetic nervous system is impaired in uremics.[196] Moreover, an increase in arterial pressure occurs in uremia at distinctly lower doses of exogenous NE compared with that of controls both in humans[195] and in the rat.[197] This exaggerated NE pressor responsiveness may well contribute to the development of hypertension in some patients with early renal failure. By contrast, a reduced pressor response to NE has been noted in patients with advanced renal failure.[198,199] There is a significant negative correlation between the changes in arterial pressure in response to NE and the blood level of parathyroid hormone (PTH) in uremic patients.[198] The administration of PTH and its amino-terminal fragment blunts the pressor effect of NE in normal rats, and this effect is completely abolished with indomethacin, implicating the modulating effect of PTH on the pressor responsiveness to NE as being mediated by prostaglandins.[200] Treatment of uremic patients with indomethacin also restores their pressor responsiveness to NE and could be useful in the treatment of patients with hypotension due to autonomic dysfunction.[198] It is of interest that even the early increased pressor response to NE infusion noted in uremic rats is restored to normal by parathyroidectomy and verapamil.[197] Thus, changes in cellular calcium transport, possibly due to secondary hyperparathyroidism, may be operative in both the increased and decreased responsiveness to NE.

The vasoconstrictor and vasopressor actions of vasopressin have also been implicated in hypertension. In renal failure, the plasma vasopressin level is elevated and apparently contributes to the hypertension, as evidenced by the reduction in blood pressure noted following the administration of synthetic vasopressin inhibitors in the rat[201] and humans.[202]

Identification of the mechanisms operative in patients with renal failure provides for a more specific and targeted approach to the treatment of their hypertension. This is a very important issue since the adequate control of hypertension during the course of renal disease will retard its progression to renal failure and end-stage renal disease.[182,203] Even when end-stage renal disease develops and the patient is started on dialysis, the control of hypertension remains important since it will reduce the risk of cardiovascular morbidity and mortality.[182]

9. The Hemopoietic System

9.1. Red Blood Cells

A hypoproliferative anemia, which is generally normocytic, is an invariable accompaniment of renal disease and is a cause of considerable morbidity, particularly in those whose disease progresses to end-stage

renal disease and who are maintained on dialysis. As a rule, the severity of the anemia is related directly to the extent of renal insufficiency.[204] The pathogenesis of the anemia is multifactorial, resulting from erythropoietin deficiency, inhibition of erythropoiesis, and shortened red-cell life-span.[204–207]

9.1.1. Erythropoietin Deficiency

The principal site of erythroipoietin (Ep) production is the kidney, and the most important cause of the anemia of renal failure is decreased Ep production as functional renal mass is reduced.[204–208] The site of erythropoietin production within the kidney remains undefined, although it has been shown that more Ep is produced in the cortex than the medulla[205] and, more recently, that Ep is present in homogenates made from tubular but not glomerular fractions of hypoxic rats.[209]

The availability of a reliable and sensitive radioimmunoassay (RIA) of Ep, which utilizes iodinated purified Ep and rabbit antihuman Ep, has considerably facilitated the study of Ep in renal failure.[204,205] Patients with renal failure have been shown to have lower levels of Ep than patients with comparable degrees of anemia but without renal failure; however, a subset of uremic patients have elevated levels of immunoreactive Ep, albeit to a level that does not show the expected correlation with the degree of anemia.[207,208] Although the failure of such patients to correct their anemia may be attributed to inhibition of erythropoiesis by uremia, the alternative possibility, that RIA is measuring immunoreactive components that are biologically inactive forms of Ep, appears more likely. A significant reduction of immunoassayable Ep has been noted to occur during a single hemodialysis.[210] Since Ep is a glycoprotein of mol. wt. about 36,000,[205] it would not be expected to cross the cuprophane membrane. Additionally, when patients with comparable degrees of uremia increase their production of Ep, as measured by RIA, because of acquired renal cysts, the red blood production is increased and occasionally the hematocrit increases to polycythemic levels.[205,211] Thus, it seems that RIA detects immunologically reactive but biologically inactive fragments of Ep which are small enough to be removed by hemodialysis and thus may give spuriously elevated values, which may be misleading. In addition, coincubation studies of human urinary Ep in the presence of uremic serum reveals marked diminution of immunoreactivity by RIA and of biologic activity, as assayed in fetal liver CFU-E growth, when compared to Ep incubated with normal human serum.[208] Thus, some alteration of Ep seems to occur in the presence of uremic serum that reduces both the immunologic and biologic activity of the hormone.[208] It seems prudent, therefore, that results noted by RIA studies be interpreted with caution.

Most prior experimental studies of the role of Ep in uremia have been negative in nature; i.e., its absence causes anemia. Recent studies, however, have been more positive in nature, demonstrating the effectiveness of Ep administration in correcting the anemia of renal failure.[212,213] The infusion of Ep-rich plasma, obtained from phlebotomized sheep, into sheep with uremia produced by subtotal nephrectomy stimulated erythropoiesis in uremic and normal sheep. Furthermore, nondialyzed uremic sheep responded as well as dialyzed animals. The total dosage of Ep necessary to correct the anemia depended on the severity of the anemia.[213] The dose–response effects obtained and the finding that normal and uremic sheep had an identical response has been construed as evidence for the absence of physicologically significant inhibitors of erythropoiesis in uremia and strengthened the possibility of Ep replacement therapy in renal failure.[213,214] This is a real, promising, and feasible avenue since the human structural gene for Ep has now been isolated and expressed,[215] thereby making the availability of biologically active Ep from recombinant sources imminent.

Small amounts of Ep can be detected in the plasma of anephric patients,[205] suggesting a source of extrarenal Ep. The isolation of Ep from liver tissue extracts and the perfusate obtained from the liver of hypoxic rats denote the liver as an extrarenal source of Ep.[216] The observation of a spontaneous increase in the hematocrit associated with episodes of viral hepatitis in dialyzed patients and the demonstration of increased Ep production in experimental hepatic damage has been construed as further evidence for the hepatic production of Ep.[205] However, the report of a patient with hepatitis whose hematocrit increased during the illness and then fell back to baseline with recovery without a change in plasma Ep[217] argues against this possibility and suggests an as yet undefined mechanism for the liver's ability to stimulate erythropoiesis.

9.1.2. Inhibition of Erythropoiesis

The results of Ep infusion studies noted above[213] notwithstanding, considerable evidence exists and continues to accrue for the presence of inhibitors or erythropoiesis in uremia.[204,205,208,210] The fact that some patients started on hemodialysis and then switched to peritoneal dialysis show an improvement of hematocrit in the absence of changes in plasma Ep levels strongly implicates the removal of an inhibitor by peritoneal dialysis.[204] The inhibitory effect seems to be exerted on the erythroid progenitors that give rise to CFU-E, the putative primary target cell for Ep. Both hemodialysis and continuous peritoneal dialysis appear to be effective in reducing, but not eliminating, the activity of uremic inhibitors from the serum,[218] which remain detectable and equally present in the

plasma of patients receiving hemodialysis and continuous ambulatory peritoneal dialysis.[210] The precise role of these inhibitors and their nature remain undefined. Evidence that the parathyroid hormone might be responsible for inhibition of red blood cell production has been advanced by some,[219] but continues to be refuted by others.[220,221] The discrepancy of *in vitro* studies has been attributed to the purity of the parathyroid hormone extract preparation used.[222] Specifically, when only partially purified extracts were used, there was inhibition of both erythropoiesis and granulocytopoiesis, suggesting a nonspecific inhibitory effect of the extract.[222] Also, the addition of 2000 pg/ml of the N-terminal or C-terminal of parathyroid hormone to bone marrow cultures resulted in no inhibitory effect of CFU-E.[220] Furthermore, no correlation could be found between serum PTH levels and the degree of inhibition of erythropoiesis in patients with uremia either before starting or after long-term dialysis treatment.[220,221]

The evidence of the polyamine spermine as the cause of erythropoiesis inhibition also remains controversial. Evidence had been advanced for [223] and against[224] elevated levels of polyamine in the sera of patients with renal failure, and its specificity to inhibit erythropoiesis has been both supported[223] and refuted.[204]

Accumulation of iatrogenic substances may contribute to the anemia of renal failure. Aluminum toxicity contributes to the anemia by altering the erythroid response because of its effect on erythroid cell hemoglobin synthesis and porphyrin metabolism.[225,226] As a result it causes a microcytic anemia with absent or markedly reduced peripheral sideroblasts.[204] Hypervitaminosis A has also been implicated as a cause of toxicity because vitamin A levels are elevated in dialyzed patients.[227]

Deficiency states that occur in renal failure also contribute to the abnormal response of the erythron. Folate deficiency may develop in some dialyzed patients despite supplements because of impaired intestinal absorption[228] and its loss during dialysis,[229] but can be corrected by adequate replacement therapy.[229] Vitamin E deficiency has also been implicated, with evidence for[230] and against[231] an improvement in hematocrit following vitamin E supplementation. Iron deficiency does occur in some end-stage renal disease patients, and the serum ferritin levels are used as an index to monitor iron stores. Although measurements of ferritin are useful, bone marrow stores cannot be unequivocally estimated from serum ferritin levels[232] and may be elevated, despite iron deficiency in individuals who receive multiple transfusions, especially in those with HLA, A3, B7, or B14 antigens.[233]

Androgenic anabolic steroids are useful in the treatment of anemia of chronic renal failure.[234] This effect appears to be mediated by an increase in Ep levels and enhancement of the erythroid progenitor's

sensitivity to Ep.[205] A significant increase in serum erythropoietin activity has been reported following a trial of prostaglandin E_2 in four patients with end-stage renal disease.[235] This observation remains to be documented and further explored.

9.1.3. Altered Metabolism and Shortened Life-Span

Red-cell survival is reduced in renal failure, to about one-half normal life-span, but this is not considered to be a major cause of the anemia.[204] The shortened survival of RBCs has been attributed to elevated PTH.[219] Evidence that PTH contributes to the shortened survival of RBCs has been advanced from studies of uremic dogs in which thyroparathyroidectomy resulted in restoration of red-cell survival to normal.[236] The shortened life-span of RBCs seems to be the result of changes in erythrocyte membrane fluidity which alter its osmotic fragility and render it more susceptible to splenic sequestration and destruction.[204,237] However, no correlation with RBC osmotic fragility after parathyroidectomy was noted in a study of uremic patients on dialysis.[238,239] Thus, the mechanism of PTH-induced shortened survival does not seem to be altered osmotic fragility.

The shortened RBC survival has also been attributed to elevated levels of neuraminidase activity in the serum of uremic patients. Cross-incubation studies of uremic serum with RBC of healthy donors was shown to result in RBC desialylation.[240] Increased neuraminidase could result in reduction of RBC sialic acid content, which would lead to their prompt removal from the circulation by the reticuloendothelial system.[240,241]

A reduction in the reducing ability of uremic RBCs would render them more susceptible to oxidant injury. The reduced RBC glutathione content and altered hexose monophosphate shunt that are present in patients with renal failure[242,243] appear to be equally corrected to normal levels by hemodialysis and peritoneal dialysis.[244]

Congenital hemolytic anemias have been shown to be associated with increased RBC pyrimidine nucleotides. An increase in purine content and abnormal pyrimidine nucleotides have been shown in RBC hemolysates of uremic subjects and may contribute to the accelerated hemolysis of chronic renal failure.[245]

The ion transport turnover rate of the erythrocyte Na–K pump is impaired in uremia by a non-ouabain-like circulating factor whose activity is diminished acutely by hemodialysis.[246] This seems to be associated with a decrease in the number of Na–K pump sites of uremic RBCs and cannot be induced in normal RBCs with cross-incubation studies of

uremic serum and normal erythrocytes.[247] However, there seems to be a significant correlation between the magnitude of weight loss during dialysis and that of increased pump activity noted after dialysis, indicating that a volume-dependent factor could be an important endogenous regulator of the Na,K-ATPase and may well play an important role in the systemic manifestations of the uremic syndrome.[246] The nature of the dialysate bath buffer also may affect the modulation of this factor. The increment in pump activity after dialysis was significantly higher with acetate as compared to bicarbonate as the buffer at comparable degrees of fluid removal during dialysis.[248] On the other hand, the prior reports of a "dialyzable plasma factor" that reduces the sodium–lithium countertransport of RBCs could not be confirmed in two recent studies.[249,250]

9.2. Hemostasis

Hemostatic abnormalities manifesting themselves as hemorrhagic diathesis develop in the course of renal failure. Dialysis therapy has resulted in the amelioration of most, but not all, of these abnormalities. As a result, whereas the mortality due to the more serious forms of hemorrhagic diathesis (GI, cerebral) has been reduced, they continue to be a serious problem in some, and a cause of morbidity in others.[251,252] Although a number of modest abnormalities in the coagulation cascade continue to be noted,[253,254] the principal cause of the bleeding tendency is that of a defect in platelet function.

Defective platelet aggregation in response to a variety of agents is the most consistent abnormality noted in studies of platelet dysfunction in uremia.[251,252] Attempts to determine the molecular basis of this defect reveal abnormalities in thromboxane formation, adenine nucleotide, storage pool, surface topography, and adenylate cyclase activation. A reduction in platelet thromboxane B_2 production in response to thrombin has now been reported by several investigators.[251,255,256] Thromboxane B_2 formation in response to thrombin and collagen is decreased by 30–50%.[256] Because of a reduction in thromboxane B_2 in response to arachidonic acid at high concentrations (>1 mM), the defect in thromboxane B_2 production has been attributed to a functional impairment in cyclooxygenase activity.[255] However, the response is normal at lower concentrations of arachidonic acid (<1 mM), leading to the countersuggestion that cyclooxygenase activity is normal.[256] The defect noted at higher concentrations of arachidonic acid has been attributed to platelet lysis at these concentrations.[256] Uremic platelets have a modest reduction (35%) in the storage pool of adenine nucleotides and of ATP

secretion (25–50%) in response to thrombin. Dialysis results in partial correction of abnormalities of aggregation and thromboxane B_2 formation but does not alter the storage pool defect.[256]

In a study of adenylate cyclase complex of platelet membrane from uremic patients, the response to PGE_1, the stimulatory response, was increased, whereas the response to catecholamine, the inhibitory response, was diminished. The density of α_2-adrenergic receptors was normal, as was the affinity of catecholamine binding, suggesting the presence of a defect beyond the receptor site, i.e., the inhibitory nucleotide binding protein.[257] This would increase intracellular cyclic AMP, which in turn would inhibit platelet aggregation. Utilizing binding studies with radioactive-labeled lectins, a defect in the surface topography of carbohydrates in uremic platelets has also been noted.[258]

PTH has been incriminated to have an antiaggregatory effect by increasing intracellular cAMP.[251] However, in *in vitro* studies PTH did not increase platelet cAMP.[259] The role of PTH as an inhibitor of platelet aggregation *in vivo* also has been questioned.[260] Whereas bovine parathyroid extract inhibited platelet aggregation, synthetic human PTH fragments and synthetic bovine PTH do not inhibit aggregation, suggesting a spurious inhibitory effect of the impurities of the bovine extract.[260] Furthermore, platelet aggregation was normal in six patients with primary hyperparathyroidism and remained unchanged after parathyroidectomy.[260]

Although the platelet count is normal in renal failure, a shortened platelet life-span has been noted.[261] This, as well as the aggregation defect, are generally corrected by dialysis. However, the dialytic procedure itself may exert acute transient detrimental effects on platelets. In a study of 14 patients, the platelet count declined during dialysis, and platelets from the effluent line were less aggregable than platelets from the arterial line.[262] The dialyzer membrane composition is a major cause of this abnormality, the platelet loss being present with cuprophane but not with polyacrylonitrile membrane dialysis.[263] Platelet damage during dialysis has been postulated as a cause of the increased antithrombin III noted during hemodialysis.[264] Deferrioxamine, used to treat aluminum toxicity, can also cause thrombocytopenia.[265]

An association between anemia and abnormal hemostasis and platelet function has been reported. A negative correlation between the log bleeding time and hematocrit, with a correlation coefficient of 0.78, was noted in 33 uremic subjects.[266] The bleeding time was normalized following transfusion, when the hematocrit exceeded 26%.[266] A defect in aggregation that is corrected in the prescence of increasing hematocrit has also been noted.[267]

The successful use of synthetic 1-deamino-8-D-arginine vasopressin

to control the bleeding diathesis continues to be documented.[268,269] Of therapeutic interest is the report of the treatment of uremic bleeding with a conjugated estrogen preparation.[270] The treatment was successful in normalizing the bleeding time after 2–5 days of premarin and was successfully used on five patients who underwent surgery.

"Platelet-activating factor" (PAF) represents a recently recognized group of phospholipids with a wide range of biologic activities including the *in vitro* activation and aggregation of platelets. The kidneys are one source of PAF. Five anephric patients undergoing hemodialysis had undetectable levels of PAF after nephrectomy.[271] The clinical implication of this observation, as well as the role of PAF, remains to be clarified.

9.3. Leukocytes

The increased propensity of uremic individuals to infection is due to abnormalities of granulocyte function and impaired immune response. The transient neutropenia noted early during dialysis has already been considered in Section 7, on the pulmonary system. The oxygen consumption and glucose metabolism of leukocytes, in response to various stimuli, is significantly reduced in uremic patients on dialysis.[272] Granulocyte adherence[279] and chemotaxis[272,274] are also impaired in these patients. The phagocytic activity, which is significantly impaired in nondialyzed uremic patients, is restored to normal during either hemodialysis or peritoneal dialysis.[275] The bactericidal capacity of these cells, however, was noted to remain abnormal after dialysis in one study,[275] but to normalize in another.[274] The impaired bactericidal activity may be due to the reduced lysosomal activity of the polymorphonuclear cells of patients with renal failure, which apparently remains abnormal during dialysis.[276]

10. The Immune System

The decreased capacity of the uremic host to mount an immunologic response, first noted in the early experiments of allograft survival in uremic recipients, has been confirmed by heterotropic heart transplantation between rats of different isogenic strains.[277] Dialysis results in amelioration of the impairment, but some defects persist while new ones appear.[278] The major detrimental result of this derangement is the increased susceptibility to infection and neoplasia of uremic patients, which accounts for much of their morbidity and mortality. Despite considerable investigative effort, the pathogentic mechanisms responsible for this de-

fect remain elusive. It is evident, however, that it is cell-mediated rather than humoral immunity which is more severely and consistently suppressed in uremic patients.[278]

10.1. Cell-Mediated Immunity

Lymphopenia is more common in renal failure,[278] and a pronounced depression in the number of lymphocytes occurs in uremic rats subjected to a variety of infections.[279] Abnormalities in lymphocyte function include depressed cell-mediated immunity, diminished lymphokine production, and impaired interferon synthesis.[278] The altered percentages of T-cell subpopulations with consequent relative imbalance of T-cell function have been implicated in the abnormal immune homeostatic mechanism. In a study of cell-mediated immunity of 76 end-stage renal disease patients on hemodialysis, there was significant diminution in the number of helper/induced (OKT4) cells, but no significant change in the number of suppressor/cytoxic cells (OKT8), resulting in a significant decrease in the helper/suppressor cell ratio.[280] Blood transfusion induced no alterations in this ratio. Uremic patients had a significant increase in the number of macrophages (OKM1 cells), but normal levels of natural killer (NK) cell activity. The majority of these patients had a delayed cutaneous sensitivity response to recall antigens, but this could not be correlated to the total circulating T cells or levels of lymphocyte subpopulations.[280] In another study of 25 end-stage renal disease patients on hemodialysis, no difference from normal of OKT4 cell levels could be detected,[281] although the suppression of mixed lymphocyte reaction was significantly higher in uremic subjects. After concanavalin A induction, the percent of T4 and particularly T8 cells expressing Ia antigen, indicating immunologic activation, was significantly higher in the uremic patients. The functional suppression in the mixed lymphocyte reaction was significantly reduced by treatment with OKT8 monoclonal antibodies. These results were taken to indicate that the reduced *in vitro* response of uremic lymphocytes may be a consequence of increased suppressor activity associated with the T8 Ia-positive subset of T cells.[281] An increase in the level of OKT8 suppressor cells has been noted in patients on peritoneal dialysis who have two or more episodes of peritonitis.[282]

The mixed lymphocyte reaction is suppressed in uremic patients who are not on dialysis.[283] In cross-incubation studies uremic serum from nondialyzed patients was shown to suppress the reaction of lymphocytes from normal donors, and the reaction of lymphocytes from uremic subjects remained suppressed following incubation in normal serum. Thus, in addition to the intrinsic defect of the cells, there appears to be an

inhibitory factor in the serum of uremics.[283] Dialysis seems to transiently improve lymphocyte function[284] and possibly remove an inhibitor, as suggested from a study in which normal lymphocytes incubated in serum from dialyzed patients retained their normal reactivity.[285] A peptide that suppresses lymphocyte proliferation has been isolated from uremic serum,[286] but its nature and cellular mechanism of action remain undefined. On the basis of circumstantial evidence, the suggestion has been made that it inhibits T-cell growth factor[287] or possibly alters the intracellular cyclic GMP/cyclic AMP ratio of lymphocytes.[288] However, the intrinsic defect of T-cell function persists after dialysis.[289] Another factor implicated in abnormal T-cell function is thymosin. Rats with experimental chronic renal failure develop marked thymic atrophy and a reduction in the number of thymic lymphocytes. Intrathymic concentration of thymosin, a potent immunomodulator, is significantly reduced in uremic rats.[290]

Nutritional deficiencies have been implicated as a contributory factor to the altered cell-mediated immunity. Both pyridoxine[291] and zinc[292,293] supplementation have been noted to improve the lymphocyte function of patients on dialysis.

Finally, whereas several studies have noted abnormalities of T-cell function, those of the NK and killer cells appear to remain normal.[294]

10.2. Humoral Immunity

Humoral immunity is affected much less than cellular immunity,[278] and acute-phase reactants remain normal in renal failure.[295] Elevated circulating immune complexes were detected in 30–40% of 200 patients undergoing maintenance dialysis in the United States or Switzerland.[296] Rheumatoid factor was present in 20% of them, and it was most of these who had elevated circulating immune complexes. This was independent of the treatment modality, and its cause remains undetermined. The possibility of enhanced activity of the alternative pathway in patients with severe renal failure has been suggested. Complement protein D is the rate-limiting protease of the alternative pathway of complement activation. It has a mol. wt. of 24,000 and is normally filtered in the glomerulus and catabolized in the proximal tubule. In 20 patients with chronic renal failure and 16 on dialysis, the concentration of the complement protein D was significantly elevated[297]

Following hepatitis B vaccination, the conversion rate of uremic subjects is less frequent than in normal controls.[298,299] The response can be enhanced by increasing the dose of vaccine, although the economics of doing so is of some concern.[300]

Given the number of drug abusers who are on dialysis and the

frequency with which blood transfusions are necessary in dialysis patients, there is a distinct possibility that patients requiring regular dialysis may be a heretofore unidentified reservoir of HTLV-III.[301] Studies to measure the seroprevalence of HTLV-III infection in patients on dialysis are underway.

11. The Nervous System

Neurologic complications are an integral component of the uremic syndrome. Electrophysiologic evaluation of the central and peripheral nervous system, using evoked potentials, reveals neurophysiologic abnormalities early in the course of renal insufficiency, often in the absence of well-defined clinical symptoms. By the time end-stage renal failure is attained, clinically manifest abnormalities of the central, peripheral, and autonomic nervous system become a principal component of the patient's symptomatology.[302]

11.1. Central Nervous System

Cerebral atrophy in the form of cortical atrophy in 46.6% and enlargement of ventricular cavities in 16.6% were noted by computed tomography of 30 patients on hemodialysis.[303] The cortical atrophy was diffuse in half the cases and predominantly frontal in the other half. Analysis of the clinical and chemical profile during the 5 years preceding the computer tomography scans revealed a decreasing correlation of cerebral atrophy with mean blood pressure, daily intake of aluminum hydroxide, cholesterol level, arterial calcification (as noted on soft tissue roentgenograms), and triglyceride level, in that order. In another study of 22 patients, aged 2–18 years, cerebral atrophy was present in 59% of the children.[304] The detrimental effect of this abnormality on children of a younger age, mean age of 18 months, was a significant reduction in head circumference, by 2–3 standard deviations below the mean, in 9 of 12 children studied.[305] Evaluation of neurologic development revealed that eight of these children were functioning in the subnormal range and one was severely retarded.

The biochemical derangements that underlie the cerebral changes continue to be explored. Brain oxygen consumption is decreased. The level of high-energy phosphates (ATP, ADP, AMP, creatine phosphate) and glycolytic intermediates (glucose, lactate, pyruvate) was normal in the brain of dogs with acute and chronic renal failure.[306] Oxygen supply was not the limiting factor to oxygen consumption since mild hypoxia (30 min, P_{O_2} 45–50 mm Hg) failed to alter the level of any of these

metabolites or the brain redox state (NAD+/NADH). These results were taken to indicate that the decreased oxygen consumption was the result of decreased demand rather than limited supply.

On the other hand, the function of synaptosomes, which are the membrane vesicles from synaptic junctions in the brain, is altered and could account for abnormal neurotransmission in the uremic state. Studies of synaptosomes from normal and uremic rats revealed increased membrane permeability to sodium and decreased Na,K-ATPase activity in the uremic rats.[307] The rate of calcium transport and equilibrium levels of calcium accumulation is increased in synaptosomes from uremic rats, apparently because of increased synaptosomal membrane permeability to calcium.[308] Brain calcium is elevated in uremic patients and experimental models of uremia, with evidence for a permissive role of PTH. In an experimental model of chronic renal failure in dogs with 5/6 nephrectomy, half of whom underwent thyroparathyroidectomy, a marked rise in calcium was noted in the gray and white matter of the brain of all the uremic animals, but the increment was significantly higher (3–4×) in those with intact parathyroid glands. Disturbances in EEG developed in the latter group, suggesting a critical neurotoxic role of PTH independent of changes in the brain calcium content.[309]

A substantial body of evidence indicates that the accumulation of aluminum in the brain also results in abnormal central nervous system function, with its own characteristic neurologic manifestations, termed aluminum encephalopathy, which are usually associated with features of bone and muscle disease.[310] Analysis of the brain of patients who had succumbed to this problem revealed significant reduction in γ-aminobutyric acid content and choline acetyltransferase activity.[311] Treatment with biotin was reported to revert some of the manifestations of the encephalopathy in nine patients who received 10 mg of biotin daily for 1–4 years.[312] Fortunately, the identification of this iatrogenic disease has resulted in its control and prevention, with fewer cases now being encountered throughout the world.

11.2. Peripheral Nervous System

The onset and progression of peripheral neuropathy correlates with the reduction in glomerular filtration rate (GFR). Electromyographic changes appear at GFR of 20 ml/min when symptoms are not yet evident. Depressed tendon reflexes, particularly the Achilles reflex, become detectable at GFR of 10 ml/min, often in the absence of subjective symptoms. Symptoms become apparent at GFR of less than 5 ml/min. The detectable lesions are more severe at the more distal sites, affecting the lower before the upper extremity.[313] Clinical and neurographic evidence

of peripheral neuropathy can be detected in over 80% of patients with renal failure. The most common abnormalities are reduced nerve conduction velocity, increased vibratory perception threshold, loss of tendon reflexes, and impaired temperature sensitivity.[314] Not unexpectedly, the peripheral neuropathy is more severe in diabetic uremic patients.[315] Another group in whom the neuropathy is more severe and rapidly progressive are patients with malignant hypertension.[316] The symptoms can be attributed to the structural changes of the lower motor unit and all the muscle fibers supplied by it.

The recording of electrical activity produced within the spinal cord, brain stem, and cerebral hemispheres in response to an external nerve stimulus, the so-called "evoked potential," is a reliable indicator of electrophysiologic activity and reveals distinct abnormalities in patients with peripheral neuropathy.[317,318] Sensory nerve conduction velocities are an easier and sensitive index to follow improvement on dialysis.[319] In acutely uremic rats, the decreased nerve conduction velocity correlates with the decrease in specific sodium permeability of the nodal membrane and the increase in axoplasmic sodium accumulation.[320] Improved nerve conduction velocity and polyneuropathy have been reported with long-term zinc therapy.[321]

11.3. Autonomic Nervous System

Derangements of autonomic function are common in uremic patients and improve with dialysis except in diabetics, in whom autonomic dysfunction persists and progresses.[322] In general, it is alterations in the parasympathetic function that are commonly present, while those in reflex sympathetic function are much less evident and confined to end-organ hyporesponsiveness.[323,324]

The circulating levels of catecholamines are elevated in uremic patients[325] and rats with experimental renal failure.[326] However, the pressor response to norepinephrine and angiotensin II is reduced in rats with experimental renal failure[327] and uremic patients[328] and may account, in part, for their autonomic dysfunction. The reduced responsiveness to norepinephrine of uremic rats can be restored to normal by the administration of indomethacin or prevented by prior parathyroidectomy.[328]

Dialysis-induced hypotension can be aggravated by the presence of autonomic dysfunction.[329]

11.4. Neurobehavioral Disorders

Depression is the most common neurobehavioral complication encountered in patients with end-stage renal disease. The frequency with

which this problem will be identified depends not only on the sensitivity of the health care team, but also on how eagerly the classical depression assessment methods used by the behavioral scientists are applied.[330] Adolescents with end-stage renal disease are particularly susceptible to adjustment problems and more prone to noncompliance with the dialysis regimen and allograft drug therapy.[331] Recovery from renal failure after a successful transplant presents a new set of difficulties and adaptive issues, particularly in the younger patient.[332]

12. The Endocrine System

The pivotal role of the kidney in maintaining the metabolic homeostasis of the body is progressively destroyed as renal failure advances. As a result, there is early loss of the role of the kidney as an endocrine organ, and as a principal site for the metabolic clearance of polypeptide hormones. As renal failure advances, the uremic environment will affect endocrine function as a whole since overall hormone synthesis and feedback mechanisms as well as end-organ responsiveness become altered. Whereas most of the derangements that ensue exert no clinically evident adverse effects, others result in significant metabolic disturbances that affect the well-being, morbidity, and even mortality of the renal failure patient.[333–335]

12.1. Carbohydrate Metabolism

Some form of carbohydrate intolerance affects over half the patients with renal failure. The principal cause is either abnormal insulin action or carbohydrate synthesis and utilization, with a contributory role of other glucoregulatory hormones that affect their balance.[335] The cellular basis of these abnormalities has been the focus of considerable work.

12.1.1. Insulin Action

The principal cause of carbohydrate intolerance in uremic subjects is a resistance to insulin action that is accounted for primarily by postbinding defects of glucose transport and metabolism. In a study of adipocytes from eight uremic nondialyzed patients, the concentration of insulin that elicited half-maximal response was 422 ± 95 pmoles/liter in uremic patients and 179 ± 38 pmoles/liter in normal subjects ($p < 0.01$). The noninsulin-dependent and the maximal insulin-stimulated glucose transport of adipocytes from uremic patients was normal. The insulin-mediated lipogenesis of fat cells from uremic patients was also depressed. Half-maximal stimulation of lipogenesis occurred at 38 ± 8 pmoles/liter

in uremic patients and at 11 ± 3 pmoles/liter in normal subjects.[336] A similar insulin resistance can be demonstrated in muscle tissue.[337] This defect in insulin resistance can be induced in normal rat adipose tissue by incubation with uremic human serum.[338] The factor(s) responsible for this defect is an acidic, heat-stable peptide of small molecular weight, less than 2000 but more than 1000, whose production appears to be caused by uremia and is reduced but not abolished in the serum of patients on hemodialysis.[339] The improvement after hemodialysis is confirmed from the study of uremic patients by the euglycemic clamp technique.[304–342] In diabetic end-stage renal disease patients, studied with a glucose controlled insulin infusion pump, the daily insulin requirements decreased from 44.8 ± 2.9 IU of insulin before the institution of dialysis treatment to 35 ± 2.3 IU after 2–3 weeks of dialysis.[343] In contrast to the marked impairment of insulin-mediated glucose uptake, insulin-mediated potassium uptake is normal in uremic subjects.[344] On the other hand, it seems that insulin-stimulated amino acid uptake by primary culture of hepatocytes is inhibited by the uremic factor responsible for inhibiting glucose transport.[339] Thus, the multiple actions of insulin on peripheral tissue can be differentially altered by the uremic state.

The glucoregulatory hormones do not cause tissue resistance since incubation of adipose tissues with insulin, glucagon, or PTH did not reproduce the resistance noted with incubation with uremic serum.[338] However, although PTH does not affect the metabolic clearance of tissue resistance to insulin, its presence appears to be necessary for the development of glucose intolerance since parathyroidectomy restores glucose metabolism to normal, as shown from intravenous glucose tolerance euglycemic and hyperglycemic clamp studies conducted in dogs with experimental renal failure, with or without parathyroidectomy.[345] This seems to be due to the ability of PTH to interfere with the capacity of β cells to augment insulin secretion appropriately in response to the insulin-resistant state.[345] The insulin content of the pancreas in uremic rats is not different from that of control rats.[346] The defect is, therefore, one of insulin secretion. That the glucose intolerance of uremic patients is consequent to an inappropriately low β-cell response has been ascertained by other studies as well.[347] The contributory role of PTH is confirmed from the study of children with chronic renal failure whose glucose intolerance was restored to normal after medical treatment of their secondary hyperparathyroidism.[348] A subsequent study by the same authors utilizing the hyperglycemic clamp technique revealed that after parathyroidectomy, the glucose metabolic rate increased by 47%, but sensitivity to insulin remained unchanged.[349] Thus, correction of hyperparathyroidism was associated with normalization of glucose metabolic rates and increased insulin secretion, but insulin resistance was not altered.

It is now increasingly evident that the glucose intolerance of renal failure is due to the production of a uremic factor that induces tissue resistance to insulin combined with an inability of the β cells to appropriately overcome the resistance by increasing insulin secretion because of an inhibitory effect of excess circulating PTH on pancreatic β cells.

12.1.2. Glucagon

Hyperglucagonemia and increased hepatic sensitivity to glucagon have been proposed to contribute to the altered carbohydrate metabolism. The hyperglucagonemia is the result of impaired degradation of the peptide by the diseased kidney.[335] In fact, the hepatocytes of uremic rats are resistant to glucagon binding, since, despite high circulating levels of glucagon, hepatocytes from uremic animals do not show the expected "downregulation" of their binding sites, presumably because of resistant to glucagon effect.[350,351]

12.1.3. Glycated Hemoglobin

The clinical utility of glycated hemoglobin measurements in patients with renal failure has been questioned because in renal failure hemoglobin A-1 is formed by binding of substances other than glucose to hemoglobin.[352] *In vitro* studies show that the addition of urea to the medium accounts for the spurious results, and that the formation of carbamylated hemoglobin is further increased by storage or heating in proportion to the amount of cyanic acid produced by the treatment of the medium after the addition of urea.[352] The falsely elevated HbA-1 levels, however, are methodologic and can be circumvented by the use of specific affinity-chromatographic measurement of glycated hemoglobin rather than the nonspecific ion-exchange chromatographic method.[353,354] Thus, affinity-column chromatography measured HbA-1 levels remain a useful adjunct to assess glycemia in renal failure.[355]

12.2. Thyroid Gland

In renal failure disturbances in thyroid function tests, due to altered thyroid hormone kinetics and binding, are common but thyroid function is normal. There is decreased thyroxine (T_4) binding to protein, poor conversion of T_4 to triiodothyronine (T_3), normal response to exogenous thyroid-stimulating hormone (TSH), but a subnormal response of TSH to thyrotropin-releasing hormone (TRH).[333,335,356] In contrast to euthyroid sick state, the serum reverse T_3 (rT_3) is low in renal failure. This is, in part, because of reduced protein binding whereas free rT_3 levels are high. Evidence that excess PTH levels may be a contributory factor

for the reduced T_3 and rT_3 level has been advanced from a study of 27 patients with primary hyperparathyroidism and normal renal function whose total T_3 levels were reduced in direct proportion to the level of PTH.[357] Despite the reduction in T_3 level, however, TSH levels are normal in uremia. This is probably a reflection of normal pituitary T_3 content as demonstrated in uremic rats,[358] since changes in TSH are a reflection of reduced intracellular rather than circulating levels of T_3. In contrast to the pituitary, the hepatic T_3 content and T_3-receptor binding capacity is reduced in uremic rats.[358] This seems to be the basis, in part at least, of the reduced hepatic messenger ribonucleic acid activity (mRNA) profile noted in azotemic rats.[359] The reduced content of hepatic mRNA of uremic rats could also be due to increased cytosolic ribonuclease activity in uremic hepatocytes.[360]

Serum thyrotropin response to TRH is blunted in uremic patients, indicating a pituitary–thyroid function abnormality.[361]

The thyroid gland volume is increased in over half of patients with renal failure.[334,362] Although a high incidence of goiter has been said to occur in urermic subjects, the increments in size noted are only modest and the incidence of true goiter in uremics is no more prevalent than in normal persons.[363] The changes in size show no correlation to the changes in thyroid hormone concentration noted in renal failure.[362]

12.3. Gonads

The reproductive organ dysfunction of patients with renal failure is due to a defect at the hypothalamic and gonadal level.

12.3.1. Men

In men, the plasma levels of testosterone (T) and dihydrotestosterone (DHT) are low. There is also a reduction in 5-α-reductase activity as evidenced by a reduced DHT/T ratio.[364] There is considerable overlap between these results on gonadal function, their functional consequence of reduced penile tumescence, and those observed in individuals with a chronic illness but with normal renal function.[365,366] However, a major component of uremic impotence is unrelated to primary testicular failure, and the 61% prevalence of erectile impotence is higher than encountered in chronic illnesses.[367] The levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH) are also significantly higher in renal failure than in other chronic illnesses.[366]

The plasma prolactin levels are elevated in renal failure and seem to correlate best with erectile impotence.[367–369] Treatment with bromocriptine results in normalization of prolactin levels and an increase

in testosterone levels, accompanied by improvement of libido and potency.[367] Not all patients respond to bromocriptine, however.[370] Those who respond have high levels of FSH and LH with testosterone levels above 1 mg/ml[371] and are hyperprolactinemic.[369] Similar results were noted during treatment with lisuride, another dopaminergic agonist, which does not induce hypotension, a significant side effect of bromocriptine.[369] Although elevated prolactin levels are present independent of serum zinc levels,[372] zinc replacement, specially in those with zinc deficiency, lowers the serum prolactin levels and improves potency.[373] The elevated levels of prolactin are due to its reduced metabolic clearance rate and increased secretion due to dysfunction of hypothalamic–hypophyseal regulation of prolactin.[374] The ratio of immunoreactive prolactin level is significantly higher, by a factor of 60%, than the active bioassayable form of the hormone.[375] Furthermore, drugs, such as methyldopa, can cause hyperprolactinemia in these patients.[376]

Although testicular atrophy can be present in humans,[334] and experimental animals,[377] Leydig cell lesions are rare. The high gonadotropin levels in patients with uremia are considered inappropriately low for the profoundly decreased levels of testosterone, implying an additional defect at the hypothalamic level.[333] In a study of testicular function in experimental uremia induced by subtotal nephrectomy in mature male rats, the predominant and early changes were those of a central regulation of pituitary LH secretion with consequent testicular and peripheral hypogonadism but initial preservation of spermatogenesis.[377] These studies in chronic uremic male rats provide convincing evidence that uremic hypogonadism is principally due to aberrant hypothalamic regulation (LHRH drive) of pituitary LH secretion, rather than an intrinsic pituitary defect.[378] A role for increased intracellular calcium consequent to secondary hyperparathyroidism has been suggested as the cause of this defect.[374]

12.3.2. Women

The reproductive dysfunction of women with renal failure is similar to that of men indicating gonadal hypofunction and hypothalamic dysfunction. The serum prolactin, FSH, and LH levels are increased, the estrogen, estradiol, and progesterone levels are low or low normal, the pulsatile release of gonadotropins is lost, and the gonadotropin response to LHRH is normal, but the estrogen rise in response to gonadotropin is subnormal.[333] The major reproductive organ dysfunction of renal failure in women is a severe impairment in ovulatory function.[379] Hyperprolactinemia is more prevalent in women (60%) than men (12.5%) who are undergoing hemodialysis.[380] As in men, women with renal fail-

ure and hyperprolactinemia have decreased libido, lower frequency of intercourse, and lower percentage of orgasm than normoprolactinemic women.[381] Uremia also affects fertility.[381] In a study of surgically induced chronic renal failure in rats, a higher percentage of the uremic rats were nonfertile and uremic mothers had litters that weighed less at birth.[383]

12.4. Adrenal Glands

In general, the plasma cortisol levels are normal in uremic subjects, although elevated plasma levels of free and total cortisol have been noted.[333,335,384] Cortisol binding to corticosteroid-binding globulin (CBG) is normal in uremia, and the distribution of free CBG-bound globulin, at equal total cortisol levels, is not different from normal.[385] However, cortisol binding to albumin is reduced in uremic plasma.[386] Measurements of cortisol levels are not affected by dialysis.[387] The metabolism of glucocorticoids is altered in renal failure. The half-life of cortisol is prolonged in renal failure, as is the half-life of prednisolone, whereas that of dexamethasone is shortened.[388]

The plasma aldosterone levels are increased in uremic subjects who are not on dialysis and variable in those on dialysis,[333] depending on the changes in the volume and blood pressure induced by the procedure.[333,335] Aldosterone and renin are not dialyzable across the membrane, whereas antidiuretic hormone is dialyzable.[389]

12.5. Growth Factors

Growth hormone (GH) levels are frequently elevated in renal failure because of impaired degradation and abnormal secretion.[335] The normal nocturnal secretion of GH is suppressed in patients on continuous ambulatory peritoneal dialysis.[384]

Serum somatomedin levels measured by radioimmunoassay and radioreceptor assay are normal or high in patients with renal failure,[390] while somatomedin activity measured by bioassay is low, but increased after dialysis.[391] Serum from uremic patients has been shown to contain high levels of a peptide of molecular weight 800–1000, normally excreted in urine, that inhibits somatomedin activity.[391] The decreased circulating somatomedin activity and impaired growth of uremic children might be due to the accumulation of this inhibitor.

12.6. Parathyroid Glands and Renal Osteodystrophy

Secondary hyperparathyroidism and renal osteodystrophy are an early and dominant problem of the patient with renal failure and one

of the most extensively investigated problems among the systemic consequences of renal failure.[333,392,393] In fact, the problem of bone disease and changes in parathyroid gland function in renal failure is of such import that in most texts and chapters they are treated as a section of their own. The fact remains that the parathyroid glands are endocrine organs. The overall disturbances in divalent ions and vitamin D metabolism and attendant renal osteodystrophy are discussed in Chapter 6 and will not be discussed in this section.

Chronic renal failure causes a hypersecretory state of parathyroid hormone (PTH). PTH levels are lower when assessed by bioassay than when determined by RIA, and the half-life of the bioactive hormone is shorter than that of the immunoreactive hormone.[394] In a study of progressive renal failure in dogs, there was a progressive rise of the circulating levels of bioactive and immunoreactive PTH with progressive deterioration of renal function, but the elevations in bioactivity were most marked in the final stages of uremia.[395] Gel filtration analysis revealed the bioactive moiety as the principal form of glandular PTH, but a smaller-molecular-weight bioactive entity was also detected in the final stages of renal failure.

Hyperplasia of the parathyroid glands is an invariable accompaniment of progressive renal failure. There is a positive relation between the total parathyroid glandular weight and that of the duration of renal insufficiency ($r = 0.71$) and of the serum level of PTH ($r = 0.67$).[396] On the other hand, in this study of 42 patients who underwent parathyroidectomy for hyperparathyroidism secondary to renal failure there was no correlation between glandular weight and clinical symptoms, radiologic evidence of bone disease, and alkaline phosphatase levels. The enlargement of the glands was mostly uniform and involved all glands. In the smaller-sized glands only diffuse hyperplasia of parenchymal cells with normal amounts of fat cells were found. With increasing glandular weight, fat cells were sparse and nodularity was common. The proportion of oxyphil cells increased in parallel with that of glandular weight.[396] Spontaneous autonomous hyperfunction of the parathyroid glands after transplantation or in dialysis patients with persistent hypercalcemia has been termed "tertiary hyperparathyroidism." A review of 128 parathyroid glands from 41 such patients revealed marked nodular hyperplasia (10- to 40-fold increase in mass) in 39 patients (95%), with a predominance of chief cells and an abundance of oxyphil cells.[397] Adenomas were found in only two patients (5%). Thus, hyperplasia continues to be documented as the predominant lesion of so-called "tertiary hyperparathyroidism"; adenomas are a rare lesion. Normally, the lower parathyroid glands are larger than the upper glands, and this differential persists in the hyperplastic glands of patients with chronic renal fail-

ure.[398] The size of the glands can be estimated by preoperative imaging techniques. In a preoperative study of 36 patients with renal failure, computer tomography detected 53.8% of all glands and 77.6% of 76 glands weighing more than 500 mg. Scintigraphy detected 51% of all glands and 77.6% of glands heavier than 500 mg. Ultrasonography detected 42.7% of all glands and 65.8% of glands heavier than 500 mg. Combination of all three methods detected 66.4% of all glands and 89.5% of glands heavier than 500 mg.[399]

Hypocalcemia of renal failure is the most important single factor responsible for the hyperplasia of parathyroid glands.[392,393] Obviously, it is the level of ionized calcium that is important. The measurement of ionized calcium is becoming increasingly available. It should be noted that a significant variance in serum ionized calcium occurs in relation to the duration and temperature of storage of the sample. Storage at room temperature for 6 hr, or longer at 4°C or −20°C, resulted in inaccuracies in 39–79% of serum samples and in 38–92% of whole-blood samples.[400] These errors were not negated by correcting the values of ionized calcium to pH of 7.4. Samples from uremic patients should be analyzed for ionized calcium within 2 hr, or within 6 hr if stored at 4°C. The total, ionized, and protein-bound calcium fractions in plasma show a significant increase following dialysis. However, when corrected for hemoconcentration due to ultrafiltration during dialysis, only the protein-bound calcium shows a significant increment.[401] This does not seem to be consequent to changes in pH, but rather appears to be due to changes in the association constant of protein-bound calcium.

Skeletal resistance to the calcemic action of PTH is one of the mechanisms responsible for the hypocalcemia of renal failure. This resistance is not limited to the skeletal system but can be shown in the renal response to PTH. Evidence for renal resistance is advanced from a study of the effect of renal function on the renal responsiveness to PTH in 19 patients with primary hyperparathyroidism secondary to renal failure.[402] There was a strong negative correlation between plasma creatinine and the cAMP response to PTH of both groups. In patients with renal failure there was marked resistance to exogenous PTH. In primary hyperparathyroid patients the cAMP responses were variable because those who had impaired renal function had an abnormally small response. In these, the recovery of responsiveness was gradual after parathyroidectomy and was never restored to normal, indicating a residual persistent resistance to PTH.[402]

Phosphate retention is another mechanism implicated in the hypocalcemia of renal failure.[392,393] As progressive renal failure develops, phosphate homeostasis is maintained by increasing phosphate excretion

per nephron,[403] as renal threshold falls in parallel with the increase in circulating PTH.[395] The reduction in the maximum tubular reabsorption of phosphate per ml GFR (TmPi/GFR) is consequent to the circulating parathyroid level and dietary phosphate intake, each of which exerts an independent and additive effect on TmPi/GFR. In 5/6 nephrectomized rats a resetting of TmPi/GFR could be demonstrated in response to both chronic dietary phosphate deprivation and acute intravenous phosphate loading independent of whether or not the animals had been parathyroidectomized.[403]

In addition to ionized calcium, there are other modulators of PTH secretion, such as ionized magnesium. In a study of 22 patients on hemodialysis the serum magnesium was elevated, but the skeletal muscles and lymphocyte magnesium concentrations were normal.[404] When 12 of these patients were dialyzed against a low dialysate magnesium concentration, the serum magnesium was normalized. Normalization of the serum magnesium was accompanied by a rise in circulating PTH levels.[404] Conversely, in another study,[405] when the magnesium concentration in the dialysate was increased form 0.75 to 1.5 mmoles/liter, the plasma magnesium concentration increased by 36% and the immunoassayable PTH fell by 23%. Thus a rise in plasma magnesium from elevated to significantly higher levels reduces circulating PTH in normocalcemic uremic patients.[405]

H_2 receptors exist on the parathyroid gland, and their stimulation could affect PTH secretion.[392] Initial reports that therapy with H_2 antagonists may be useful in the treatment of secondary hyperparathyroidism have not been substantiated and continue to be refuted.[406,408] Neither cimetidine nor ranitidine has an effect on PTH concentration, urinary cAMP excretion, or the renal threshold for phosphate absorption.[406]

In patients who fail to respond to conservative treatment of the ravages of secondary hyperparathyroidism, parathyroidectomy provides the ultimate cure. The responsiveness to changes in calcium in acute studies can help predict whether medical therapy or surgery is indicated. Patients in whom calcium infusion (4 mg/kg per hr for 4 hr) does not suppress PTH may have true parathyroid autonomy and require early surgery.[407] Both total and subtotal parathyroidectomy with autotransplantation of a gland segment in the forearm have proven effective in reducing renal osteodystrophy and nonvisceral soft tissue calcification.[409–411] Cryopreservation of parathyroid tissue for subsequent autotransplantation has also been successful.[412,413] Recurrences of hyperparathyroidism necessitating reexploration or reoperation occur but are rare.[409,413] A major complication of parathyroidectomy is hypocalcemia.

The fall in serum calcium is almost immediate, but attains its nadir 4.4 ± 2.7 days after surgery.[414] The magnitude of the postoperative drop in serum calcium is the best indicator of osteoclastic activity present at the time of surgery.

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13

Nutrition in Renal Disease

Wilfred Druml and William E. Mitch

1. Introduction

In the last 2 years, there has been a renaissance of interest in low-protein diets in the treatment of chronic renal failure (CRF), due mainly to suggestions that a low-protein intake may slow or even halt progression of renal insufficiency. It also has become apparent that other modifications of the diet, including phosphate restriction, altering the proportions of fatty acids, or adding polyunsaturated fatty acids, might have considerable impact on the course of the disease and the metabolism of the patient. Unfortunately, the optimal intake for most nutrients in renal failure, including amino acids, vitamins, and trace elements, remains undefined. Many descriptive studies have appeared, but our understanding of the pathophysiology is still limited.

Despite the limited options for treating diabetic nephropathy and the potential for nutritional intervention, this field remains the "Cinderella" of nutritional therapy. Similarly, little new information is available concerning nutritional intervention in childhood CRF or in patients with the nephrotic syndrome or posttransplant. Despite the high incidence of acute renal failure (ARF) in critical care units and the many unresolved nutritional problems of this patient group, little progress has been made in the clinical nutrition of this syndrome. Several experi-

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mental studies have investigated the metabolic impact of ARF. In this chapter, we shall review new developments that could have an impact on the treatment of patients with renal disease.

2. Progression of Renal Insufficiency

The effects of dietary manipulation on the course of renal insufficiency remain one of the most exciting nutritional topics. Enthusiasm derives from reports indicating that low-protein diets will maintain nitrogen balance and can slow progression.[1] Fortunately, compliance can be monitored using simple methods.[2] We shall review reports investigating how dietary manipulation affects progression and results from clinical studies. Regarding the enthusiasm, a word of caution is appropriate since many of the clinical studies lack the controls necessary to prove that protein restriction slows progression. In fact, Alvestrand *et al.*[3] have reported in abstract form that frequent physician visits, emphasizing control of blood pressure and phosphorus intake but without lowering dietary protein, may slow the decline in creatinine clearance of patients with progressive renal disease. To establish the importance of this therapy, multicenter trials are currently underway in the United States and Europe.

2.1. Progression in Experimental Renal Disease

The influence of glomerular hemodynamics on renal damage has been studied in rats with different types of renal injury. Remuzzi *et al.* injected rats intravenously with Adriamycin and compared groups fed 6.6 or 20% casein.[4] In rats fed 20% protein, the drug produced proteinuria and glomerular foot process fusion after 14 days without any substantial change in glomerular filtration rate (GFR) or renal blood flow (RBF). The low-protein group did not develop proteinuria and had less severe histologic damage. Thus, a very low-protein diet can ameliorate the glomerular damage of a nephrotoxin, as well as that associated with subtotal nephrectomy. With regard to the latter, Kenner *et al.* fed partially nephrectomized with 14% or 37% protein for 6 months and confirmed experiments reported 50 years ago that a high-protein diet is associated with a higher mortality, more rapid loss of renal function, and more proteinuria.[1,5] They also provided fascinating scanning electron microscopic pictures of tubular dilatation, interstitial inflammation, and glomerular damage associated with the higher protein intake.

Glomerular sclerosis associated with chronic renal disease has been linked to dietary protein-induced intraglomerular hypertension.[1] To study

the mechanism for the glomerular hemodynamic responses to protein, Seney and Wright[6] evaluated the tubuloglomerular feedback (TG) mechanism in rats following subtotal nephrectomy. The rats were fed isocaloric diets containing 6% or 40% casein for 10 days before the experiment; effects due to differences in extracellular volume were excluded on the basis of similar hematocrits and blood pressures in the two groups. The 40% casein diet decreased the sensitivity of the TG feedback by 40%–50%, leading to the conclusion that a high-protein diet causes failure of the normal mechanisms controlling glomerular function. How dietary protein interferes with the TG mechanism is unknown. To examine this question, Brezis *et al.* measured the effects of different mixtures of amino acids on the function of isolated, perfused rat kidneys.[7] Renal vascular resistance was substantially reduced by 8 mM combinations of amino acids or by 2 mM glutamine plus glucose. The hemodynamic change was linked to increased oxygen consumption, suggesting that the change was due to tubular metabolism of amino acids. This was confirmed by showing that amino acid-induced vasodilatation was blocked when mitochondrial function was inhibited with antimycin or rotenone, or when the kidney was perfused with α-aminoisobutyrate or other amino acids that are transported but not metabolized. It was concluded that the lower renal vascular resistance was due to improved metabolic function of the kidney.

Anderson *et al.*[8] used another method to investigate how altered glomerular hemodynamics cause damage. They used the converting enzyme inhibitor enalapril to lower glomerular capillary pressure and studied rats at 4 and 8 weeks after subtotal nephrectomy. After 4 weeks, the blood pressure and intraglomerular pressure of the treated rats were reduced to normal. In spite of the lower pressure, SNGFR was supranormal because of the higher glomerular capillary flow related to the lower renal vascular resistance. Untreated rats had increased systemic and intraglomerular pressure accounting for the increase in SNGFR. After 8 weeks, blood pressure in the treated group remained normal and proteinuria was minimal. Enalapril also caused striking differences in pathology; in untreated rats, 21% of glomeruli had sclerotic changes compared to only 6% in the enalapril group. Comparison of changes in weight and BUN indicated that dietary intake could not account for these findings.

The possibility that abnormal glomerular hemodynamics cause pathologic damage has also received attention in diabetic nephropathy because of the high GFR and RPF of this condition. Zatz *et al.*[9] showed that streptozotocin-treated rats with persistent, moderate hyperglycemia (achieved by small, daily insulin doses) develop glomerular lesions proportional to their dietary protein. Diabetic rats fed a high-protein (50%)

diet had higher values of GFR, SNGFR, glomerular plasma flow, and transcapillary hydraulic pressure than rats fed lower (6% or 12%) amounts of protein. During a year-long feeding period, the 50% protein diet also caused progressive albuminuria and more severe histologic damage; almost 20% of the glomeruli of this group showed evidence of sclerosis compared to about 2–3% of the low-protein groups. Thus, modification of glomerular hemodynamics by varying dietary protein also protects rats against the development of diabetic nephrosclerosis. Likewise, in type I diabetic patients, Mogensen and Christensen have linked abnormal glomerular hemodynamics to progressive renal dysfunction.[10] They reported that an abnormally high GFR in diabetic patients with subclinical proteinuria served as a predictor for future clinical proteinuria. Forty-three patients with high GFRs studied between 1969 and 1976 because of microalbuminuria (>15 mg/min) subsequently developed >500 mg protein/day or >150 μg/min. Patients with more normal GFRs (averaging 134 ml/min) did not develop more severe proteinuria. Since Mogensen previously noted a relationship between the presence of diabetic microalbuminuria and the development of renal failure and mortality, abnormalities in glomerular hemodynamics in diabetes appear to be linked to progressive renal damage.[11]

2.2. Nonprotein Dietary Factors

Other dietary constituents that could cause progressive renal damage include energy intake and phosphorus. Laouari and Kleinknecht studied the influence of energy intake in rats following subtotal nephrectomy.[12] They varied the intake of cornstarch or glucose while maintaining protein content (13%) and mineral intake constant simply by selectively reducing carbohydrate intake to 63.5% of the energy intake of the CRF rats fed *ad libitum*. Both groups developed early, severe hypertension; no differences in renal function were detected until the fourteenth week, when the *ad libitum*-fed rats were noted to have increasingly severe renal insufficiency and histopathologic renal damage. They concluded that restriction of glucose, but not complex carbohydrates, led to less deterioration of renal function, less severe histologic damage, and improved survival. They also mentioned that indomethacin treatment did not protect the kidney. The mechanism for this effect of excess glucose remains obscure.

Lipid is another dietary component that could contribute to the pathogenesis of progressive renal insufficiency. Barcelli and Pollak have emphasized that manipulating dietary lipids can change prostaglandin production and affect the renal function of rats with immunologic or ablative renal damage.[13,14] Recently, this group fed mice different quan-

tities of linoleic acid for 4 weeks before and during the development of immune complex nephritis induced by intraperitoneal injection of aproferritin.[15] Compared to a high-linoleic-acid diet, a lower intake of this fat was associated with more severe proteinuria and histologic damage. The high-linoleic diet also protected mice against progressive renal insufficiency. Others have not found this beneficial effect. Hirschberg *et al.* varied the triglyceride and essential-fatty-acid intake of both subtotally nephrectomized rats and the NZB/NZW mice strain that spontaneously develops lupus nephritis.[16] An increased essential-fatty-acid diet did not change the outcome of either type of renal lesion; the degree of proteinuria, changes in serum urea and creatinine, and histopathology were the same as in control animals. In discussing these differences, Barcelli and Pollak[13,14] pointed out that dietary-induced changes in prostaglandin production are complex, and that the desired effect on prostaglandins must be documented. For example, feeding *cis*-linoleic acid stimulates, while a *trans*-linoleic-acid-rich diet inhibits, the conversion of essential fatty acids to arachidonic acid. Regardless, the effects of dietary fat on progression of renal failure must be regarded as unproven.

2.3. Progression in Humans

Several investigators have suggested that the response of RBF and GFR to protein feeding is absent or blunted in CRF patients. Bosch *et al.* fed a meal containing 70–80 g protein to normal subjects, uninephrectomized patients, and patients with various degrees of renal insufficiency who were eating a normal diet containing 1–1.5 g protein/kg per day.[17] The protein-rich meal increased the creatinine clearance of normal subjects by 28%, but caused little or no change in the renal function of CRF patients. Based on their results, they introduced a "renal reserve" term calculated as the difference between the baseline and postmeal creatinine clearance. The average renal reserve of normal subjects was 34 ml/min; patients with a creatinine clearance <40 ml/min had no renal reserve. Similar findings were reported by Rodriguez-Iturbe *et al.*[18] To examine the long-term effects of protein feeding, Bergstrom *et al.* fed a high-protein (2 g/kg per day) or low-protein (0.3 g protein/kg plus essential amino acid tablets) diet to eight normal subjects and measured their inulin, creatinine, and PAH clearances in response to a meat meal.[19] The basal GFR was 12% higher with the high-protein diet, but the increase in GFR (20%) in response to a protein load was the same with both diets. This was interpreted as showing that there is no functional renal reserve; instead, the response to a high-protein meal is uniform regardless of the basal value. The test meal also was associated with a change in hormones, but except for insulin, the rise in hormone levels

occurred after the increase in GFR. They concluded that glucagon and growth hormone could not account for the effects of protein feeding. In a similar study, Hirschberg *et al.* measured the change in creatinine clearance of nine vegetarian subjects fed a high-protein meal.[20] They found that five subjects had a large increase, but four others had no change or a decrease in creatinine clearance. Finally, terWee *et al.* tested the effects of an intravenous infusion of amino acids on [^{125}I]iothalamate and [^{131}I]hippurate clearances. The average increase in GFR of normal subjects and uninephrectomized patients was 10%, but CRF patients with a GFR <30 ml/min had no rise in GFR.[21] Thus, a protein meal or infusion of amino acids can increase the GFR of normal subjects to a variable degree by some as-yet-unidentified mechanism. The link between this and progressive renal disease is unclear because the response of GFR is lost in patients with established CRF.

The importance of dietary phosphorus in the progression of renal insufficiency of rats was largely discounted based on analysis of feeding studies. A recent study of humans with CRF, however, suggests that phosphorus retention should not be ignored. Barsotti *et al.* followed 26 CRF patients (creatinine clearance <30 ml/min) for an average of 20.8 months while they were eating 0.6 g protein/kg per day and a normal phosphorus (12 mg/kg per day) diet.[22] These patients were compared to 29 patients with renal insufficiency eating the same amount of protein but only 6 mg/kg per day phosphorus. The dietary difference was achieved by preparing diets with a special pasta made from starch and egg white. Progression of CRF was virtually halted in patients eating the very-low-phosphorus diet; the decline in creatinine clearance changed from -0.9 to 0.07 ml/min per month ($p < 0.001$). The higher-phosphorus diet had only a small benefit: the loss of creatinine clearance decreased from 0.79 to 0.53 ml/min per month. Other studies have suggested that dietary phosphorus is less important. Alvestrand and Bergstrom found that progression was slowed in some patients fed a low-protein diet supplemented with amino acids in spite of an increase in serum phosphorus and the calcium × phosphorus product.[23]

2.4. Therapeutic Trials in Progressive Renal Disease

During the last 2 years, reports of several trials have supported the conclusion that dietary protein restriction can improve the course of renal insufficiency. In a trial from the Netherlands, 149 patients were studied for at least 18 months after random assignment to low-protein diets or control groups.[24] In patients with creatinine clearances between 10 and 30 ml/min per 1.73 M^2, the treatment consisted of 0.4 g protein/kg per day; diets containing 0.6 g protein/kg per day were assigned to

patients with creatinine clearances between 31 and 60 ml/min per 1.73 M^2. In terms of changes in serum creatinine, patients under 41 years of age progressed faster, and there were no apparent adverse effects of the diets on weight, serum calcium, albumin, and alkaline phosphatase. It was concluded that dietary protein restriction significantly slowed progression compared to patients whose diet was unchanged. Importantly, it also was noted that low-protein diets seemed to decrease proteinuria at least in some patients. In evaluating these data, some caution is warranted. First, some of the patients were given a diet containing less than the minimum daily requirement for protein, so that their nutritional status might be compromised during long-term therapy. Second, it is difficult to detect a decline in renal function at low values of serum creatinine.[25] If the rate of change in $1/S_{Cr}$ averaged -0.01 dl/mg per day, it would take 30 months to progress from a serum creatinine of 1.5 to 2.6 ml/dl, a difference that would be difficult to detect.

In two other reports, the impact of dietary protein restriction on progression in different types of renal disease has been tested. Oldrizzi *et al.* prescribed a low-protein (~0.6 g/kg per day) diet for 33 patients with glomerulonephritis, 17 with polycystic disease, and 28 with pyelonephritis.[26] A control group without dietary manipulation consisted of patients with all three diseases. The degree of renal insufficiency was similar (serum creatinine 1.7–4.1) in all groups. Patients with hypertension or the nephrotic syndrome had significantly faster progression, as analyzed by changes in $1/S_{Cr}$ with time, but all three protein-restricted groups had slower progression than the control patients who were eating an average of 70 g protein/day; 51% of those with glomerulonephritis, 41% of polycystic patients, and 68% of pyelonephritic patients had no progression while following the diet. The diet appeared to be most effective in pyelonephritis. A similar conclusion was reached by El-Nahas *et al.* in a cross-over study of 12 patients in which they compared a diet containing 0.5 g protein/kg, 700 mg phosphorus, and 1.5–2 g calcium/day to an unrestricted control diet, with GFR ([^{51}Cr]EDTA) and [^{131}I]hippuran clearances measured at the start and end of each diet. Unfortunately, little or no information was given on the composition of the unrestricted diet. No patient experienced a significant change in GFR, though RPF was higher with the unrestricted diet. Those with the greatest percent decrease in RPF in response to the low-protein diet had the most improvement in the decline of $1/S_{Cr}$. It was concluded that patients with interstitial nephritis had the most benefit, those with glomerulonephritis had marginal benefit, and there was no effect of the diet on hypertensive nephrosclerosis. These results seem contradictory and need confirmation.

Patients with more advanced kidney disease also respond to dietary protein restriction. We studied 17 patients who had well-defined prior

rates of progression, as determined by a linear decrease in $1/S_{Cr}$ with time.[28] Ten (59%) exhibited a significantly slower rise in serum creatinine than predicted during an average of 20 months of therapy; no patient exhibited a faster rise than predicted. Seven of the seventeen patients began treatment before serum creatinine reached 8 mg/dl, and six of the seven exhibited arrested progression for an average of 2 years at the time this study was reported. It appears that this regimen, if initiated before advanced renal insufficiency occurs, might halt the progression of the disease in a majority of patients for at least 2 years.

In summary, the mechanism by which dietary protein changes the progression of renal insufficiency in humans has not been identified, but it appears that protein restriction may have different degrees of benefit in different types of renal disease. Additional studies will be required to determine how frequently progression can be slowed. At present, there are no studies examining whether protein restriction will benefit patients with progressive diabetic nephropathy.

3. Metabolism in CRF

3.1. Carbohydrate Metabolism

CRF causes insulin resistance in peripheral tissues characterized by decreased glucose uptake by skeletal muscle and adipose tissue, both in the basal state and during insulin stimulation.[29–32] Except for a single study that demonstrated decreased insulin binding to erythrocytes, it is generally agreed that insulin binding and insulin receptor number are not influenced by CRF.[33–35] Consequently, a postreceptor defect must be responsible for the altered insulin response seen in CRF. Pederson *et al.* also concluded that postbinding defects accounted for abnormal insulin-stimulated glucose transport and metabolism in adipocytes from uremic patients.[34] In fact, their data show that insulin sensitivity was abnormal, so this conclusion is based solely on their finding that insulin binding to uremic adipocytes was normal. It should be emphasized that their results differ sharply from other experiments which have shown that insulin sensitivity is unaffected by uremia while the maximal insulin response is depressed.[32,35] These latter results, but not those of Pederson *et al.*, are consistent with postbinding defects in insulin-mediated metabolism.

Although hyperglucagonemia occurs in CRF because of decreased renal catabolism of this peptide hormone, hepatic glucose release is unresponsive to glucagon in spite of normal or increased binding of glucagon to hepatocytes.[35,36] Thus, there seems to be no important role for glucagon in the glucose intolerance of CRF. In contrast to the apparent

resistance of the liver to the effects of glucagon in CRF, insulin reportedly suppresses hepatic gluconeogenesis normally, and glucose release by the liver is said to be normal in uremia.[35,37] This is difficult to understand because alanine extraction by splanchnic tissues and gluconeogenesis from amino acids is reported to be increased substantially by CRF.[35] These apparently contradictory findings have not been resolved. The conclusion of Kalhan *et al.*[37] is also surprising because they found increased recycling of glucose carbons and decreased total body glucose oxidation in uremia, apparently due to inhibition of pyruvate oxidation. Decreased glucose oxidation suggests that pyruvate and lactate release by nonhepatic tissues in increased in uremia. This effect, like the increased recycling of amino acids to glucose production, should increase hepatic glucose production.

Insulin resistance in peripheral tissues may be due to a circulating factor since resistance can be induced in normal adipose tissue by incubation with uremic serum.[38] Such a factor has been partially purified[39] and reported to be a heat-stable protein of mol. wt. 1000–2000 daltons. The protein inhibits the capacity of adipocytes to oxidize glucose or incorporate glucose into lipids. Since initiation of dialysis was associated with a fall in levels of the protein, it was suggested that this is the mechanism for the improved glucose utilization attributed to dialysis.[32,39]

Another potential contribution to glucose intolerance in CRF is hyperparathyroid-associated metabolic changes. Glucose intolerance does not develop in dogs with CRF unless their parathyroid glands are intact.[40] Parathyroid hormone (PTH) does not affect the metabolic clearance of insulin nor does it cause tissue resistance to insulin. Instead, PTH interferes with the ability of beta cells to augment insulin secretion appropriately in response to hyperglycemia; normalization of glucose metabolism in the absence of PTH is due to increased insulin secretion.[40]

3.2. Amino Acid and Protein Metabolism

It is generally agreed that plasma amino acid concentrations and intracellular amino acid pools are altered by uremia. Since amino acid pools can be affected by the severity and duration of uremia, the nutritional state of the patient, the diet, and other therapies including hemodialysis, the pattern of amino acid abnormalities can vary widely. There are several proposed mechanisms for the changes in amino acid pools, including impaired synthesis of amino acids because of reduced kidney mass (serine, tyrosine, arginine), inhibited enzymatic conversion of amino acids (phenylalanine–tyrosine or citrulline–arginine interconversion), changes in metabolism (splanchnic extraction of valine), malnutrition, and dialysis losses.[41] Elimination of amino acids is altered in uremia. The clearances of phenylalanine, valine, proline, alanine, his-

tidine, and arginine are decreased in patients with stable CRF, while in hemodialysis patients, the clearances of lysine, methionine, aspartate, and serine are increased, and proline elimination is reduced.[41] The interorgan exchange of amino acids also is altered by uremia. For example, in examining the arteriovenous difference in amino acids across different organs, Alvestrand noted decreased release of phenylalanine and tyrosine from the leg and suggested that muscle proteolysis is not accelerated in uremic patients on a normal diet.[42] In a similar study, Tizianello *et al.* reported that branched chain amino acid (BCAA) release from the leg of uremic patients is decreased and that hepatosplanchnic extraction of glutamine, serine, and valine is decreased, while proline and histidine extraction is increased.[43] How these observations contribute to the amino acid abnormalities of CRF is unknown since different authors report different results and there are abnormalities of amino acid handling by the kidneys and the brain in CRF.[43] There is little evidence that insulin resistance directly accounts for changes in amino acid metabolism. For example, Alvestrand *et al.* used the insulin-clamp technique to demonstrate that plasma concentrations of BCAA decrease normally in response to insulin.[35,42]

Besides amino acid metabolism, protein synthesis by the liver is changed by CRF. Zern *et al.* investigated the molecular mechanisms for decreased hepatic protein synthesis in uremia to extend their earlier findings that decreased albumin synthesis is associated with disaggregated, membrane-bound polysomes.[44] They reported that liver polysomes and albumin messenger RNA content were increased in uremic rats, but in spite of this, albumin synthesis by a cell-free system was reduced owning to degradation of albumin messenger RNA by increased ribonuclease activity. In summary, additional, carefully controlled studies in patients and experimental animals will be necessary to understand the mechanisms for the abnormalities in lean body mass, protein turnover, and amino acid metabolism that are associated with uremia.[29–31] At present, it is difficult to integrate the available reports to provide a unifying metabolic profile.

3.3. Lipid Metabolism

Altered lipid metabolism in chronic, nonnephrotic CRF patients is mainly the result of defective catabolism of triglyceride-rich lipoproteins.[31] In contrast to older studies, it was reported recently that fractions of postheparin lipolytic activity (PHLA) and lipoprotein lipase (LPL), as well as hepatic triglyceride-lipase (HTGL), are decreased by uremia.[45] One explanation for this newer finding may be the difference in the experimental design. For example, in ARF patients, it was shown that following heparin, the peak activity of each enzyme occurs at a different

time.[46] Consequently, an accurate estimate of the activation state of both enzymes cannot be made from a single measurement. Since Chan *et al.* measured PHLA activity 15 min rather than 10 min after heparin, they were able to distinguish a decrease in both HTGL and LPL. Besides PHLA, LCAT activity is reduced 29% by CRF, even though the distribution of LCAT is normal [90% of total activity is found in high-density lipoprotein (HDL) and very-low-density lipoprotein (VLDL)].[47] The significance of this impairment in LCAT activity is marginal, since there is no correlation between LCAT activity and total plasma triglycerides.[47]

The most likely cause of impaired lipolysis in CRF is the presence of a circulating inhibitor of lipolysis, since plasma from uremic patients can inhibit lipoprotein lipases in adipocytes taken from normal animals.[48] The inhibitor can be removed by dialysis, is present in the nonlipoprotein fraction of plasma, and has a mol. wt. of 10,000 daltons. This is too large to be classified as middle molecule, but its removal could account for the amelioration of uremic hyperlipidemia by long-term hemofiltration therapy.[49] Besides circulating inhibitors, the metabolic acidosis and hyperinsulinemia associated with uremia can profoundly depress lipoprotein lipase activity,[50] and heparin given during dialysis could cause depletion of lipolytic enzymes, thereby reducing the capacity for fat clearance.[51] Last, but not least, there may be structural abnormalities of lipolytic enzymes or of the lipoprotein activators and/or inhibitors of these enzymes. For example, it has been shown that lipoproteins isolated from uremic patients contain excess sialic acids.[52] Such an abnormality could contribute to the reduced fat clearance of uremic patients.

Whether increased production of fatty acids or triglycerides adds to the hypertriglyceridemia in CRF remains controversial. Increased triglyceride production by uremic rats was not found in one recent study,[53] but the high incidence of hyperlipidemia in continuous ambulatory peritoneal dialysis (CAPD) patients and nephrotic patients indicates that renal disease or its treatment can be associated with increased triglyceride synthesis.

In addition to hypertriglyceridemia, HDL formation is decreased and the HDL formed appears defective since transport of cholesterol from HDL to VLDL or low-density lipoproteins (LDL) is 50–60% lower in uremic patients.[54] Finally, uremia impairs LDL catabolism. Defective LDL catabolism has been attributed to an abnormal interaction of LDL with its receptors on fibroblasts.[55] Suggested mechanisms for the abnormal receptor interaction include carbamylation of LDL or the receptor, the presence of middle molecules, or triglyceride enrichment of LDL, plus altered apoprotein B catabolism, leading to a change in the configuration of LDL.

Two questions influence the decision to treat hyperlipidemia. First, does disturbed lipid metabolism affect the development of or progres-

sion of renal insufficiency? There is, as yet, no substantial experimental or clinical evidence that hyperlipidemia affects renal damage directly, but there are studies suggesting that changing dietary lipids can influence the course of experimental renal disease (see Section 2.1). This has been attributed to interference with prostaglandin synthesis rather than correction of abnormal triglyceride or cholesterol metabolism. Second, is uremic hyperlipidemia an independent risk factor for atherosclerosis and can reduction of plasma lipids influence cardiovascular mortality or morbidity? Although arteriosclerosis and cardiovascular mortality are increased in hemodialysis patients, the evidence that hyperlipidemia is an independent risk factor is slim. There are few, mostly short-term studies showing a weak correlation between triglyceridemia and cardiovascular mortality.[31] In addition, large epidemiologic studies indicate that hypertriglyceridemia *per se* has only a minor influence on cardiovascular morbidity. Any lipid-related increased risk of arteriosclerosis in CRF may be due to a low HDL level and impaired cholesterol transport rather than hypertriglyceridemia.[56] In fact, it was suggested that uremia may protect against the development of arteriosclerosis by decreasing the release of platelet-derived growth factor, plus increased prostacyclin and decreased thromboxane production in vessel walls.[56] In summary, the question of whether to treat nonnephrotic, non-posttransparent hyperlipidemia is difficult to answer. The available data indicate that therapy should be restricted to patients with hypercholesterolemia or severe hypertriglyceridemia.[56]

3.4. Treatment of Hyperlipidemia

In dialysis patients, the initial enthusiasm for bicarbonate dialysis as a treatment for hypertriglyceridemia has not been fulfilled. Bicarbonate dialysis has no clear advantage over acetate dialysis in reducing plasma triglyceride levels,[57] but long-term hemofiltration may have some benefit by eliminating an inhibitor of lipoprotein lipase, leading to a lower triglyceride level.[49]

Drugs such as beta blockers, androgens, estrogens, and glucocorticoids can contribute to hyperlipidemia and should be withdrawn if possible.[58] Drugs used to treat hyperlipidemia in renal failure, clofibrate, bezafibrate, and acipimox, have been shown to lower plasma triglycerides and cholesterol in uremic subjects.[31] Any benefit, however, may be outweighed by potential toxicity due to the changes in pharmacokinetics caused by renal failure.

Regular exercise can change the lipid metabolism of hemodialysis patients. Goldberg *et al.* enrolled 14 patients in a 12-month exercise program and noted that plasma triglyceride concentration decreased 33%, HDL cholesterol increased from 31 to 37 mg/dl, plasma insulin

dropped slightly, and blood pressure was lower.[59] Unfortunately, it appears to be difficult to achieve compliance with an exercise program. Shalom *et al.* found that only 7 of 174 chronic dialysis patients would participate in a 12-week exercise program.[60]

An important aspect of therapy is dietary manipulation. Low-protein diets have no influence on plasma lipids, apoproteins, or lipolysis.[61] However, supplements of 24 capsules of fish oil containing predominantly eicosapentaenoic acid effectively reduced total triglyceride, cholesterol, and phospholipid concentrations and caused a small, but significant, reduction in diastolic blood pressure.[62] Unfortunately, no lipid-lowering therapy has demonstrated a beneficial influence on the morbidity and mortality of uremic patients.

3.5. Carnitine

The quartenary ammonium carnitine is an essential cofactor for transport of fatty acids into mitochondria, and in dialysis patients, carnitine levels are low.[31] Dialytic loss of carnitine is at least one cause, since the plasma level falls during dialysis and increases to predialysis values within hours, suggesting a shift of carnitine from muscle to the plasma pool. However, dialysis losses are less than the urinary losses of normal subjects, and there is no evidence for impaired carnitine absorption in the intestine.[63] Thus, carnitine synthesis from methionine and lysine must be impaired in dialysis patients.[64] It is unlikely that carnitine deficiency is a major cause of abnormal plasma lipids in CRF because plasma carnitine is normal in undialyzed, stable CRF and ARF patients who have qualitatively similar changes in lipid metabolism. Moreover, the major defect causing abnormal lipid metabolism occurs before utilization of fatty acids, i.e., at the breakdown of triglyceride to glycerol and free fatty acids.

One way to test whether carnitine deficiency causes lipid abnormalities is to provide carnitine supplements. Results of such studies have been inconsistent. Some have shown a decrease in plasma triglycerides, but only during the first weeks of therapy.[31] Alternatively, one study of 3 g/day of *l*-carnitine reported a paradoxic increase in plasma triglycerides from 180 to 219 mg/dl.[65] It was suggested that this occurred because fatty-acid synthesis rose as the supply of acetyl-CoA increased because the acetylcarnitine shuttle was stimulated. In rats with experimental CRF, carnitine did not change the plasma lipid profile, and caused only a slight acceleration of lipid removal during an intravenous fat-tolerance test.[66] In summary, there is little evidence for a beneficial effect of carnitine supplementation in dialysis patients, and the drug does have the potential for serious adverse effects.[65]

4. Nutritional Assessment and Monitoring of Protein Intake

To administer nutritional therapy successfully, nutritional status must be evaluated repeatedly. This can be difficult in the renal failure patient because the standards of several parameters used to assess nutrition are unreliable. Plasma concentrations of short-lived proteins, such as retinol-binding protein and prealbumin, are elevated in patients with renal failure owing to decreased tubular degradation. Tests of immunocompetence are of limited value because immune function can be altered by renal disease independently of the nutritional state.[67] In spite of the many descriptive studies examining methods of measuring nutritional status in renal failure patients, few firm conclusions can be reached. Few studies have critically evaluated single tests and investigated the sensitivity and specificity of the methods. The demonstration of a difference in any test between CRF and control subjects may not necessarily reflect differences in nutritional status.

Berkelhammer *et al.* compared conventional tests of nutritional status with a test of skeletal muscle function in response to electric stimulation.[68] In a well-nourished group of CRF patients with near-normal nutritional parameters, muscle force and the maximal relaxation ratio were not different from those of control subjects; in malnourished CRF patients, these measures of muscle function were reduced. Since both groups had CRF, this test appears to be influenced more by the nutritional state than by azotemia. In nearly all studies of hemodialysis patients, there is a high frequency of abnormalities of nutritional status even when subjects with multiorgan disease are eliminated.[69] However, it is difficult to decide whether these abnormalities are clinically important because the specificity and sensitivity of the tests have not been critically evaluated. Thunberg *et al.* reported that in more than 69% of dialysis patients, triceps skinfold thickness, plasma transferrin, and total lymphocyte count were subnormal, whereas height/weight ratio, body mass index, serum albumin concentration, and arm muscle circumference were within normal limits.[70] In their longitudinal study, little change in these parameters was observed. Wolfson *et al.* also found multiple abnormal tests of nutritional status in dialysis patients, including decreases in body weight, triceps skinfold thickness, midarm and biceps muscle circumference, subnormal plasma concentrations of total protein, albumin, transferrin, immunoglobulin, and complement, plus reduced lymphocyte transformation.[71] There was a tendency to a higher mortality in patients with the most abnormalities.

Panzetta *et al.* measured total-body water, extracellular water, the exchangeable potassium pool, and alkali-soluble nitrogen in skeletal muscle of stable CAPD patients.[72] Total-body water was normal and correlated with body weight, but the distribution of water was abnormal,

suggesting cellular overhydration. This means that extracellular water was low in contrast to CRF patients treated by other means. They also reported that alkali-soluble nitrogen in muscle was low in five of nine CAPD patients, but at the same time, there was little or no reduction in exchangeable potassium. During a 14-month period, no consistent changes were observed. This report seemingly indicates that protein nutrition is distinctly abnormal in CAPD. It will be interesting to determine whether similar changes occur in other CAPD patients.

The protein intake and compliance to prescribed diets can be monitored accurately in CRF and dialysis patients using simple tests. In nonnephrotic CRF patients, the urea nitrogen appearance rate correlates closely with dietary nitrogen intake. To increase the accuracy of estimating total nitrogen excretion, Maroni *et al.* measured the non-urea nitrogen excretion of nonnephrotic CRF patients and showed that it did not correlate with protein intake and averaged 0.031 g N/kg body weight per day irrespective of dietary treatment.[2] Consequently, total nitrogen excretion can be calculated as urea nitrogen plus 0.0031 g N/kg body weight per day. When this value was used in hospitalized CRF patients undergoing nitrogen balance studies, it was found that the calculated nitrogen excretion was indistinguishable from the measured nitrogen excretion. Using this method, nitrogen balance of CRF patients can be estimated if nitrogen intake is known. To monitor compliance with a low-nitrogen diet, nitrogen excretion should be compared with the prescribed dietary nitrogen. If they are different, then dietary counseling or investigation into causes of abnormal protein metabolism is indicated.

Several studies have shown that patients on hemodialysis frequently eat less than recommended.[69,70] In these patients also, a simple method for estimating protein intake would be useful. Urea kinetics, based on the blood urea before and after dialysis treatments and the change in body weight, has been widely used to give a rapid and easy calculation of urea appearance.[73] This value, plus an estimate of non-urea nitrogen losses, can be used to assess dietary compliance, just as with nondialysis patients. A microcomputer program for this calculation has been published by Davidson and Davidson.[74]

5. Trace Elements and Vitamins

5.1. Trace Elements

There are many reports of abnormal plasma concentrations of trace elements in uremia,[75] but the reports are often contradictory because of differences in analysis and standardization. An abnormal plasma concentration may not identify a change in pool size or a deficiency state since the plasma level can be affected by changes in compartmentali-

zation induced by uremia and/or intermittent hemodialysis. In fingernail and hair samples, increases in manganese, arsenic, and copper levels were found in stable CRF patients and of zinc and vanadium in hemodialysis patients.[76] Such reports must be interpreted with caution unless the composition of shampoos, hair dressings, etc. has been carefully controlled.

5.2. Zinc

Zinc levels in plasma, hair, and fingernails are low in stable, undialyzed CRF patients, but are normal or increased in hemodialysis or hemofiltration-treated patients.[76,77] It also has been reported that the zinc content of heart, spleen, bone, plasma, leukocytes, and erythrocytes of uremic subjects is abnormal.[77] One suggested mechanism for this abnormal distribution is that a cytosolic protein, metallothionein, capable of binding trace elements is synthesized in response to stress, endotoxin, leukotrienes, low-protein diets, food deprivation, and other factors.[77] Intracellular accumulation of this protein could shift trace elements from plasma to cells. This would not necessarily change the pool size but could account for the finding that animals fed a low-protein diet develop low plasma zinc levels that are unresponsive to zinc supplements.[78] Another mechanism proposed for the changes in plasma zinc in CRF is impaired intestinal absorption.[79]

Zinc deficiency has been suggested as the cause of certain uremic symptoms, including apathy, loss of taste and/or olfaction, anorexia, dermatitis, impaired leukocyte and lymphocyte function, anemia, and sexual dysfunction.[77] Supplemental zinc reportedly improves taste, increases appetite, and stimulates cell-mediated immunity in dialysis patients.[77] Sexual dysfunction, however, is not explained solely by zinc depletion since supplements of zinc did not improve the sexual function of dialysis patients in a controlled, double-blind study.[80]

5.3. Iron

CRF patients usually have a hypoproliferative anemia with increased iron stores. Total iron turnover, measured as the disappearance of injected ^{89}Fe, is increased because of uptake by tissues other than erythrocyte precursors.[81] Hence, iron therapy does not cause a large rise in the hematocrit but can cause an iron overload syndrome, especially with repeated tranfusions. To diagnose iron overload or deficiency in dialysis patients or those eating very low-protein diets, the serum ferritin level is useful.[82–84] A serum ferritin below 100 ng/ml is compatible with iron deficiency. Iron therapy should be given orally if possible, since par-

enteral administration favors tissue iron deposition in organs such as the liver and spleen rather than the bone marrow.[84]

5.4. Nickel

Nickel in uremia has not attracted much interest until recently. Hopfer *et al.* compared the plasma nickel concentrations of nondialyzed CRF and dialysis patients with controls and found that only one of seven stable CRF patients, but all 65 dialysis patients, had massively increased plasma nickel levels.[85] The water supply was incriminated in hypernickelemia because changing to a new water purification system dropped plasma nickel by 75% after 5 months.

5.5. Cobalt

Based on a postmortem finding of increased myocardial cobalt levels, Clyne *et al.* suggested that cobalt caused myocardial dysfunction in uremia.[86] More recently, it was reported that plasma cobalt is high in uremia and is directly related to the degree of left ventricular dysfunction, possibly by inhibiting certain critical enzymes and antagonizing calcium effects.[86] Interestingly, a low-protein intake was considered to potentiate the absorption and toxicity of cobalt in subjects with beer potomania.[87]

5.6. Selenium

Plasma selenium is depressed in hemodialysis and stable CRF patients, possibly owing to decreased intake, impaired intestinal absorption, and/or increased losses during dialysis. Recently, it was suggested that a low plasma selenium might activate experimental carcinogens and contribute to the increased frequency of malignancy in uremic patients.[88] Obviously, this will require further study.

5.7. Vitamins

Plasma concentrations of water-soluble vitamins are generally reported to be low in uremic patients, and a decreased oral intake, decreased intestinal absorption, and increased loss during dialysis treatment have been implicated as causes of these findings.[31,89] A recent study investigating the concentrations of water-soluble vitamins in plasma, erythrocytes, and granulocytes, however, questions the concept of decreased water-soluble vitamin pools in hemodialysis patients.[90] In plasma, only the concentration of vitamin C was reduced; biotin, riboflavin, and pantothenate concentrations were increased. In erythrocytes, no vitamin

deficiencies were observed, the concentrations of vitamin B_{12}, riboflavin, biotin, and pantothenate being increased. In granulocytes, thiamine, riboflavin, vitamin B_6, and B_{12} levels were within normal limits, pantothenate and biotin were increased, and only vitamin C was reduced. Excessive supplements of water-soluble vitamins, therefore, are not indicated in hemodialysis patients and may even cause serious problems, since neurologic dysfunction has occurred during high-dose vitamin B_6 therapy and hyperoxalemia can occur with excessive vitamin C.[91,92]

Of the fat-soluble vitamins, vitamin D metabolism is discussed in Chapter 6. The plasma vitamin A level is uniformly high in uremia because degradation of its transport protein, retinol-binding protein, is impaired.[31] Normally, degradation takes place in renal tubular cells, so renal damage increases the half-life of retinol-binding protein, leading to hypervitaminosis A. Despite losses of vitamin A and retinol-binding protein during CAPD, plasma concentrations are elevated even in these patients.[93] As discussed previously,[31] vitamin A may contribute to the anemia,[94] pruritus, dry skin, anorexia, weight loss, and hepatic dysfunction of uremia, so there is no reason to give supplemental vitamin A.

6. Oxalate

Oxalic acid is a metabolic end product that is excreted by the kidneys[95–97] and is derived mainly from the metabolism of glycine and, most important, from the catabolism of ascorbic acid. Vitamin C accounts for about 50% of daily oxalate formation.[98] Only about 10% of oxalate production is derived from dietary oxalate because the compound is poorly absorbed. However, a diet consisting of large amounts of certain oxalate-containing foods, such as spinach, rhubarb, and chocolate, or large quantities of ascorbic acid will raise plasma oxalate. Oxalate elimination during a single dialysis averages about 3 times the normal daily oxalate excretion, suggesting that endogenous formation and/or intestinal absorption must be increased in uremia.[99]

The pathophysiologic importance of oxalate accumulation in uremic patients has not been emphasized until recently. In several European reports, it was suggested that certain carbohydrates used in parenteral nutrition, such as xylitol, may increase oxalate formation and calcium oxalate deposition in soft tissues.[100] The case against xylitol is not proven, however, because retrospective analysis indicates that at least in some instances, excessive vitamin C and/or vitamin B_6 deficiency may have been the reason for increased oxalate levels.[101]

In renal failure, secondary oxalosis can be aggravated by pyridoxine deficiency, since low levels of vitamin B_6 decrease the catabolism of glyoxylic acid, the precursor of oxalic acid.[102] Hyperoxalemia in CRF can cause precipitation of calcium oxalate in soft tissues, including the

kidneys, pancreas, blood vessels, and brain. A clinically important site is cardiac deposition, and several cases of congestive heart failure have been reported.[103] Whether secondary hyperoxalemia influences the progression of renal disease is unknown, but in autopsy series, >90% of patients with end-stage renal disease had calcium oxalate deposition in their kidneys.[104] Moreover, secondary oxalosis in nonrenal diseases can cause acute interstitial inflammation, or even ARF due to massive interstitial oxalate deposition.[101]

There are three therapeutic approaches to hyperoxalemia in renal disease. First, the intake of oxalic acid and its precursors should be reduced; spinach, rhubarb, and chocolate should be omitted, and vitamin C should be limited to a maximum of 100 mg/day.[98] Limiting dietary protein as well will reduce the intake of glycine and decrease oxalate formation.[105] Second, endogenous oxalate production can be inhibited by pyridoxine, which increases the degradation of the oxalate precursor, glyoxylic acid.[102] Third, oxalate elimination can be increased by more frequent dialysis, but this is effective only if approaches one and two are used.[99,106,107]

7. Nutrition in Childhood Renal Failure

Balancing the nutritional objective of minimizing waste product production while supplying adequate amounts of nutrients is especially difficult in children with CRF. Since catchup growth may not occur after hemodialysis or transplantation therapy is begun, it is critical to optimize the nutritional status of children at the earliest stage of renal disease.

Broyer *et al.* compared the nutritional efficiency of three diets, a low-protein diet containing human milk, a diet with half the milk protein replaced by essential amino acids, and a third, milk-based diet in which five of the essential amino acids were replaced by their α-ketoanalogs.[108] Six infants less than 24 months old with creatinine clearances of <6 ml/min were studied by giving the diets sequentially, in part by nasogastric tube to ensure comparable intakes. Weight gain and estimated nitrogen balance were highest with the milk protein diet supplemented by essential amino acids and lowest when the α-ketoanalogs were given. Urea appearance and the BUN were highest with the milk diet and lowest with the milk-protein-plus-ketoacid regimen. Even though the urea appearance per gram of nitrogen intake was lowest during the ketoacid diet, any improved efficiency of nitrogen utilization was not clinically apparent, since weight gain and nitrogen retention were also low.

The fact that growth retardation is especially prominent in uremic infants less than 12 months of age and is resistant to nutritional intervention was reemphasized in a retrospective, long-term study by Rizzoni *et al.*[109] Only 3 of 42 children, aged 1–15 years (average 6.7 years), with

a GFR <70 ml/min per 1.73 M^2, exhibited significant growth retardation during an observation period averaging 4.3 years. In contrast, three of five infants found to have CRF during the first 6 months of age had significant growth impairment. It can also be difficult to achieve normal growth in children with CRF. In 14 CRF children with a mean age of 9.9 years and growth retardation, Sigstroem *et al.* investigated the effects of a low-protein, high-energy diet supplemented with essential amino acids.[110] The diet reduced the BUN by 50%, and 10 of the children achieved normal linear, but no catchup, growth. There was no benefit of the regimen on uremic osteodystrophy. Unfortunately, the subjects did not receive the same attention during a control period. Thus, an easily demonstrated benefit of nutritional therapy in childhood CRF has not been uncovered.

8. Nutrition and Renal Transplantation

Despite the potential benefits of nutritional intervention in decreasing steroid-induced protein wasting, ameliorating posttransplant hyperlipidemia, and preserving renal function, nutritional therapy of transplanted patients has received little attention.

Alterations in lipid metabolism after renal transplantation differ fundamentally from those observed in CRF patients.[111,112] In the patients investigated by Kobayashi *et al.*, hypertriglyceridemia disappeared after transplantation, but hypercholesterolemia of the type II variety developed.[111] Likewise, 66% of transplanted children developed either an isolated type IIa hypercholesterolemia or a mixed type IIb hyperlipidemia.[112] Corticosteroids are usually cited as the leading cause of hyperlipidemia, but the abnormality can be aggravated if renal function is reduced or there is heavy proteinuria.

Alternate-day steroid treatment and dietary intervention offer the most important therapeutic options for these patients. In a study of 12 hyperlipidemic transplant recipients, Shen *et al.* investigated the influence of dietary modification based on a calorie intake according to the Harris Benedict standard (<500 mg cholesterol/day, <35% of calories from fat, <50% calories from carbohydrates, PS ratio >1).[113] After three months, body weight fell in 11 of 12 subjects, and cholesterol and triglyceride levels decreased to normal in eight of nine patients with mixed hyperlipidemia; in three patients who had elevated cholesterol levels before therapy, the level decreased but remained supranormal. HDL cholesterol increased in all 12 patients and became normal in 11. In 11 control patients, plasma lipid concentrations were unchanged.

Steroid treatment can have other prominent metabolic effects after renal transplantation. Acceleration of protein breakdown appears to be dose-related and is most pronounced in the first weeks after transplan-

tation when the steroid dosage is still high.[114–116] The calculated protein catabolic rate increased by an average of 0.8 g/kg per day for 2–4 days after transplantation and rose by 0.3 g/kg per day for 5–10 days after intravenous steroids were given for rejection.[115] These values suggest that transplanted patients are probably in 20–40 g/day negative protein balance (corresponding to 3.2–3.6 g N/day). Cogan *et al.* reported that increasing protein intake to about 1.3 g/kg per day and calorie intake to about 33 kcal/kg per day permits nitrogen equilibrium in the immediate posttransplant period in patients being hemodialyzed and receiving pharmacologic dosages of glucocorticoids.[114] These results may not apply to all patients, since Whittier *et al.* found that a higher protein intake (210 g protein/day) was required to achieve balance. They compared the high-protein diet with an isocaloric diet containing 70 g protein in a controlled study.[117] Patients ingesting the high-protein diet achieved positive nitrogen balance, whereas five of six of those fed 70 g protein/day were in negative balance. Cushingoid side effects were not observed in any of the high-protein diet subjects but were said to develop in four of six patients of the control group. In spite of these encouraging results, it remains to be shown that a high-protein diet will restore immunocompetence, decrease infectious complications, and accelerate wound healing or recovery of renal function following renal transplantation.

9. Nephrotic Syndrome

The nephrotic syndrome is usually defined as proteinuria of more than 3.5 g/day, though protein losses may be much greater. Generally, a high-protein diet (up to 3 g/kg body weight per day) is recommended, but its usefulness is controversial.[118] In one study, Manos *et al.* found that a diet containing 1 g protein/kg pre-illness weight per day plus 1 g protein/g proteinuria and 200 kcal/g N can produce a positive nitrogen balance in nephrotic patients.[119] Increasing calories above 200 kcal/g N or increasing protein intake further did not yield additional benefit. If these findings are confirmed in further studies, they might provide a rational basis for therapy and avoid the less palatable, very-high-protein diets. There are recent, preliminary reports that low-protein diets reduce proteinuria without causing hypoalbuminemia. These reports require confirmation, especially since nitrogen balance was not measured.

Hyperlipidemia in the nephrotic syndrome is distinctly different from that of nonnephrotic CRF. Cholesterol, triglycerides, and phospholipids, VLDL and LDL lipoproteins are all increased, and IDL and lipoprotein remnants may accumulate.[120,121] Plasma HDL is usually normal, but cases with HDL levels up to 93 mg/dl have been observed,[122] while other cases with heavy proteinuria have low levels due to excretion of HDL.[123] Hyperlipidemia is due mainly to increased hepatic lipopro-

tein secretion triggered by hyopalbuminuria and/or plasma oncotic pressure or plasma viscosity.[121,124] Lipid removal also can be abnormal. This is caused by low activity of lipases due to loss of lipase activators such as apoproteins, HDL, or the complete enzyme, lipoprotein lipase. CRF can aggravate abnormal lipolysis.[125]

Therapy for hyperlipoproteinemia of the nephrotic syndrome includes cholestyramine, tryptophan, and clofibrate.[118] There is considerable risk of clofibrate therapy since albumin binding is decreased in nephrotic patients; Bridgeman *et al.* noted that five of six patients treated with clofibrate developed muscle pain and stiffness.[126] Because of side effects, drugs are not used to treat hyperlipidemia in the nephrotic syndrome since Wass *et al.* reported that there was no increase in cardiovascular disease in 159 patients with the nephrotic syndrome.[127] The better approach is a diet low in cholesterol and saturated fat, combined with protein modification as described by Manos *et al.*[119]

10. Acute Renal Failure

Our understanding of the metabolic abnormalities associated with ARF is mainly derived from studies of experimental animals. In considering therapy, it must be remembered that ARF occurs as a complication of many disorders that affect nutrition, so that no single nutritional regimen will be suitable for all patients with ARF.

10.1. Protein Metabolism

As with other catabolic processes, ARF is characterized by the internal redistribution of amino acids from peripheral tissues (mainly skeletal muscles) to support gluconeogenesis and protein synthesis in the liver.[128] Amino acid release from muscle is excessive in ARF and is due to an acceleration of protein degradation.[129] Insulin suppresses muscle proteolysis in ARF, but does not return it to normal levels. Protein synthesis in muscle is relatively unaffected by ARF until uremia is advanced and suppresses protein synthesis.[129] Signals that have been proposed to account for this metabolic change include release of interleukin-1, prostaglandin, glucagon, and proteases, plus the response to abnormal cellular energy metabolism.[129–131]

Studies of hepatic protein synthesis in ARF have yielded conflicting results; in cell-free systems with added RNA, protein synthesis is unimpaired.[132] In animals with experimental uremia, hepatic extraction of amino acids is high,[133] and therefore, intracellular amino acids should not be rate-limiting for protein synthesis. Consequently, defective hepatic protein synthesis in ARF must be due to impaired RNA synthesis

or transcription. In fact, there is evidence for a defect in RNA turnover in the liver of rats with chronic uremia.[44]

It is not surprising that defective protein turnover in muscle and liver should be associated with changes in amino acid pools and metabolism. In patients with ARF, plasma amino acid concentrations were found to be abnormal; phenylalanine and the sulfur-containing amino acids methionine, cystine, and taurine were increased, while leucine and valine were decreased.[134] The impact of these abnormalities on protein turnover in ARF is unknown.

10.2. Glucose Metabolism

Glucose intolerance in ARF is caused by reduced insulin-mediated glucose uptake in peripheral tissues; hepatic glucose metabolism is relatively unimpaired. ARF does not decrease the insulin sensitivity of glucose metabolism in muscle since the insulin concentrations that produced half-maximal stimulation of glucose uptake, glycogen synthesis, and glucose oxidation were similar to normal values.[135] There was, however, a decreased insulin responsiveness of muscle glucose metabolism, suggesting that postreceptor events are responsible for the ARF-induced defects in glucose metabolism. It was proven that ARF caused postreceptor defects in muscle when it was shown that ARF inhibited the ability of insulin to stimulate glycogen synthase activity.[135] Besides causing post receptor defects in insulin action, ARF also increased the proportion of glucose taken up by muscle which is shunted into glycolysis. This suggests less efficient use of glucose. Interestingly, when the ratio of glycolysis to glucose uptake was plotted against the rate of protein degradation in individual muscles, the two were found to be highly correlated.[131,135] The demonstration of a similar strong correlation in muscles of fed and starved rats and rats responding to the catabolic stress of thermal injury[136] indicates that glucose and protein metabolism in muscle are closely linked. It is possible, therefore, that correction of abnormal muscle glucose metabolism in ARF might improve protein metabolism.

10.3. Fat Metabolism

In ARF, as in CRF, a type IV hyperlipoproteinemia with lipoprotein abnormalities, including a decrease in apoprotein AI, AII, and B, is usually present.[137] Total triglycerides and the triglyceride content of LDL and VLDL are increased; total cholesterol and the cholesterol fractions of LDL and especially HDL are decreased. In exploring the mechanisms of these defects, it was shown that abnormal hepatic synthesis of fatty acids and triglycerides does not play a major role in the hyperlipidemia of ARF.[138] As in CRF, the most important cause of altered lipid metabolism appears to be impaired catabolism of triglyceride-rich li-

poproteins because of decreased activity of lipoprotein lipase; the activities of both fractions of PHLA, HTGL, and LPL are consistently depressed in ARF.[46] Although acidosis alone can inhibit PHLA activity and may contribute to impaired fat removal in ARF,[50] PHLA activity in ARF patients is low, even when their blood pH is near normal. Other potential causes of these defects in lipid transport include increased triglyceride binding capacity of serum and decreased surface tension of lipoprotein complexes.[139]

10.4. Nutrition, Renal Regeneration, and Function

It has been suggested from studies of ARF in rats that amino acids could enhance renal phospholipid deposition and protein synthesis and thereby accelerate tissue regeneration after a nephrotoxic or ischemic insult, but this is controversial.[29,140] For example, recent reports indicate that an intravenous amino acid infusion can decrease renal function and increase protein excretion in rats.[141] When amino acids were infused simultaneously with a toxin or during ischemia, tubular cell degeneration was increased.[142] The mechanisms proposed for this toxic effect include a high intraluminal concentration of basic amino acids causing decreased protein reabsorption[143] and increased energy consumption by tubular cells related to amino acid reabsorption.[144]

The clinical implication of these animal studies is that amino acids probably have no obvious beneficial effect and should be withheld during a nephrotoxic or ischemic insult. Second, amino acids should be infused over 24 hr to avoid high plasma levels of total amino acids, and imbalances of plasma amino acid concentrations (e.g., high concentrations of basic amino acids) should be avoided.

10.5. Parenteral Nutrition in Acute Renal Failure

What amino acids should be given in ARF? Evidence suggests that a mixture of essential (EAA) and nonessential (NEAA) amino acids, rather than only the original eight EAA alone, should be used.[129] Histidine is now regarded as essential, and several studies indicate that this may also be true for arginine.[145] A parenteral nutrition regimen for adults lacking arginine could impair the ability to detoxify ammonia, leading to hyperammonemia, metabolic acidosis, and coma.[146] Moreover, arginine is reported to improve immunocompetence and decrease infectious complications.[147] Besides arginine, tyrosine should be given because conversion of phenylalanine to tyrosine is impaired in ARF, and tyrosine could become limiting for protein synthesis or for the production of catecholamines and thyroid hormone.[134] Finally, NEAA that are interconverted, such as glycine and serine, may become limiting for

protein synthesis during infusion of a mixture of EAA alone.[148] These considerations must be balanced against the theoretical possibility that NEAA will not be limiting for protein synthesis, at least for normal subjects. In addition, it has been shown in normal and CRF rats that provision of EAA plus NEAA improves growth compared to EAA alone.[149] Finally, in healthy adults, nitrogen balance was improved when NEAA were included in the diet.[150]

Three controlled studies have compared the effects of EAA with a complete amino acid solution.[151–153] In all three studies, neither supplement improved survival. But survival may not be the best endpoint for evaluating a nutritional study because of the influence of factors other than optimal nutritional support in determining mortality. In the first study, urea production was higher and nitrogen balance more negative with a mixed solution of 42 g of EAA plus NEAA compared to 21 g of EAA.[151] In a second study of nondialyzed, ARF patients, Mirtallo *et al.* found no difference in urea appearance or nitrogen balance in patients receiving 21 g EAA compared to patients receiving 21 g of EAA plus NEAA.[152] Finally, preliminary data from Feinstein *et al.* comparing the influence of 21 g EAA with a regimen of EAA plus NEAA at a dosage depending on urea nitrogen appearance (to a maximum of 15 g nitrogen) showed that those receiving the total mixture experienced an increase in urea appearance of 7.5–14.0 g N/day, though there also was a trend toward greater nitrogen retention.[153]

In what proportions should amino acids be supplied? EAA solutions have a composition based on the recommended "safe intake" for healthy young men, as determined by Rose.[154] This mixture is almost certainly not optimal for ARF patients. First, modern studies indicate that requirements are different, even for normal subjects; lysine requirements are about 50% higher than the Rose estimate.[155] Second, the clearance of amino acids in ARF is distinctly abnormal, and infusion of EAA, according to the "safe intake," can cause an imbalance syndrome.[41] For example, phenylalanine and methionine in amino acid solutions are high, even though phenylalanine clearance is decreased 50% by ARF.[134] Consequently, therapy with available solutions leads to a large increase in plasma phenylalanine and sulfur-containing amino acids and an even greater imbalance in the phenylalanine/tyrosine ratio. Mixtures of EAA and NEAA appear to have a less pronounced effect on the plasma aminogram and theoretically would be preferred to commercial solutions containing EAA only.[41]

What is the optimal nitrogen intake in ARF? Reports of nitrogen balance measurements in which nitrogen intake is systematically varied are not available. It is unlikely that they would provide important data, however, because of the heterogenity of patients with ARF. One con-

clusion seems clear: the minimal nitrogen requirement of healthy young men and stable CRF patients will be insufficient for hypercatabolic, ARF patients.

10.6. Energy Substrates

Mault *et al.* have shown that the oxygen consumption of ARF patients is about 35% above normal; if the energy supply was inadequate, there was a higher mortality compared to patients placed in a positive energy balance.[156] In surgical patients with ARF, Spreiter *et al.* measured nitrogen balance while energy intake was varied from 25 to 55 kcal/kg body weight per day. At 45–50 kcal/kg per day, positive nitrogen balance was achieved in several, but not all, patients; the authors concluded that in ARF, energy intake should be at this level.[157]

The proportion of glucose and lipid used to meet energy requirements is unknown. The glucose intolerance caused by ARF suggests that more lipids should be used, yet there are often contraindications to intravenous fat infusions, including hypotension, shock, abnormalities of the microcirculation, and disseminated intravascular coagulation. Moreover, evidence from a fat tolerance test shows that lipolysis is reduced by ARF since the elimination half-life of lipid following an infusion of Intralipid was doubled from 14 to 28 min.[158] To minimize complications, lipid infusions should be given over 24 hr and triglyceride concentrations monitored daily, especially in sepsis, trauma, or postsurgery states when rates of lipolysis vary widely.

In summary, no single nutritional regimen will be suitable for all ARF patients. Available evidence suggests, but has not yet proven, that a mixture of NEAA + EAA might be more suitable than EAA alone for catabolic patients. Energy requirements can be met by a mixture of glucose and lipids. Careful measurement of changes in urea appearance should be used to indicate the suitability of different nutritional regimens.

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14

Dialysis

Lee W. Henderson

1. Introduction

I have reviewed the published work on hemodialysis (broadly interpreted) for the 2-year period beginning January 1984. What follows is a personal impression of the important advances that have occurred in that time. My effort, as in the past, is directed at being selective rather than all-encompassing.

2. Shortening Treatment Time

Shortening the time spent on hemodialysis has been our goal since the inception of maintenance dialysis. Time on treatment has decreased steadily to its present empirically arrived-at level of 4–5 hr three times weekly using conventional flow rates for blood and dialysate with a 0.8–1.5 m^2 nominal membrane area. While efforts to reduce treatment time even further have been reported (see, for example, work by Cambi *et al.*[1] and others), there has never been a widespread acceptance of these shorter protocols. This, I suspect, is because of anxiety about the adequacy of such treatments. As previously commented on, the demonstrated importance of time on treatment[2] as a significant variable in the

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treatment prescription supports this anxiety of the Dialysis Unit Director and has, in large measure, offset the enthusiasm brought to the judgment by the maintenance dialysis subject. The recent reduction in the amount of reimbursement offered by the government for maintenance dialysis will no doubt bring increasing pressure on the Unit Director to reexamine this option to permit across-the-board reduction in personnel costs. Let us examine the most recent spate of publications that address this topic to see what new information may be gleaned both technically and theoretically to help with this decision.

von Albertini *et al.*[3] and Miller *et al.*,[4] working at Wadsworth Veterans Administration Medical Center, have clinically applied a concept previously put forward by Cheung *et al.*[5] that involves using two large (1.8 m^2) hollow-fiber membrane modules in series. Blood and dialysis fluid flow in countercurrent directions at higher-than-usual rates, i.e., 500 and 1000 ml/min, respectively. In addition to this large-surface-area (3.6 m^2), high-flow-rate dialysis, they have adjusted the transmembrane pressure gradients to favor filtration in the first (upstream with respect to the direction of blood flow) and reabsorption in the second (downstream) membrane. By so doing the first membrane acts as a hemofilter with a convective plasma water clearance that is equal to the ultrafiltration rate for solutes small enough to pass across the membrane unimpeded (i.e., a clearance of 115 ml/min in this circumstance). The second membrane, with its casing to blood path pressure gradient, acts in essence as an infusion port taking dialysate that is sterile and pyrogen free and reverse filtering it into the blood path in a volume equal to that lost across the first membrane. As this internal filtration and "reabsorption" occurs at a rate of 115 ml/min, it is apparent that ultrafiltrate generated into the dialysate path is readily swept to drain by the 1000 ml/min flow rate of dialysate. Furthermore, this high flow rate of dialysate entering the casing of unit two provides an ample source of dialysis fluid, free of uremic solutes, to reconstitute the extracorporeal blood volume after its reduction in unit one. Fluid balance is thus maintained or electively unbalanced in an amount necessary to restore total body water to normal. Both units contribute to conventional diffusion-based mass transfer. A bicarbonate-based dialysate containing 140 meq/liter of sodium is employed. As a safety measure, dialysate is sterilized and rendered pyrogen free by an additional membrane before entering the casing of the second unit. As the solute cutoff for the cellulose acetate membrane used is well below that of both particulates, such as bacteria, and soluble bacterial wall fragments, such as pyrogen, the system provides for a redundancy in its protection from possible intravenous infusion of these noxious substances.

Using this system, which clearly requires custom-made fluid-cycling

and volume-monitoring equipment, this group reports reducing treatment time to 2 hr in four stable maintenance dialysis subjects for a study period of 6 weeks.

In a parallel line of investigation, Keshaviah *et al.*[6] studied 10 patients with shortened treatment schedule by increased membrane area. Two cuprophane hollow-fiber dialyzers were used either in series or in parallel for a total membrane area of 2–2.5 m^2. Although not stated in the article, a saline rinse formaldehyde storage protocol for membrane reuse was employed. Blood and dialysate flow rates were approximately 400 and 500 ml/min, respectively, in countercurrent mode. Of interest in this protocol was the clear confirmation of the earlier observation of Graefe *et al.*[7] that switching to bicarbonate as the alkalinizing agent for large-surface-area dialysis reduced the morbid events occurring during treatment. Reduction in treatment time was accomplished gradually to "the limit of the patient's tolerance" as identified by an increase in intratreatment symptoms on acetate dialysis. Reduction in treatment time by 1/3, i.e., to approximately 3 hr, was possible using this approach.

Third, Rotellar *et al.*[8] from Barcelona report on 6 hr/week dialysis using 5-m^2 membrane area (two 2.5-m^2 dialyzers configured in parallel) and bicarbonate dialysate run in countercurrent mode with a sodium concentration of 138 meq/liter. The membrane used was not specified but probably was cellulosic. Three programs were offered with 25 patients on a thrice-weekly 2-hr schedule, 10 patients on a twice-weekly 3-hr schedule, and six patients on a single 6-hr treatment each week.

What may we learn from these studies? First, the work from Wadsworth[3,4] must be judged more as a feasibility study than a clinical trial. There are, however, at least three noteworthy elements to this study. First is the demonstration that 2.6 ± 1.3 liters of excess total body water can be removed in a 2-hr treatment time with a significant reduction in the number of episodes of symptomatic hypotension (from 1.2 ± 1.1 to 0.4 ± 0.6 episodes/treatment). This observation was made in spite of very high urea clearance values (407 ± 15 ml/min). This alone is newsworthy, as a real limit heretofore on shortening treatment time with hemodialysis has been an increase in patient morbidity. Second is the accomplishment of very high clearance values for small molecules for which urea may be considered surrogate and for large solutes owing to the convective mass transfer that will remove all solutes up to the starting cutoff of the membrane at 115 ml/min. They have selected a membrane for this feasibility study that is not particularly open either for water flux or for solute transport. Presumably, by a more judicious selection of the membrane, even higher "internal" filtration rates can be achieved with even larger solutes passing unimpeded into the dialysate and moving to drain. The implications for adequacy of treatment ren-

dered will be commented on subsequently. Third, the technical demonstration that fluid balance can be maintained is noteworthy with what must be considered a relatively complex and unstable system when compared with hemodialysis, and for that matter even with hemofiltration.

The work of Keshavia *et al.*[6] may be considered a preliminary report on an ongoing clinical trial. What is impressive about this work is their utilization of conventional fluid-cycling equipment and membranes to accomplish their goal. Unlike the Wadsworth study, however, these workers appear to be operating near the limit of their patient's tolerance with respect to intratreatment morbidity. This is true in spite of reusing their cuprophane membrane with a protocol that others have demonstrated to be enhancing to membrane biocompatibility (as assessed by measuring the activation of the complement system) and the use of a high-sodium dialysate. Switching to a bicarbonate from an acetate bath, however, appears to give more "room" to reduce treatment time. By contrast, the Wadsworth group, using a bicarbonate bath, a 140 meq/liter "dialysate" sodium, a larger membrane area of cellulose acetate, i.e., a membrane that is more activating of complement than reused cuprophane (saline rinse formaldehyde storage), and with urea clearances of 407 ml/min rather than the 280 ml/min reported from the Minnesota study, appear to have greater cardiovascular stability even at 2 hr of treatment time. The unproven, but strongly suggestive message with respect to the etiology of symptomatic hypotension that flows from these studies is that although the sodium concentration in the dialysate, high urea clearances, and complement activation by the membrane may all play an etiologic role, the predominant event that governs cardiovascular stability during treatment must be tied to the presence of the significant convective loss of solute inherent in the Wadsworth system and/or the presence of sterile pyrogen-free "dialysate."

The work from Barcelona,[8] although uncontrolled in terms of dietary intake and different in the use of a high dialysate glucose concentration (5–10 g/liter), may be considered a clinical trial, having been conducted for a year or more in 41 patients. The largest subset studied was the 25 subjects relegated to the 2-hour thrice-weekly protocol. Close comparisons of the two prior studies with this work and the incidence of symptomatic hypotension is not possible because of these important differences. In this study, pre- and postclinical chemistries and conventional clearances of urea, creatinine, uric acid, phosphate, and vitamin B_{12} were measured. In addition, neurologic outcome parameters were assessed (nerve conduction velocities, EEG, and visual evoked potentials). In light of the relatively insensitive nature of these measures of outcome and the small number of patients studied (by contrast, for example, with the National Cooperative Dialysis Study, where 68 patients in each subset

were followed), it is not possible to accept the presence of adequate therapy for each of the protocols on the basis of clinical demonstration. Table I compares some of the operating parameters for these studies on shortened treatment time.

A common thread in all three studies is a concern for offering adequate treatment. Each achieves that adequacy slightly differently. The Wadsworth group, leading from the National Cooperative Dialysis Study (NCDS), focuses on achieving a small-molecular-weight clearance that is at least comparable to, if not more than, that offered by the clinically best treated of the NCDS subgroups, i.e., subgroup I. Recognizing that time on treatment is a variable in the prescription that is surrogate for middle molecules, they have added a convective solute transport component at approximately 100 ml/min that will generously exceed what may be obtained by simple diffusion. They argue for (and I would agree), but have not clinically demonstrated, that they are offering "state-of-the-art" or better adequacy as can be judged theoretically in comparison with the NCDS.

In like token, the Minneapolis team have paid close attention to sustaining comparability in their short treatment schedule to that in their conventional 4-hr protocol at the molecular size both of urea and of vitamin B_{12}. In doing so, they have used both the information from the NCDS on urea and that from the clinical studies of Scribner in qualifying the dialysis index. The Barcelona study is comparable to the Minnesota study in this regard. The Barcelona study with its three separate protocols, the most radical of which is the single 6-hr treatment each week, raises questions as to the space of distribution and/or site of generation of uremic toxins and the respective mass transport of these toxins within the compartments of the body. The Wadsworth team have looked at the rebound of urea as one important index of internal mass transport resistance and identified that the magnitude of the rebound is greater when a 2-hr treatment is given. They note that when they reduce treatment time by half, they must increase urea clearance by more than 2 times in order to remove the same amount of urea as they would with a full 4-hr treatment. Said another way, because urea clearance is doubled, it reduces its plasma water concentration more swiftly, so that the driving gradient for urea is less at any given time during a 2-hr treatment than when contrasted with a comparable fraction of the treatment time during a 4-hr treatment (e.g., 30 min of a 2-hr treatment compared with 1 hr of a 4-hr treatment). While these events may be measured for urea, it is not possible to do so for all uremic toxins. As such, it is not presently possible to state with confidence that the short treatment regimes prescribed by any of the three studies noted above will provide better or even comparable net clearance from the body when contrasted with 4-

Table I. Comparison of Studies on Shortening Treatment Time

Author (ref.)	Q_B	Q_D	Membrane area (m^2)	Dialysate		Hr/week	Treatments/ week	C_{urea} (ml/min)	$C_{B_{12}}$	Acetate or HCO_3	Membrane reuse	No. patients	Duration of study (months)
				Membrane	Sodium								
von Albertini (3)	500	1000	3.6	Cellulose acetate	140	6	3	407	115[b]	HCO_3	No	4	1 1/2
Keshaviah (6)	400	500	2–2.5	Cuprophane	140	8.25	3	280	7.5[c]	HCO_3	Yes	10	2
Rotellar (8)	500	1000	5.0	?[a]	138	6	3	456	98	HCO_3	?	25	12
						6	2					10	12
						6	1					6	12

[a] Unspecified, likely to have been 2.5 m^2 C-DAK regenerated cellulose hollow-fiber membrane from Cordis Dow Corp.
[b] Assumes a sieving coefficient of unity for this membrane.
[c] Calculated B_{12} clearance of 32 liters/week, giving a dialysis index of >1.0.

hr hemodialysis for an unspecified uremic toxin that dwells within the cell water. In conclusion, there is much to be learned from these studies, but as of the moment, shortened treatment time must still be considered clinical investigation, not routine therapy.

I note the work of Chang *et al.*[9] in preliminary studies in four patients on shortening treatment time by one-third by combining routine hemodialysis with hemoperfusion of microencapsulated carbon. These studies are based on the hypothesis that treatment time may be shortened by improving the removal of middle molecules. Although I think this is probably correct, these experiments are too preliminary to permit critical judgment.

3. Quantitation of Treatment

There has been a modest flap over the last two years concerning two elements of urea kinetic modeling. First, the appropriateness of using a single pool model for urea, and second, whether direct measurement of urea in collected dialysate is a more accurate technique than measuring blood side clearances for urea and multiplying by time of treatment to arrive at the mass removed. Ellis *et al.*,[10] Ilstrup *et al.*,[11] and Aebischer *et al.*[12] conclude that total collection of the dialysate with measurement of its urea concentration is less subject to error than employing a clearance-times-treatment-time computation. Both Ilstrup *et al.* and Tsang *et al.*[13] in following the urea rebound note that a two-pool model fits the data most satisfactorily. However, the single-pool, variable-volume model popularized by Gotch and Sargent, with data on mass transfer obtained from clearances obtained from arteriovenous blood path concentration differences and blood flow rate as used in the NCDS, has in the recent past dominated the scene. The "errors" reported by these more recent workers in, for example, computing the volume of distribution for urea within the body are on the order of 15–20% with single-pool, clearance-based-volume estimates coming in too high.

Does this mean that we must throw out the results of the NCDS as somehow in error by 15–20% in its quantitation of the therapy delivered? I think none of us doubt the presence of urea on both sides of the cell wall, rather the underlying question is how fast it traverses that barrier. The duration of the observed rebound, i.e., 20–30 min (see Tsang *et al.*) argues, in my mind, for that transfer rate to be slower than would permit fitting the data to a single-pool model but fast enough to point away from the catabolic stimulus of dialysis[14] as being the underlying event. If the rebound was not movement of urea from cell to plasma water, but rather an upturn in the tempo of catabolism induced by

dialysis, it should, in my judgment, continue for an hour or more after the procedure is concluded. The difficulty here, I believe, is the tradeoff between the rigor of the model and the practicality of using it. A two-pool model requires more blood sampling to characterize patient performance and more complex computation than a single pool. The single-pool model is a "reasonable" fit, and the errors in estimation of the parameters bear an orderly, if not constant, relationship to those derived from a two-pool model. Furthermore, the single-pool model has the considerable advantage of having strong clinical correlations established by the NCDS for its derived parameters, such as the volume fraction cleared (Kt/V). Part of the discrepancy shown between the clearance of urea derived from the arteriovenous extraction ratio and that from measuring the urea in collected spent dialysis fluid relates to this "reasonable" approximation. For the purist, then, or the clinical investigator in pursuit of an absolute value for the volume of distribution of urea or the amount of urea removed per treatment, a two-pool model with additional blood samples taken at 5- to 10-min intervals after the conclusion of dialysis to characterize the rebound and compute the average transcellular mass transfer coefficient is the way to go. I note in particular that those investigating short treatment time would do well to pay attention to these absolute values, as the rate of movement across body compartments may provide a limiting constraint on reducing treatment time, especially for solutes larger than urea. For those wishing to assure adequate treatment as qualified by the NCDS, the single-pool blood-clearance-based methodology is adequate. As noted by Gotch and Sargent in response to Ilstrup's concerns[15] about methodologic inaccuracies, there is room for random as well as systematic errors by the investigating team in determining dialyzer clearance. I note that these errors are magnified by the calculations employed.

Finally, the paper by Gotch and Sargent[16] on an alternative ("mechanistic") analysis of the results from the NCDS is complex in its reading but makes an important point, namely, that the study design by the NCDS team did not permit an independent assessment of the impact of low-protein catabolic rate and amount of dialysis given, because no patient with low-protein catabolic rate (i.e., <0.8 g/kg per day) was treated with a high fractional urea clearance. The outcome of this strategy is not known and cannot be accurately surmised. That is, one must view the results of the NCDS in terms of the two levels of treatment given over the range of protein catabolic rates between 0.8 and 1.4 g/kg per day as a step function and not a smooth curve. A relationship of the step-function sort does not permit any confidence in an extrapolation beyond that which has been experimentally demonstrated. We are therefore cautioned against assuming, for example, that increasing protein intake and/or dialyzer clearance on a thrice-weekly schedule with the

regenerated cellulose membranes used by the NCDS will assure lower morbidity in the patient.

4. Treatment of Acute Renal Failure

The evolution of the continuously applied slow-flow techniques has been rapid in the last 2 years and is far from over. Continuous arteriovenous hemofiltration (CAVH), as popularized by Peter Kramer,[17] has given rise to the following variants and/or embellishments:

1. Continuous arteriovenous hemodialysis[18]
2. Continuous arteriovenous hemodiafiltration[19]
3. Predilution continuous arteriovenous hemofiltration[20]
4. Slow continuous ultrafiltration[21]

In addition, these techniques may be employed with or without pumps on the blood path, with or without pumps on the ultrafiltrate/dialysate side, and with or without electronic monitoring of fluid balance.

The number of study subjects for all but the original technique of CAVH is small, i.e., ten or less per reported series, and as such these are more feasibility studies than full clinical reports. It follows that no clear choice can be made on the basis of reported experience. Several useful bits of information may guide us on what are probably the important advances.

For example, we must be indebted to Geronemus and Schneider[18] for calling attention once again to the importance of diffusion in the removal of urea. One real limitation of CAVH is its inability to cope with the large urea loads of the postsurgical highly catabolic patient. Employing a regenerated cellulose membrane (i.e., lower hydraulic permeability than the Amicon polysulfone membrane employed most commonly for CAVH, but a better diffusion membrane because it is thinner than the "spongy" XP-50) and using a gravity feed infusion of peritoneal dialysate at 15–20 ml/min, he reports clearances of 13–16 ml/min of urea and 12–14 ml/min of creatinine, i.e., better than with conventional CAVH (see Table II). A urea clearance of 13 ml/min would translate into approximately 18 liters of clearance per day, or 131 liters/week, as contrasted with a 4-hr dialysis offered 3 times per week with a membrane that clears urea at 170 ml/min which offers 122 liters/week; i.e., they are quite comparable but with the intermittent techniques, 300 liters of unsterile dialysate is used whereas 150 liters of sterile pyrogen-free dialysate is used for the continuous arteriovenous hemodialysis. The limitation in this technique appears to be fluid removal, which at maximum is reported to be 100 ml/hr. This 2400 ml/day limit would permit little flexibility in providing for hyperalimentation, either intravenously or orally.

Table II. Comparison of "Continuous" Techniques

Technique	C_{urea}	Fluid requirements
CAVH	10.6 ± 0.3[a]	678 ± 26 ml/hr sterile pyrogen-free solution
CAVHD	13–16	900–1200 ml/hr sterile pyrogen-free solution[b]
CAVHDF[d]	15–20	2.4–6.4 liters/hr of dialysate[c] and 600 ml/hr of sterile pyrogen-free infusion solution
Predilution CAVH	12.5 ± 0.2[a]	825 ± 14 ml/hr sterile pyrogen-free solution

[a] I have chosen Kaplan's CAVH data for comparison as it was collected in cross-over study predilution CAVH in the same patient.[20] Average clearance values for CAVH will differ in different series.
[b] Sterile pyrogen-free peritoneal dialysate was used for this study,[18] but it is not at all clear that conventional dialysate mixed from concentrate might not equally well be used, as demonstrated for CAVHDF.
[c] Computed for the range of dialysate flow rates that do not reduce net filtration flow rate (see text).
[d] These figures are unaugmented by pumps on the blood or dialysate/ultrafiltrate lines.[19]

The techniques described by Ronco *et al.*[19] (continuous arteriovenous hemodiafiltration) and Kaplan[20] (predilution CAVH) both offer significantly higher urea clearances as well (Table II). Both, however, involve more in the way of technical complexity. Kaplan introduces diluting fluid upstream (in terms of the direction of blood flow) of the filter, so that whole blood enters the filter diluted and is reconstituted to starting volume by the filtration process. Augmentation of the urea clearance comes both from the recruitment of urea from the red-cell water and from the increase in filtration volume possible when oncotic force is reduced by dilution to below normal at the same hydrostatic driving pressure (mean arterial blood pressure plus the height of the filtrate column hanging below the filter and above the collection vessel) commonly employed for CAVH. Predilution against arterial pressure requires a pump to deliver the solution. Ronco, using combined convection and diffusion (hemodiafiltration), requires both a dialysate flow that, by gravity, can be put by the outside of the membrane so that diffusional solute driving gradient may be sustained, as well as the use of sterile pyrogen-free replacement solution to restore fluid balance in a manner comparable to Kramer-style CAVH. Of note is the maintenance of pump-free simplicity with this system. The tradeoff for this simplicity appears to be a reduction in filtration flow rate at much over 100 ml/min of gravity flow dialysate.

Finally, slow continuous ultrafiltration[21] is aimed purely at fluid removal in patients who would tolerate such net negative fluid balance with conventional dialysis poorly from the cardiovascular standpoint.

In looking at these various techniques it is easy to think that adding a pump or vacuum source or automatic fluid-balancing equipment would be an advance, but I do not feel that it necessarily would be. It seems to me that the tradeoffs here relate to sustaining a technical simplicity that permits ready application by nursing personnel with the basic professional skills of the intensive-care nurse, without the need for technician support, versus safe control of uremia in most instances. That is, 10–15% of the hypercatabolic acute renal failure patients will require more efficient continuous therapy or adjunctive intermittent hemodialysis, a position comparable to that of peritoneal dialysis for treatment of acute renal failure. Prior to clinically demonstrating superiority of one or another system, I would identify that employing both diffusion and convection continuously appeals to me most, i.e., CAVHemodiafiltration. I have two reasons for wishing to hold in place a significant convective component to the overall transport transaction: first is the vascular "space" that such filtration creates for use by the clinician in nourishing his patient, and second is the possible augmentation of the quality of the treatment accomplished by mimicking the solute clearance profile of the normal kidney more closely, i.e., my long-standing prejudice.

I feel that applying filtration membranes purely for fluid removal will, in most cases of acute renal failure, be a waste of the professional time invested given the opportunity to use one or another of the previously described techniques, which by their very inefficiency with respect to solute removal (when compared with acute intermittent hemodialysis) would be expected to be as "kind" to the patient's cardiovascular stability as simple fluid removal might be.

If the continuous technique to be applied has a diffusional component and a convectional component, it seems to be the wise choice to select a membrane that has both the required high hydraulic permeability, i.e., comparable to the polysulfone membrane of Amicon, and the short diffusion path (thin wall) typical of cuprophane but not found with the asymmetric membranes such as polysulfone. At present, the membrane technology is at hand to design such a membrane, but one has yet to be supplied to the clinician/investigators for evaluation.

As these techniques spread, the importance of work such as reported by Golper *et al.*[22] becomes apparent, as it will be needed to permit rational prescription of drugs lost (with varying degrees of efficiency) during these continuous treatments.

Finally, there continues to be uncertainty in my mind about the level of caloric intake and the quantity and quality of protein needed to treat patients with acute renal failure. What is more clear is that we have been really short of the mark in relying on intravenous replacement con-

strained by our concern about fluid overload in the oliguric postoperative acute renal failure patient. Studies such as reported by Mault *et al.*[23] and Fienstein *et al.*[24,25] point to major, as yet unmet, needs. As noted earlier, the continuous therapies share the common ground of creating space for nutritional supplementation.

5. Peritoneal Dialysis

The report of Gutman *et al.*[26] on the Veterans Administration Multicenter Study comparing home-care intermittent peritoneal dialysis (IPD) with home-care hemodialysis (HD) over a 12-month time span is, I believe, consonant with the general clinical wisdom that in most cases IPD is a less satisfactory home maintenance technique than HD in spite of the shorter training time for IPD, i.e., 1.8 versus 3.9 months. The higher hospitalization rate for those on IPD and the longer duration of hospital stay is ameliorated only slightly by the finding of fewer serious cardiovascular events occurring in the IPD study population.

There continues to be the sharp upswing in application of CAPD (4.5% of the dialysis population in 1980 and 11.9% in 1983).[27] Data on CAPD from the NIH-underwritten registry are now being reported, as well as clinical and investigative experience from individual centers. CAPD continues to be plagued with a high dropout rate per year, either from death (15%) or from transfer to another treatment modality (22%). It is of interest that the influence of patient age (>60 years) and the presence of diabetes do impact on drop out from mortality but do *not* seem to influence very much the transfer to other modalities.[28] Patients from the more than 7000 registered spent 22 days/year in the hospital and had 1.7 episodes of peritonitis per patient year. These are higher figures than reported from groups with large experience and/or special interest in CAPD. That they are probably reflective of the common experience is spoken to by the similarity of the figures for patient survival and survival of the CAPD technique given for the NIH Registry and by registries reporting from Europe,[29] New Zealand and Australia,[30] and Canada[31] (see Table III).

New information here may be broadly cast into technical ("contectology related") information and pathophysiologic information. Cited by way of example of the former are the significant reduction in infection rate noted with improved connection methodology. The study of Fenton *et al.*[32] showed a fourfold reduction in infection rate using the Oreopoulos–Zellerman connector over prospectively matched randomly selected control patients. All 27 adult study subjects were drawn from a high-risk subset of the CAPD population which had had three or more episodes of peritonitis over the year or two episodes over the prior 6

Table III. CAPD Registry Data for Patient and Technique Survival (%)

	Patient	Technique	No. patients
Europe	92	67	705
New Zealand	79	37	509
Canada	80	52	596
United States	73	61	7295

months prior to entry into the study. Hamilton *et al.*[33] demonstrate a reduction by half in the peritonitis rate in 31 adult study subjects using the Dupont sterile connection device. Each subject acted as his/her own control. This 31-subject group was identified as high risk because they had had an episode of peritonitis during the control period. Of interest is a lack of significant reduction in incidence when all study subjects ($n = 73$) undergoing the protocol were examined. This probably reflects the need for a larger study group to achieve significant differences in a population that is at "average" risk for developing peritonitis.

Probably more important than the technical differences between the Dupont "hot-knife" device and the Oreopoulos–Zellerman Betadine-containing bell covering for the spike is the fact that technology plays a significant role in reducing peritonitis in the high-risk patients and by definition in the total population, even though a large cohort would be needed to demonstrate the point. It also identifies that a predominant role is played by organisms entering the peritoneum via the catheter lumen as contrasted by those migrating either across the bowel wall or down the exterior of the catheter. Identifying critically important trade-offs to the reduced incidence of infection, such as patient acceptance/convenience and additional cost, must now be examined in addition to rigorously comparing the different technologies to determine which offers the minimum incidence of infection. That is, it will take time and further clinical experience to determine what is better for whom.

The risk factors for the development of peritonitis have been examined using data from the NIH CAPD Registry.[34] The demographic and nosologic characteristics shown to have importance as increasing the risk were extremes of age, i.e., <20 and >60 years old, presence of diabetes mellitus, and prior therapy for end-stage renal failure. Black patients living with family appeared to have an increased risk of infection, whereas the reverse was true for white patients.

Several miscellaneous reports that have bearing on the pathophysiology of infection-related events within the peritoneal space bear comment. The first is the report from Keane *et al.*[35] showing that low opsonic

activity found in spent dialysate correlates with the incidence of peritonitis from *Staphylococcus epidermidis*. Opsonic activity is, of course, crucial to the efficient implementation of microbicidal activity by the phagocyte, in this instance the peritoneal macrophage. In their series of 17 subjects, there was a 10-fold higher incidence of *S. epidermidis* peritonitis in those with low (17%) opsonic activity as contrasted with high (46%) levels. They show that opsonic activity in spent dialysate is only 1/50 to 1/100 that observed in normal pooled plasma to begin with, a value at the lower limit required to facilitate monocyte ingestion. The opsonin was primarily of the heat-stabile variety of IgG. The degree to which IgG enters the peritoneal space was considered to be the probable underlying event in the variable defense against peritonitis noted. Two points of importance should be taken from this work: first, that there are differences between patients in their defenses against infection that are independent of technique-related events; second, that measurement of opsonin concentration in spent dialysate shows sufficient separation between low and high values that it might well be used to select patients who would truly profit from the use of more expensive connection technology, i.e., fiscal triage. The report of Piraino *et al.*[36] showing a correlation between low percent eosinophil count in the dialysate in the first 1.5 months on CAPD and an increased incidence of peritonitis reiterates the genetic predisposition noted above, but the scatter in the data is sufficiently wide to make its use as a screening test problematic.

Several articles aimed at characterizing the transport across the peritoneal membrane need comment. Viewing the peritoneum as a passive semipermeable membrane, Hirszel *et al.*,[37] using polydisperse neutral dextran, have shown that molecules as large as 50,000 daltons do move by diffusion from plasma water to dialysate across the membrane, albeit slowly. They note that there is a size-independent transport process that operates to take up dextran above that molecular size from the peritoneal space, probably uptake by the lymphatic system. There is an interesting and quantitative description in the rabbit of transport for a wide spectrum of homologous neutral solutes that we have not had heretofore. They argue from a linear regression analysis of their dextran clearance versus molecular weight curve that an estimated maximal pore radius of the peritoneum is about 50 Å. As the diffusivity of dextran in dialysate is not taken into account in this analysis, it seems unlikely that this is a correct figure.

Flessner *et al.*[38–43] have approached the peritoneum not as a semipermeable membrane, but rather as a series of capillaries embedded in a matrix, i.e., a distributed model of transport. Projections from their theoretical description (model) are surprisingly consonant with existing measurements on blood to dialysate transport. This distributed model

is particularly relevant to the use of chemotherapeutic (anticancer or other) agents topically in the peritoneal space, as it permits descriptions of concentration profiles within the tissues as one moves from the peritoneal surface back into the capillary lumen. They have measured tissue concentrations of macromolecules such as radiolabeled albumin and polydisperse neutral dextran in rats. This work also indicates the lymphatic uptake of dextrans of 39,000 daltons and greater in size noted by Hirszel *et al.*[37] What is vexing about this "lymphatic" (pinocytotic?) uptake is that even larger solutes, such as radiolabeled albumin, larger-molecular-weight dextran, and even particulate material such as autologous red blood cells, cannot be used as "dye dilution" volume markers to track changes in peritoneal volume for solutions that are other than isotonic. Further investigation with the distributed model will be necessary, especially with other than isotonic solutions, to determine whether this model is more truly reflective of peritoneal membrane transport than the semipermeable membrane model commonly used.

6. Blood–Membrane Interaction and First-Use Syndromes

At last writing[2] there had been an upswing in the identification of dialyzer-associated symptoms when new, rather than reused, membranes were employed. In addition, the activation of the complement cascade and the presence of residual sterilant (ethylene oxide) was considered as possibly contributory to the allergic manifestation associated with first-use syndrome-1 (as distinguished from FUS-2, i.e., the increase in incidence of any morbid event, allergic or not, associated with the dialysis treatment).

In update with respect to the activation of the complement system by dialysis membrane, there is work from Hakim *et al.*[44] that reports a higher level of complement activation (as measured by plasma $C3a_{des\ arg}$ and $C5a_{des\ arg}$ levels) in six patients undergoing adverse symptoms with first use of cuprophane membrane than in a control group of 10 patients who were symptom free. The peak values for C3a of 8533 ± 157 and C5a of 106 ± 4 ng/ml were significantly higher than values of 2907 ± 372 and 34 ± 4 ng/ml, respectively, in the control subjects. This observation is of high interest as it is the first prospective study that correlates complement activation with untoward clinical events during clinical dialysis. Selection of the patients for subgrouping fell to the nurse/technical staff and unit director who diagnosed the FUS simply on the basis of an increase in symptoms during the first 15–30 min of dialysis noted when new cuprophane membrane was used. The description of their cases shows that both the florid allergic type FUS-1, with angioedema, flushing,

and dyspnea, was grouped with those showing symptoms of back pain and chest pain with dyspnea, i.e., nothing pathognomonic of an allergic event.

In vitro study of the serum from these first-use reactors by measuring the level of $C3a_{des\ arg}$ generated in response to the addition of an alternate pathway activator, yeast, to the plasma also showed higher levels than for the control subjects, indicating that their complement system was "set high" either genetically or by prior conditioning. This would provide one potential explanation as to why only a certain few patients react with symptoms to first use of the membrane whereas most are symptom free. As to whether a genetic predisposition or a conditioned event underlies this high set, it is noteworthy that studies by Volanakis *et al.*[45] of the serine protease complement protein D show that this enzyme that is rate limiting for alternate-pathway activation of complement is higher (less likely to be rate limiting) in patients with chronic renal failure and correlates ($r = 0.75$) with the plasma creatinine. At plasma creatinine values of 4 mg/dl and above, the correlation appears to be lost. It is three times higher (unlikely to be rate limiting) in those uremic subjects on maintenance dialysis (with suponified cellulose ester membrane) and slightly but significantly higher in dialysis subjects just following treatment, i.e., 1.53 ± 0.39 mg/dl predialysis versus 1.74 ± 0.37 mg/dl postdialysis. Complement protein D with an approximate molecular weight of 24,000 is readily lost across the glomerular basement membrane and reabsorbed and metabolized by the proximal tubular epithelium, and hence one would expect to see elevated levels in chronic renal failure. The profound jump in concentration noted between undialyzed chronic uremic patients and maintenance dialysis subjects who use a complement activating membrane coupled with the postdialysis elevation suggests that serial dialysis may be an important "conditioning factor." Finally, with regard to this paper, the spread in measured concentrations of D for the 16 dialysis subjects (10–25 g/liter) is far wider than that of the healthy controls (1–3 g/ml) and overlaps that for nondialyzed chronic renal failure subjects (1–12 g/ml). Again, there seems to be an underlying genetic component as well.

These observations on protein D should be coupled with further observations by Hakim *et al.*[46] on the effect of serial activation of complement on ambient levels of anaphylatoxin ($C3a_{des\ arg}$ and $C5a_{des\ arg}$). They show that both the peak level and rate of metabolic degradation increase, in proportion to the degree to which the new membrane on first exposure activates complement; that is, cuprophane membrane, which shows a peak increase over predialysis values of 309% at the beginning of the study, increases to a peak value of 471% after 1 month of dialysis with this membrane. Cellulose acetate showed a similar in-

crease. The rate of degradation as identified by the faster rate of fall from peak values as well as lower predialysis values indicates that the complement system is adaptive to its recurrent activation.

The presence of an elutable and "toxic" substance from new dialyzers has been addressed by Pearson *et al.*[47] and Henne *et al.*[48] There is agreement that there is material present in and elutable from the blood path of cuprophane hollow-fiber membranes that is derived from the cotton linters that occur in production of the cellulose that composes the membrane, that is not pyrogenic, but that does give a positive test for limulus amebocyte lysate (LAL reactive). This nonpyrogenic LAL-reactive material has been shown to be antigenic in New Zealand rabbits.[49] Of sera from seven patients showing an adverse reaction to dialysis, however, only one showed a borderline positive response [RadioAllergo Sorbent Test (RAST)]. The RAST test method involves adsorbing the allergen, for example, the LAL-reactive material, to an inert particle. The patient's serum is added to the particles, and these are subsequently washed with saline. If the test serum contains IgE antibody against the ethylene oxide antigen, it will form an immune complex on the particle. By adding radiolabeled antibody to IgE one may then measure, in a very sensitive manner, in the patient serum, the presence of IgE against a specific antigen. The additional finding that most of these reactive patients had normal IgE levels points away from a classical type 1 hypersensitivity mechanism as underlying the severe early adverse reactions to first use of a dialyzer.

With respect to residual ethylene oxide as etiologic in these first-use reactions, it is apparent from work by Henne *et al.*[50] and Lee *et al.*[51] that polyurethane potted hollow-fiber membranes cannot readily be "degassed" because of residual ethylene oxide that remains in the potting compound and moves out into the blood path slowly. The fiber itself (cuprophane) "deaerates" reasonably swiftly (9–10 days) and must be considered an unlikely source for residual sterilant. Priming solution left standing in the blood path is reported to contain milligram quantities of ethylene oxide from this source. In conjunction with this observation, we should recall earlier work that correlates the degree of eosinophilia in maintenance dialysis patients with the duration of hemodialysis therapy[52] and at least one paper in which a positive correlation between eosinophilia and symptoms during treatment is made.[53] Our colleagues in industry would do well to reduce the quantity of polyurethane used or swap it for a nonpermeable material.

Dolovich *et al.*[54] report a positive RAST reaction for ethylene oxide-related antibody in 22 of 27 patients with "acute allergic-type reactions shortly after onset of dialysis" with only 5 of 37 showing a positive RAST who had no clinical reactions. RAST study for ethylene oxide in 24

peritoneal dialysis patients was uniformly negative. A lower incidence (4 of 25) of positive reactions was reported by Lamke *et al.*[55] using similar allergosorbent methodology. Interestingly, five of Dolovich's study subjects were selected because of "isolated eosinophilia" (not because of allergic-type reactions) and only one proved to be borderline RAST reactive for ethylene oxide.

The study of Ward *et al.*[56] aimed at determining whether different preparation techniques for the dialyzer could be correlated with anaphylatoxin (C3a, C5a) formation, predialysis eosinophil counts, or plasma levels of IgE was negative. His preparation techniques involved a standard 1-liter saline rinse/prime of the blood compartment; a 1-liter saline rinse/prime but with a 10-min recirculation of the blood compartment prime through a 0.45-particle filter; and a 1-liter saline rinse/prime but with a slow (3–4 hr) reverse filtration of 1 liter of saline from casing to blood path and hence to drain. Ethylene oxide-sterilized cuprophane membrane was used in all protocols and was produced with a freon wash of the fibers to remove isopropylmyristate necessary in the manufacturing process. A last group was studied with the standard 1-liter saline rinse/prime but was produced with not only a freon but a 2-propanolol rinse. My worry about this negative study is that with only 11 study subjects undergoing a 6-week exposure to each protocol, the statistical strength of the negative answer is not very great given the wide scatter normally present in the outcome parameters measured; i.e., I think some of these factors will prove to be important in larger and more prolonged studies. Useful review articles on the membrane characteristics that result in complement activation and the impact on the polymorph are provided by Chenoweth[57] and Craddock and Hammerschmidt.[58]

The studies of Camussi *et al.*[59] and Horl *et al.*[60] point to what will probably prove to be one of the outcomes of the "new look" at blood membrane interaction. That is, given the complexity of whole blood, it is likely that there will be many more tests of biocompatibility (see, for example, Ref. 61) and that phenomena now considered to be reasonably well understood, such as hemodialysis leukopenia, will prove to be far more complex than is presently appreciated. More specifically, Camussi *et al.*[59] show that neutrophil cationic protein released when the polymorph degranulates plays a role in leukocyte aggregation in conjunction with $C5a_{des\ arg}$. The degranulation reaction of polymorphs will now need to be more fully understood and may serve as another sensitive index of blood–membrane interaction, i.e., at the blood cell level in addition to surface-sensitive plasma proteins, such as the complement system and the clotting cascade. The work of Horl *et al.*[60] shows that the release of granulocyte elastase, a neutral proteinase that is probably instrumental in causing tissue death because of its capacity to attack a broad spectrum of substrates, depends on the dialyzer membrane to which the granu-

locyte is exposed. The elastase was measured in conjunction with the alpha-1-proteinase inhibitor. They establish an "activity series" (least to most likely to release elastase) that goes polysulfone (Fresenius FRG) polyacryonitrile (Hospal, France), ethylene–vinyl alcohol copolymer (Salvia, FRG), cuprophane (Fresenius FRG), polymethylmethacrylate (Toray, Japan), and cellulose hydrate (Secon, FRG). What is of interest in addition to identifying a polymorph/dialysis membrane incompatibility is that this "activity series" is different than that noted for complement activation, which usually runs polyacrylonitrile (Hospal, France), polymethylmethacrylate (Toray, Japan), cellulose acetate (Dow Cordis, USA), cuprophane (ENKA, FRG), and cellulose hydrate (Secon, FRG); i.e., there is a sharp difference in where the polymethylmethacrylate membrane falls, indicating that activation of plasma complement is mediated by different membrane parameters than is polymorph/dialysis membrane interaction. Life is never simple. Finally, polymorph chemiluminescence that is presumably complement mediated is sharply increased when blood is exposed to cuprophane but not polyacrylonitrile (Hospal) membrane.[61]

What may we learn from this welter of information that is at times conflicting? It is my best perception that blood–membrane interaction will continue to be studied and become increasingly complex and that these studies will show increasing test system-specific correlation with clinically relevant sequelae.

These sequelae will probably surface as an increase in chronic morbidity, e.g., carpal tunnel syndrome, excess protein catabolism with intercurrent infection, and death,[62] and to a lesser extent will correlate with acute intratreatment symptoms; the acute intratreatment symptoms of which FUS (defined as allergic-type reactions in the first 15–30 min of treatment) will probably prove to be multifactorial, with the following components playing a role to a variable degree in any given individual: "genetic predisposition," IgE-mediated allergic response to both ethylene oxide and/or limulus lysate-positive cotton linter-derived nonpyrogenic material, complement activation by the membrane with anaphylatoxin generation, and possibly cell release of such powerful mediators as platelet-activating factor, the thromboxanes, and granulocyte elastase. Many of these components will also play a role in the FUS, defined simply as an increase in symptoms during treatment allergic or not occurring at any time during dialysis.

7. Access/Anticoagulation

Reviewing the published work on vascular access left me with two conclusions of note, both negative. The first was the unfavorable report

on the hemocite device by Barth *et al.*[63] Infection and thrombosis occurred in the 14 patients under study with roughly twice the frequency as in the 28 patients with polytetrafluoroethylene grafts. The authors conclude that this device should not be used until these problems are overcome. The second is a report from Raja *et al.*[64] comparing double-lumen Shiley–Vascath (n = 47/171 patient insertions) versus single-lumen (n = 46/52) catheters. They find in favor of the single-lumen catheter as having fewer problems of inadequate flow and infectious complications. This kind of study emphasizes the need for good-quality control information in order to make comparative judgements, otherwise the study deteriorates into a less informative feasibility report, such as that by Tapson *et al.*[65] on the same device used successfully in some 30 patients.

The reports from Schrader *et al.*[66] and Ljundberg[67] on the use of a low-molecular-weight fraction of heparin sound promising, as some of the undesirable side effects of unfractionated heparin appear to be ameliorated. A dose of low-molecular-weight heparin that was only half of that required for the unfractionated product produced the same plasma level of anticoagulation (i.e., elevations of Factor VIII and fibrin monomers) and by contrast produced only a slight increase in plasma thromboplastin time and thrombin time and only marginal stimulation of lipoprotein lipase. Low-molecular-weight heparin may reduce the risk of bleeding in our patients.

8. Vascular Refilling Rate/Colloid Osmotic Pressure

The work of Koomans *et al.*[68] adds a new dimension to my thinking about the changes in vascular volume that occur during hemodialysis. They point out that in his 21 study subjects using radiosotope dilution methodology, the degree of hydration present in the interstitial space (i.e., bromide space minus plasma volume) is a crucial determinant of the vascular refilling rate and, for that matter, of the plasma volume after steady state has been attained after (24 hr) the perturbation induced by dialysis. Overhydration of the interstitial space correlates with swifter vascular refilling and higher postequilibration plasma volumes. Interestingly, both total plasma protein and plasma albumin left the vascular space in response to ultrafiltration and returned during vascular refilling, although a statistically significant repletion of mass was observed only for plasma albumin. Postulated but not measured in the present study were changes in the pre-to postmicrovascular resistances. These workers note, as have most clinicians, that certain patients show hypotension with restoration of normal total body water even when interstitial volume is

well above normal, indicating that further, as-yet-undescribed mechanisms are at work.

Rodriguez *et al.*,[69] in one element of their study, have made formal measurements of colloid osmotic pressure during both routine hemodialysis and isolated ultrafiltration in five study subjects. They note no difference between these subjects, pointing away from differences in oncotic force as being explanatory of the differences in blood pressure stability noted between these two techniques. We are again left with the need to identify another mechanism(s).

9. Reuse

The practice of reuse, whether economically or scientifically driven, continues to spread (in 1980 approximately 18% of centers reused, rising to 52% in 1983).[70] The work of Robson *et al.*[71] in particular, so far presented in abstract only, supports the contention (with a large study population) that dialyzer reuse is associated with lower intradialytic morbidity, i.e., fewer episodes of symptomatic hypotension, chest and back pain, dyspnea, and chills. This may well relate to the washout of limulus lysate-reactive material and/or a reduction in the burden of ethylene oxide delivered to the patient and/or improved "biocompatibility" as assessed by complement activation by mechanisms discussed in Section 6. Kaye *et al.*[72] contributes to the work already in existence supporting lack of change in transport characteristics for urea, creatinine phosphate, and vitamin B_{12} in cuprophane hollow-fiber units reused up to 30 times. They note that a 1% hypochlorite rinse employed in their manual reuse procedure (2.6% formalin storage) retained the advantage of sustaining an unchanged neutrophil count in a manner analogous to a saline rinse, formalin storage protocol and in contradistinction to their automated reuse procedure, which utilized a 4.3% hypochlorite rinse. Deane *et al.*,[73] in prospectively examining six different reuse machines with cuprophane hollow-fiber dialyzers, note that on average each of the symptoms studied showed a significant reduction in incidence over first use, i.e., back pain, cramps, nausea, pruritus, chest pain, fever/chills, dyspnea, headache, and medication utilization. The scatter of the data was wide, however, with different symptoms scoring higher with some machines than others.

Peracetic acid has entered the field as a bactericide to replace formalin, but with a mixed review.[74] On one hand, less hypotension and headache occurred during dialysis than occurred with formalin; on the other hand, the ultrafiltration rate for the unit fell significantly (5–10%) between the first and second use of the cuprophane hollow-fiber units

studied, and clearances for urea, creatinine, and vitamin B_{12} were significantly lower after the eighth reuse, unlike formalin-processed units in which no such changes were noted. While there are documented ill effects from "trace" formalin reinfusion, there is no such body of information on peracetic acid. Further study seems appropriate.

Finally, the deaths resulting from nontuberculous mycobacterial organisms in hemodialysis patients reported out of Louisianna[75] and the 83% incidence of the presence of these bacteria in water from 115 dialysis units examined have focused attention on the need for 4% formaldehyde or equivalent disinfectant, as 2% is not germicidal for these organisms.[70]

10. Hemofiltration

Several papers characterize the mass transport properties of hemofiltration membranes over a broader range of molecular size than has been available previously.[76–78] Use of polydisperse neutral polymers as test solutes permits characterization of transport in the range from 2000 daltons up to protein-sized molecules. Leypoldt *et al.*[77] and Feldhoff *et al.*[76] both show a striking difference in membrane permeability between manufacturers. Leypoldt *et al.* used neutral polydisperse dextran, whereas Feldhoff *et al.* used maltodextrin. An important conclusion drawn from this work and subsequently demonstrated more elegantly[78] is that plasma protein interacts with many of the commonly used hemofiltration membranes in such a way as to reduce their openness. More specifically, the polysulfone membrane from Amicon, the polyacrylonitrile (PAN) membrane from Asahi and to a much lesser extent the PAN membrane from Hospal, and the cellulose D-6 membrane from Fresenius all showed an across-the-board reduction in sieving coefficients on exposure of the membrane to plasma protein. The message from these papers is, of course, that an evaluation of membrane transport performance, if carried out *in vitro*, should be conducted with biologically relevant solutions, i.e., plasma or whole blood. A second message relates to a striking difference in the degrees of protein fowling of the membrane that occurs with different membranes. For example, Asahi PAN membrane shows extensive reduction in transport at virtually all tested molecular weights, whereas the PAN membrane from Hospal is far less affected. As to why transport of larger molecules is important, I would point to my long-standing prejudice in this regard.[2] In addition, the identification of a likely link between the acute-phase-reactant β_2 microglobulin (11,800 daltons) and carpal tunnel syndrome[62] adds another reason, in addition to the inferences drawn from the NCDs, for using both more open and more biocompatible membranes.[2]

Schneider and Streicher[79] introduce what appears to be a new and highly promising polysulfone membrane (F-60) (Fresenius) that on initial reporting shows remarkably high clearances for large solutes like inulin (5200) and β_2 microglobulin. At flow rates for blood of 200 ml/min and for dialysate of 500 ml/min with a zero ultrafiltration rate, the clearance values were obtained for blood urea nitrogen 189 ± 5, inulin 85 ± 10, and β_2 microglobulin 56 ± 14 ml/min. Studies by Schmidt *et al.*[80] identify a significant "internal" convective mass transport that occurs for this membrane as it is operated clinically even at zero net ultrafiltration rate, i.e., Starling-like filtration and reabsorption. In a careful study of the pressure (oncotic, osmotic, and hydraulic) gradients across this membrane with its very high hydraulic permeability, they identify an "internal ultrafiltration" in the zero net fluid balance condition that resulted in the addition of 20 ml/min of convective transport under clinically relevant operating conditions. Even backing this convective element out of the 56 ml/min for β_2 microglobulin clearance still leaves a remarkable 36 ml/min value for this 1.25 m^2 hollow fiber membrane. The value is sufficiently high so as to need confirmation by other workers.

An interesting paper by Picca *et al.*[81] compares the respective contributions of sodium balance and blood temperature change on the improved vascular stability noted with hemofiltration in a study population of seven subjects. Each underwent in random sequence a hemofiltration procedure (postdilution with 1.2 m^2 polysulfone membrane, Amicon) in which (1) blood temperature returning to the patient was held "normal" at 36.4–36.7 or cooled by 0.6–0.8°C. This degree of cooling produced clinical chills in the patients. Two sodium balance protocols based on interdialytic sodium intake were employed. The first involved sodium removal during the hemofiltration session equal to that taken aboard dietarily in the prior interval between treatments, but employed a reinfusion fluid sodium concentration higher than plasma water sodium concentration. The second protocol involved removal of sodium during therapy by equating the sodium concentration of the reinfusion solution to that in the ultrafiltrate. They establish that with normal temperature hemofiltration, sodium balance modulates the degree of vascular stability in the expected manner. Cold hemofiltration, however, was preeminent in sustaining vascular stability, with no modulation of mean arterial pressure by the concentration of sodium used in the reinfusion solution. This would place temperature variation higher on the list of factors influencing vascular stability than sodium concentration. The problem remains as to whether temperature change underlies the observed difference in vascular stability between hemodialysis and hemofiltration, even accepting the observation above and the well-known cardiovascular stabilization of hypothermia.

Maggiore *et al.*[82] have extended their study of temperature effects on cardiovascular stability comparing hemofiltration with hemodialysis by distinguishing between net calorie gain or loss across the arteriovenous lines (i.e., equipment gain or loss) and endogenous up or down turn of the body's metabolic thermostat. In a study of 18 patients on hemodialysis, they have shown that even by blocking any calorie transfer to or from the hemodialysis equipment, rectal temperature rises by 0.67 ± 0.4°C (1 SD), a figure outside that expected for the changes of circadian rhythm. Of high interest is the failure to block the temperature rise either by giving aspirin (1 g pre- and 4 mg/min during dialysis i.v.) or using sterile dialysate. These findings point away from this temperature rise being mediated by interleukin-1 or via a path involving the arachidonic acid pathway. Can it be that uremic toxin removal underlies this observation, and if so, by what mechanism?

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Renal Transplantation

Terry B. Strom

1. Introduction

Incredibly high rates of engraftment are now routine in many centers. At the August 1986 meeting of the International Transplant Society, it became obvious that we have entered a new era in clinical transplantation. Unsensitized, i.e., patients with sparse anti-HLA antibody reactivity, recipients of primary cadaver donor renal transplants have a 90%+ chance of retaining their graft for at least 1 year in many centers. The concept that the various immunosuppressive drugs can be used interchangeably is giving rise to a remarkable and newfound therapeutic flexibility. This does not necessarily mean that it is time to discard the staple items. Azathioprine is back in vogue, at least as part of trendy multidrug regimens. Not suprisingly, the best results are obtained in well-matched and transfused recipients. While many transplant surgeons regard HLA typing as an altogether avoidable nuisance, which may threaten to necessitate organ sharing, the continuing value of HLA typing cannot be denied. Some problems persist in regard to the typing effect; the effect is extremely powerful among certain patient populations and weak among other subsets. The transfusion effect may also be weaker than in years past. On the other hand, donor-specific transfusions may be a discard item.

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Scientific advances have enabled a truly molecular understanding of transplant antigens and the rejection process. Finally, new therapies, focused on the cells actually involved in rejection, are supplementing traditional drug-based approaches. The pan-T-cell monoclonal antibody OKT3 is a very effective antirejection agent. A very refined form of monoclonal antibody sharp-shooting, which selectively targets transplant-activated lymphocytes, has been proven to be of value in mouse, rat, and subhuman primate models of organ transplantation. Over the past decade we have witnessed a complete turnabout in transplantation. While the concerns of the past were high mortality and uncertain engraftment, the government is now alarmed by a fall in organ donation and sequestration of ideal transplant candidates in dialysis units.

2. Immunogenetics

2.1. Molecular Biology

The tools of molecular biology are being used to good advantage to explore the molecular organization and the source of antigenic diversity of HLA molecules. As reviewed in the last volume of *Contemporary Nephrology*, class I major histocompatibility complex (MHC) HLA-A,B,C and class II MHC (HLA-DR, -DP, -DQ) glycoproteins have been extensively characterized at the biochemical level.[1,2] Class I molecules are comprised of a polymorphic 45,000-dalton heavy chain that is noncovalently associated with β_2 microglobulin (light chain), a non-MHC gene product. Class II molecules are comprised of a 34,000-dalton heavy chain and a 29,000-dalton β chain. The recent advances in molecular genetics pertain largely to a dissection of the organization of class II molecules. The polymorphisms (antigens) can be detected by serologic (typing sera) and mixed lymphocyte culture (MLC or Dw typing) reactions. Three well-defined subregions have been identified in the HLA-D region and are designated HLA-DR, -DP, and -DQ.[1] Within the DR subregion, one α- and three β-chain genes have been discovered; the α-chain and two of the three β-chain genes, DR β_1 and DR β_2, are expressed.[2] DNA sequence analysis has revealed that DR β, but not α-chain, gene, is highly polymorphic; hence, the antigenic specificities can be traced to the β chains.[1,2] Indeed, DNA sequencing techniques reveal that the variability among DR β_1 molecules is restricted to the region around amino acid 70.[3] Interestingly, three-dimensional modeling of DR structure predicts that this region contains the only α helix within the first domain of the glycoprotein. The DQ region contains two sets of α and β chains, DX and DQ α and β; however, the DX genes may not be expressed.[4] DQ

α-chain genes show intense diversity, but the β chain is polymorphic. The DP subregion also contains two sets of α and β chains. A secondary MLC has been used to define the HLA-DP locus.

Molecular genetic approaches are providing means to more precisely identify HLA-D region polymorphisms (Table I). In the DNA-to-DNA hybridization technique described by Southern,[5] DNA is extracted from tissues and treated in such a manner that single-stranded DNA is bound to filters. Various radiolabeled DNA probes of known sequence are applied to separate samples of this filter paper-bound DNA material. Owing to the complementary, double-stranded nature of DNA, homologous radiolabeled DNA probes, but not nonhomologous probes, will bind to the filter paper-bound DNA sample. Typically, the DNA extract is enzymatically cut into fragments by use of DNA sequence site-specific restriction endonucleases. For example, the enzyme BamH1, which is often used in these analyses, recognizes and cuts at a unique six base-pair sequence of GGHTTC, which is randomly found every 4096 nucleotides. Thus, BamH1-derived DNA fragments will vary in length from individual to individual. These variations in size reflect DNA polymorphisms. Radiolabeled DNA probes can be utilized to identify DNA fragments containing homologous DNA sequences. The variations in the size of the identified fragments are called restriction fragment length polymorphisms (RFLP). Fortunately, homologies between class II α and β chains of a given locus are extensive enough so that a cDNA probe of one DR beta gene, for example, can hybridize to all kindred genes; i.e., a cDNA probe for one DR β-chain gene will bind to any DR β-chain gene. Currently, cDNA probes to all known class I and class II genes are available for use.

While serologic identification of class I molecules is definitive and

Table I. Revised HLA-D Region Nomenclature

Revised locus designation	Previous locus designation	Defined using	Genes within region
HLA-DP	HLA-SB	Primed lymphocyte testing (PLT)	DR alpha 1, DR alpha 2, DR beta 1, DR beta 2
HLA-DQ	HLA-DL(MB,SB)	Serologically	DQ alpha, DQ alpha DX alpha, DX beta
HLA-DR	HLA-DR	Serologically	DR alpha 1, DR alpha 1, DR beta 2, DR beta 2
HLA-DP	HLA-D	Mixed	?

readily performed, application of molecular genetic techniques to class II genes has revealed heretofore unappreciated complexities, as indicated by the identification of new subregions and new genes segregating within these regions. Insofar as class II molecules are constitutively expressed on a minority of cell types while class II genes are, of course, present on virtually all nucleated cells, RFLP typing has some practical advantages over classical typing techniques. Several laboratories have utilized RFLP techniques to first identify and then sequence DNA restriction fragments associated with given HLA-typing designations. This approach enables accurate molecular genotyping. The chains, domains, and even exact amino acid sequences giving rise to HLA-typing specificities have been identified. Moreover, RFLP allows more incisive typing than classical techniques; certain inherited polymorphisms unappreciated by classical techniques can be detected by RFLP.

2.2. Dynamic Expression of HLA Molecules and Graft Rejection

The cellular components of a kidney were not created equal in regard to their ability to sensitize the host and elicit rejection. All major pathways of immune reactivity toward allogeneic tissues, i.e., delayed-hypersensitivity-like phenomena, activation of allospecific cytotoxic cells, and elaboration of high-titer, high-affinity alloantibody, require participation of alloactivated helper T cells. As reviewed in Volume 3 of *Contemporary Nephrology*, activation of alloantigen-specific helper T cells requires stimulation by histoincompatible class II MHC molecules; therefore, the most potent immunogenic cellular components of the graft express class II MHC molecules. Most renal parenchymal cells do not constitutively (in the unstimulated state) express class II MHC molecules. In essence, only two cell types within the graft express class II MHC molecules at the time of transplantation: bone marrow-derived "passenger leukocytes," especially dendritic cells, and some endothelial cells. Clearly, class II-bearing dendritic cells are potent stimulators of the allograft response.[6] Indeed, some maintain that a graft is immunogenic only if it bears dendritic cells.[6–8]Nonetheless, certain endothelial cells express class II antigens[9–11]; endothelial cells stimulate powerful allogeneic responses *in vitro*.[12] The minor controversy as to which class II antigen-bearing cell is the most immunogenic has become somewhat sterile, as it is now apparent that the magnitude of expression of MHC antigens on most cell surfaces is not constant. MHC antigen expression is a dynamic phenomenon dependent on the immune status of the host and milieu of the tissue. Class II-negative macrophages express class II antigens *de novo* following exposure to lymphokines.[13] γ-Interferon was identified as the lymphokine responsible for inducing expression of class II anti-

gens upon macrophages,[14,15] monomyelocytic leukemic,[15] and endothelial cells.[16] It is now clear, as predicted in Volume 3 of *Contemporary Nephrology*, that *de novo* expression of HLA-DR is a hallmark of rejection.[17]

Induction of class II antigens upon endothelial cells in transplanted heart[18] and skin[19] has been noted during rejection. Similarly, in experimental models of bone marrow transplantation, epidermal keratinocytes and intestinal epidermal cells, normally negative for class II antigens, become positive during the graft-versus-host response.[20,21] *De novo* induction of DR molecules has also been observed in renal transplants. During rejection, the renal vascular endothelium and epithelial cells of the proximal and distal tubule express class II antigens *de novo*[17,22]; however, DR induction is a consequence of immune activity and not a specific consequence of rejection. For example, induction of DR upon renal tissue is a usual consequence of CMV infection.[23] Moreover, the degree of immune activation necessary to induce expression of class II antigens may be less than that necessary to cause rejection.[24] Thus, class II antigen induction regularly accompanies, but is not diagnostic of, rejection.

2.3. Clinical Applications

Two very large collaborative studies, the International Collaborative Transplant Study directed by Dr. Gerhard Opelz[25,26] and Terasaki's UCLA Collaborative Study,[27] have thoroughly documented the usefulness of HLA typing in renal transplantation. A powerful effect is exacted upon short- and long-term graft survival; the influence of histocompatibility is most powerful in those receiving repeat transplants.

The recent widespread deployment of cyclosporine has impelled the transplant community to reevaluate the role of HLA typing among recipients receiving this powerful new agent. Several early reports, often from single centers, questioned the value of HLA typing in cyclosporine-treated patients. While these studies cannot be wholly dismissed, the reports often contain a variety of troubling features, such as a small number of subjects, short follow-up periods, and actuarial rather than actual statistical analysis. In contrast, the two large collaborative studies are able to analyze the data generated from thousands of transplants.

An opportunity to evaluate the impact of HLA matching on important patient subsets is enabled by the sheer wealth of these data. These data undermine the concept that HLA typing has outlived its usefulness. Among primary allograft recipients, Opelz[25,26] demonstrated that HLA-DR and B locus typing exerts a very powerful effect among patients treated with or without cyclosporine in a study that analyzes

data from over 200 centers. A group of patients with 0 mismatches for HLA-B and -DR had a 1-year graft survival of 86 ± 3%, a far higher rate of survival than observed in the European[28] or Canadian[29,30] multicenter cyclosporine trials. In striking contrast, a 67% ± 4% 1-year graft survival rate was noted in patients receiving HLA-B and -DR totally mismatched grafts[25,26] ($p < 0.0001$). A gratifying dose–response effect for each matched antigen was noted for grafts with implanted intermediate histocompatibility scores.[25,26] The improvement of ~20% in the success rate of HLA-B and -DR matched grafts as compared to HLA-B and -DR mismatched grafts was also demonstrated in patients not receiving cyclosporine (75 ± 2% versus 57 ± 3%; $p < 0.0001$).[25,26] The most recent data from Opelz (June 1986 *Newsletter*) uphold the strong influence of HLA-B and -DR typing in primary graft recipients, but also note that the typing effect is strongest in patients presensitized to >50% of a large random panel of individuals.

The other major collaborative study, the UCLA Study, also shows a similar additive effect for matching individual antigens in cyclosporine-treated patients.[27] This study corroborates, in a North American patient population encompassing over 100 centers and 5000 patients, the additive effects of HLA-B and -DR typing, but also demonstrates the additional benefits derived from HLA-A locus typing. This study also demonstrated a 67% 1-year graft survival in cyclosporine-treated recipients, with six HLA-A, -B, -DR mismatches, while an incredible 93% 1-year graft survival was detected in patients with 0 mismatches.[27]

As the clinical experience with cyclosporine mounts, the rate of 1-year engraftment with this drug is improving among recipients of primary cadaver donor grafts. As the learning curve is extended, results improve yearly.[25–27] It is notable that the effect of cyclosporine on repeat transplants is not improving, over time, at the same rate as noted in primary grafts.[25,26] Consequently, it is especially important to note that HLA matching is more important in the difficult circumstance of a repeat transplant that in primary grafts.[25–27] While these effects were first noted in azathioprine-treated patients studied by both large collaborative studies, new data from Opelz[25,26] demonstrate that the HLA typing effect is magnified in repeat cadaver grafts.

Also noteworthy is the compilation by Krakauer[31] of the data available through the large ESRD registry at the Health Care Financing Administration in which the benefits of HLA typing are amply demonstrable in American cyclosporine-treated cadaver graft recipients. Only the Scandinavian Multicentre Trial,[32] which analyzed DR matching in 139 patients with just 6 months actuarial cadaver survival rates, showed a 77% success rate in 71 patients with 0 DR mismatches and a 69%

success rate in 46 patients with 1 DR mismatch; an 82% success rate in 22 patients with 2 DR mismatches fails to find a typing effect.

The long-term effects of tissue matching on graft survival in cyclosporine-treated patients have not been well studied; however, both Dausset[33] and Festenstein *et al.*[34] have demonstrated that the beneficial effects of HLA typing become magnified in azathioprine-treated hosts with time. Hence, relatively small, but significant, benefits observed 1 year posttransplantation become remarkably powerful when analyzed 5–8 years postgrafting.

The generally hapless situation of the highly presensitized transplant candidate improved, with the demonstration that, in many cases, a donor organ could be successfully engrafted despite a strongly positive cross-match with past serum if the current serum lacked donor T-cell-specific cytotoxic antibodies.[35] The possibility that sensitization engendered by an allograft is more deleterious than sensitization owing to a transfusion was suggested by a recent survey of the American Society for Histocompatibility and Immunogenetics.[36] Although the concept that past positive–current negative cross-matches do not mitigate strongly against a successful transplant when the "past and present" cross-match circumstances relate to a previous rejected transplant, successful retransplantation occurred in only 6 to 15 cases.[36]

3. Immunosuppression

3.1. Cyclosporine

3.1.1. Mechanism of Action

The mechanism by which cyclosporine blocks T-cell proliferation has been extensively studied. Cyclosporine is not a lymphocidal agent; nonetheless, cyclosporine blocks T-cell proliferation at a step that precedes DNA synthesis (reviewed in Refs. 37,38). The minimum requirements for T-cell activation include dual activation of the T3–T cell for antigen complex (T3–TCR) and activation by the monokine interleukin-1.[39] Following this dual activation, the T-cell growth factor interleukin-2 is released and interleukin-2 receptors are synthesized.[39] Cyclosporine has been previously shown to block interleukin-2 production but preserves the ability of previously activated T-cells to proliferate in the presence of interleukin-2 (reviewed in Refs. 37,38). T-cell proliferation is believed to be preceded by hydrolysis of membrane inositol phospholipids, resulting in a rise in cytosolic Ca^{2+} and activation of protein kinase C.[40] However, cyclosporine does not block mitogen-stimulation, phos-

phoinositide breakdown, or Ca^{2+} mobilization in T or B cells.[41,42] Nonetheless, cyclosporine may block activation of another second messenger system related to activation of the interleukin-2 gene because cyclosporine has been demonstrated to block elaboration of the interleukin-2 encoding messenger RNA.[43–47] In the presence of cyclosporine, activated T lymphocytes fail to transcribe messenger RNA for interleukin-2 and several other lymphokines.[43–47] While activation of several lymphokine genes is blocked by cyclosporine, the drug does not block all elements of T-cell activation. Indeed, the drug is not an antimetabolite because many species of mRNA stimulated *de novo* by T-cell activation, such as the interleukin-2 receptor gene, are not blocked by cyclosporine.[43–45] Insofar as both antigen activation and interleukin-1 are required for T-cell activation,[39] and insofar as we[49] believe that activation of the T3–TCR complex is linked to inositol phospholipid hydrolysis and a sharp rise in cytosolic calcium, I hypothesize that cyclosporine interrupts a second messenger activated by macrophages or interleukin-1. Cyclosporine has been reported to bind to calmodulin.[49] Nonetheless, it is difficult to reconcile this observation with cyclosporine's target cell specificity for lymphocytes, since calmodulin is ubiquitous in eukaryotic cells and the lymphocyte calmodulin protein is not distinctive.

3.1.2. Clinical Applications

Over the past several years, cyclosporine has become the linchpin of immunosuppressive antirejection protocols. The prior claims of potent immunosuppressive efficacy and warnings related to the drug's dreadful nephrotoxicity have proven correct. The results of 11 controlled studies carried out worldwide are shown in Table II. The mean improvement in graft survival is 15% better in patients receiving cyclosporine than in patients given azathioprine (Table II). There is no doubt that the use of maintenance cyclosporine plus corticosteroids yields a superior rate of engraftment at 1–3 years than azathioprine plus prednisone, although patients in the cyclosporine group have evidence of nephrotoxicity. Nonetheless, there is no consensus that the cyclosporine-plus-corticosteroids regimen is the best long and short solution toward achieving optimal rates of engraftment or avoiding long-term nephrotoxicity. Before I attempt to review some of the pertinent (although already outdated) data, let me simply state my unproven beliefs relating to the use of cyclosporine-based regimens.

First, I believe that many of the principles of cancer chemotherapy may be usefully applied to immunosuppressive protocols. At our disposal are three drugs, i.e., corticosteroids, whose major therapeutic target is the macrophage (reviewed in Ref. 59); cyclosporine, whose major target

Table II. Results of 11 Studies with Cyclosporine[a]

Study	No. of CsA-treated	Addition of steroids/ALG in control population	Pt. survival (1 year)		Graft survival (1 year)		Randomized study	Reference	Comments
			CsA (%)	AZA (%)	CsA (%)	AZA (%)			
European multicenter trial, 1983	117	− ±	94	92.2	72[b]	52	+	28	
Canadian multicenter trial, 1984	142	+ ±	No data		78[b]	69	+	29,30	
Pittsburgh, 1983	191	+ −	91	85	81	50	−	50	
Hannover, 1985	169	+ −	96	92	80	63	−	51	
Munich (2nd series), 1984	205	+ −	97	93	80	50	−	52	
Houston, 1984	103	+ −	96	83	81	50	+	53	
Australia, 1985	34	− +			72	75	−	54	
Minneapolis, 1985	9	+ +	92	95	87	80	+	55,56	Includes LRDs, diabetics, etc.
Japanese trial, 1985	28	+ −	97	94	93	77	−	57	Includes LRDs, diabetics, etc.
Cambridge, 1984	7	− −	88	76	77	62	−	58	
Oxford, 1985	6	− −	92	95	73[b]	58	+	59	

[a] CsA, cyclosporine; AZA, azathioprine; ALG, antilymphocyte globulin; LRD, living related donors.
[b] Statistically significant difference.

is lymphokine-secreting T cells (reviewed in Ref. 38,60); and azathioprine, an antimetabolite (reviewed in Ref. 60), as well as biologic immunosuppressives, including polyclonal and monoclonal antilymphocyte antibodies. Each of these agents has a distinct mode of action and side effects. Oncologists long ago learned the advantages of piecing together regimens in which diverse and effective, but not thoroughly salubrious, agents are used in multidrug protocols. Each drug is administered in reduced dosage—as compared to single-drug regimens—thereby greatly reducing toxicity. The potential benefits of using cyclosporine, a dose-dependent nephrotoxin, in reduced doses are obvious. We do not have an effective tool by which toxic doses can be avoided inasmuch as circulating drug levels of this extremely hydrophobic drug do not adequately reflect therapeutic or toxic drug levels. Moreover, combination therapy using azathioprine plus cyclosporine plus corticosteroids may add to effectiveness. While cyclosporine and prednisone are very effective in unsensitized, primary-graft recipients, sensitized patients remain at high risk to reject a graft.[25–27] Hence, we now use "triple therapy" in all high-risk situations.

Another explicit principle of cancer chemotherapy, but often unstated principle of immunosuppression, relates to differences in "induction" and "maintenance" requirements. It does not take as much immunosuppression to prevent rejection 1 year following transplantation as was required 1 week following transplantation. While most attempts to rapidly switch from cyclosporine have proven troublesome,[61–63] it may be quite safe to delete cyclosporine from so-called triple-drug, i.e., cyclosporine, azathioprine, and prednisone, at 1 year post-transplantation. In short, I believe that multidrug, flexible protocols can be tailored for many patients, especially patients at high risk to reject a graft, that will add to immunosuppressive effectiveness without creating overwhelming immunosuppression. It is possible, but not altogether certain, that these triple-therapy protocols will also be useful in patients at lower risk to reject a graft because these multidrug, low-dose cyclosporine protocols may be an efficient means to obviate cyclosporine's nephrotoxic effects. Indeed, our initial experiences support this prejudice.

Follow-up analysis of the European[64] and Canadian[30] controlled, multicenter studies show that the superiority of cyclosporine-based over conventional regimens is maintained throughout 3 years of follow-up. The enormous database available to Opelz and his co-workers,[25,26] Terasaki and colleagues,[27] and H.C.F.A.[31] give further validity to the concept that cyclosporine-based regimens are superior, at least after 1 year of follow-up, to conventional regimens. Analysis of these very large databases has indicated an advantage for cyclosporine-based regimens in regard to the rate of engraftment at 1 year following transplantation.

To cite the absolute numeric superiority in the 1-year rate of engraftment of cyclosporine-based protocols (10–20%) may understate the advantages of the drug in our present circumstances. Krakauer[31] has demonstrated that the American primary cadaver graft recipients given conventional regimens have a 65% rate of engraftment at 1 year while similar patients treated with cyclosporine have a 74% rate of engraftment; however, in the centers that gained experience with the drug in the prelicensing era, the rate of engraftment for such patients was 82%. Krakauer's analysis[31] also reveals that cyclosporine treatment obviates some, if not all, of the excess risk factors for graft failure formerly noted in diabetic, black, and older recipients. As noted previously, each of these three very large studies shows that cyclosporine does not, however, obviate the advantages of good tissue matching or pretransplant transfusions. While the transfusion effect is lessened with use of cyclosporine,[26] there is absolutely no lessening of the effect of good HLA matching in patients given cyclosporine-based regimens taken as a group. Indeed, cyclosporine-treated first cadaver graft recipients given HLA-A,B,DR matched grafts have an astounding 93% rate of engraftment at 1 year, while totally mismatched recipients have a 67% rate of engraftments.[27] Furthermore, Terasaki estimates that a national network of organ sharing would enable 20% of recipients to receive phenotypic HLA identical grafts.

It is regrettable that the excellent rate (by yesterday's standards) of engraftment enjoyed by many units since the licensing of cyclosporine has prompted a marked decrease in organ sharing. A frequent justification for the decrease in organ sharing is the published findings of Canadian Multicentre Study[29] in which long perfusion (cold ischemia) times were correlated with an abrogation of the advantages of cyclosporine over conventional therapy. These data gave rise to the view that it was imperative to transplant a graft so quickly as to essentially preclude organ sharing. Subsequently, evaluation of these data revealed that prolonged intraoperative, warm ischemia times, and not long cold ischemia, are correlated with an abrogation of the cyclosporine effect. Recent studies[65,66] reveal that cyclosporine use tends to prolong periods of initial anuria, but the overall incidence and eventual outcome of such bouts of anuria are ultimately unaltered by cyclosporine use. The collaborative transplant study[25,26] headed by Opelz has now also demonstrated that prolonged cold ischemia does not adversely affect survival rates in cyclosporine-treated patients. When the advantages and disadvantages of allocating kidneys by tissue-typing criteria, and hence delaying engraftment, are weighed, an advantage is seen for grafting well-matched kidneys preserved for up to 48 hr by cold Collin's solution preservation (Collaborative Transplant Study, November 1985 *Newsletter*).

The difficulties encountered in patients with initial anuria are being approached in some centers by total avoidance of cyclosporine during the period of anuria. Especially impressive are the results obtained in several units in which polyclonal antilymphocyte antibodies are administered; sometimes with azathioprine; cyclosporine is initially withheld until a diuresis ensues.[67–70] Hospitalizations are shortened; prolonged dialytic requirements are obviated. The rate of engraftment in these uncontrolled studies is also unusually high. The "quadruple-therapy" approach is interesting and warrants rigorous testing.

While avoidance of cyclosporine during brief early periods of anuria may prove a practical resolution to the early nephrotoxicity, manifestations of chronic cyclosporine-induced nephrotoxicity remain a difficult problem. While repetitive and very expensive circulating drug measurements have been heralded by some as an adequate means to avoid toxicity,[71] many have found that circulating drug levels do not adequately discriminate between rejection and drug toxicity.[63,72–75] In my view, almost all patients taking doses of 6–8 mg/kg per day of cyclosporine experience drug toxicity. Very high drug levels can be used as an "excuse" to hasten the drug taper, but there is no evidence that drug level measurements accurately discriminate between toxicity and rejection.[72–75] It is often difficult to discriminate between rejection and nephrotoxicity. The classical signs of rejection, i.e., fever, graft pain, and renomegaly, are often absent in cyclosporine-treated patients undergoing rejection. Hypertension may be present in either rejection or nephrotoxicity, but the failure to detect rises in blood pressure, in my experience, mitigates against rejection as the diagnosis. A very rapid deterioration in glomerular filtration is far more likely to be due to rejection than toxicity. Another fundamental problem exists, however, in making neat distinctions between rejection and nephrotoxicity; the two entities can coexist.

Cyclosporine blood levels do tend to be higher in patients experiencing nephrotoxicity than rejection; however, the overlap in drug levels between the patients experiencing nephrotoxicity and rejection is substantial.[73–76] Hence, in an individual case, the drug level is not of diagnostic value.

Owing to the inability of noninvasive studies to yield a precise diagnosis of cyclosporine nephrotoxicity, we and others frequently obtain a renal biopsy to aid in making a diagnosis. The primary goal in analyzing renal morphology is establishing or refuting a diagnosis of allograft rejection. Inasmuch as cyclosporine nephrotoxicity is not associated with an interstitial nephritis (reviewed in Ref. 38,72), a dense and diffuse mononuclear leukocytic cellular infiltrate is characteristic of rejection.[38,72,73,77,78] Within the infiltrate, activated interleukin-2 receptor-

positive lymphocytes and procoagulant-positive activated macrophages are plentiful.[78] In addition to the mononuclear leukocytic infiltration of the graft interstitium, cellular rejection is characterized by edema, hemorrhage, and vascular endothelial-cell proliferation,[73] infiltration of the arterial wall,[73,77] and glomerular infiltration primarily by T4+ T cells.[77] Does cyclosporine nephrotoxicity give rise to a distinctive morphologic pattern? In the most florid cases, protein deposits permeate the arteriolar wall, narrowing the vascular lumen.[79] Electron microscopy reveals that the protein deposits replace necrotic myocytes. These lesions have been seen primarily in patients receiving the very high-dose, intensive cyclosporine regimens that have not been abandoned. Hence, this distinctive lesion is now rather uncommon. As a consequence, while the diagnosis of rejection has a morphologic basis, cyclosporine nephrotoxicity is a diagnosis of exclusion.

In very toxic patients taking high-dose cyclosporine[80] and in patients receiving polyclonal antilymphocyte antibodies plus lower doses of cyclosporine,[81] glomerular capillary thrombi are seen in concert with endothelial swelling. The pattern is reminiscent of the hemolytic–uremic syndrome. While the etiology of cyclosporine nephrotoxicity is uncertain, these morphologic observations suggest that the endothelium, rather than the renal parenchyma, is the target of the toxic drug effects. Neild *et al.*[80] suggest that abnormalities in arachadonic acid metabolism indirectly leading to an inability to elaborate prostacyclin may underlie these morphologic alterations. Defective prostacyclin synthesis would result in endothelial cell damage, intraglomerular thrombosis, and renal ischemia.[81a,82] The prominent interstitial fibrosis seen with chronic toxicity[76,83] may result from ischemia.

The recent development of fine-needle aspiration biopsy (FNAB) by Hayry and von Willebrand[74,84] in providing a cytologic methodology for monitoring renal transplants appears promising. This entirely safe means of transplant monitoring may be best suited for monitoring during the first month to 6 weeks posttransplantation. I suggest a cutoff at about 1 month because of the inability to examine intact vascular surfaces; insofar as rejection-mediated humoral injury becomes an ever-increasing problem with time, FNAB may find its greatest utility in aiding with the difficult differential diagnosis of early acute renal failure versus rejection versus nephrotoxicity versus various combinations of the above. Many laboratories[85–87] have now corroborated the findings of Hayry and von Willebrand that rejection can be accurately diagnosed by FNAB as manifested by an increasing accumulation of inflammatory cells, lymphoblasts followed by macrophages, that appear in the aspirate. Hence, FNAB and renal biopsy are able to detect cellular rejection. Obviously, this is the most urgent diagnosis to identify in the differential diagnosis

of nephrotoxicity versus rejection. Hayry and von Willebrand[74,84] claim that acute tubular necrosis is characterized by the appearance of swollen tubular cells in the aspirate while an "isometric" vasculation of tubular and endothelial cells accompanies cyclosporine nephrotoxicity. Criteria for the adequacy of FNAB samples have been established and agreed upon by a large number of workers. These criteria include ≥ 7 parenchymal cells/100 inflammatory cells and ≥ 0.25 tubular cells/hpf (400). This technique is widely practiced in continental Europe but has not received sufficient attention in the United States. Indeed, I have not been able to convince my own pathologists to jointly pursue this technique.

3.2. Anti-T-Cell Monoclonal Antibodies

3.2.1. OKT3

3.2.1.1. The T3–T-Cell Receptor Complex. Thymus-derived lymphocytes play a quintessential role in the rejection of organ transplants. As a consequence, there is considerable interest in utilizing agents that specifically target T cells for destruction or inactivation. As the T-cell receptor (TCR) for antigen protein is uniquely expressed on mature T cells, the TCR is an interesting target for immunotherapy. Over the past several years, the structure and genetic organization of the TCR have been elucidated.[88,89] The heterodimeric TCR protein is physically linked with a series of proteins termed the T3 complex on the T-cell membrane.[89–93] The TCR–T3 complex is comprised of at least five different polypeptide chains: the clone-specific (clonotypic) alpha and beta chains serve as the T-cell antigen receptor, while the three invariant proteins of the T3 complex, designated gamma, delta, and epsilon, appear to play a role in transduction of activating signals emitted from the TCR to the cell interior.[40,94] These proteins are inscribed exclusively on the surface of mature T cells. The antigen receptor is a sulfhydryl-linked heterodimer consisting of the glycosylated alpha and beta chains and ranging in molecular mass from 32 to 50 kD (reviewed in Ref. 40). The genes encoding the alpha and beta chains bear considerable homology to human immunoglobulin genes (reviewed in Ref. 95). Constant, variable, diversity, and joining segments are present as in the case of immunoglobulin genes.[95] Thus, the genetic diversity for TCR, enabling a vast repertoire of antigen-binding proteins, is assembled through somatic gene rearrangements ("gene shuffling") for the separate genes encoding variable, diversity, and joining segments. These gene segments are drawn from gene pools that are large and diverse. Hence, the extraordinary

demands for distinct receptor proteins able to meet the needs for a multiplicity of foreign antigens is enabled by the recombinatorial capacity of these distinct gene segments. Formal evidence demonstrating the necessity of the TCR alpha and beta chains in antigen binding has been achieved. Fusion of complementing pairs of nonfunctional mutants which have lost TCR alpha or beta chains to antigen-specific cloned T cells (T-cell hybridomas) gives rise to T-cell populations with restored antigen-binding function.[88] Similarly, transfection of alpha- and beta-chain genes from one cytotoxic T-cell clone to another confers the antigen-binding properties of the gene donor to the cells receiving the TCR genes.[96]

The three T3 polypeptide chains have molecular weights of 25 kD (T3-gamma chain) and 20 kD (T3-delta and -epsilon chains).[40,94] The T3-gamma and T3-delta chains are glycoproteins, whereas the T3-epsilon chain does not contain any detectable oligosaccharide. Several monoclonal, e.g., OKT3, Leu-4, 64.1, and UCHT-1, antibodies reacting with the human T-cell-specific T3 antigen complex define one of the 20-kD T3 components. A physical interaction of T3 with TCR was initially suggested by the finding that incubation of T cells with antibodies directed against either structure causes disappearance ("modulation") of both TCR and T3 as well as a loss of antigen-specific functions.[90–92] When anti-T3 antibodies are removed from the culture, concomitant reexpression of TCR, T3, and antigen-specific functions is noted within 48 hr. Recent data have formally demonstrated that the proteins are physically linked on the cell surface.[93] and that antibody-induced modulation of TCR–T3 results in translocation of TCR and T3 as well as antibody into multivesicular bodies which eventually fuse with lysozomes.[40]

A study of T-cell-leukemia mutants indicates that the chains of the TCR–T3 complex obey "one for all and all for one" rules because cell surface expression of the TCR alpha and beta proteins and T3-gamma, -delta, and -epsilon chains requires the presence of all five components. For example, in the event that messenger RNA for one of the five chains is lacking, the TCR–T3 complex, in its entirety, is not expressed on the cell surface.[40,97] A similar circumstance pertains to the intrathymic differentiation from premature to immunocompetent T cells. The earliest pre-T cells contain messenger RNA for the T3-delta and -epsilon chains, but the delta and epsilon proteins accumulate in a perinuclear distribution.[98] Surface expression of any component of the TCR–T3 complex awaits until the mature T-cell differentiated state appears in which messenger RNA for each of the TCR–T3 components is transcribed.

3.2.1.2. OKT3: Therapeutic Effects. As outlined in Volume 3 of *Contemporary Nephrology*, several murine anti-human-T-cell monoclonal antibodies have been utilized to abort rejection episodes in renal allograft

recipients. The long-awaited introduction of anti-T-cell monoclonal antibodies follows demonstrations that polyclonal antilymphocyte globulin is more effective than corticosteroids in aborting acute rejection episodes in renal allograft recipients.[99,100] If polyclonal antibodies are effective, why is the transplant community so interested in monoclonal antibodies? First, polyclonal antibodies are "unnecessarily" toxic because many side effects result from inclusion of antilymphocyte antibodies that "cross-react" with determinants expressed on nonlymphoid tissues. Indeed, T-cell- or lymphocyte-specific antibodies constitute a minority of the antibody specificities included in polyclonal antilymphocyte globulin preparations. As a consequence, granulocytopenia and thrombocytopenia as well as serum sickness are common side effects observed in patients treated with antilymphocyte globulin. Obviously, truly T-cell-specific or lymphocyte-specific monoclonal antibodies can completely obviate the problems of granulocytopenia and thrombocytopenia if not serum sickness. Even the difficulties associated with serum sickness can be diminished by relying on monoclonal rather than polyclonal antibody preparations. Because polyclonal preparations contain many antibodies that cross-react with nonlymphoid cells, a large quantity of antibody must be given in order to target lymphoid cells with a therapeutic concentration of antibody because nonlymphoid cells tie up antibody intended to be targeted to lymphocytes. In other words, the therapeutic effect on lymphocytes of a given quantity of antibody protein should be higher with a lymphocyte-specific monoclonal antibody than an antibody that binds to many tissues, including the lymphocyte. Thus, large quantities of antilymphocyte globulin (up to 30 mg/kg per dose) are routinely administered.

While both polyclonal and monoclonal preparations are comprised of heterologous proteins, the likelihood that therapeutic doses of protein in monoclonal preparations will be less than in polyclonal preparations has raised expectations that serum sickness—a major side effect with polyclonal preparations—may prove less vexing with monoclonal antibodies. In this context of hope and anticipation, two "pan-T-cell" monoclonal antibodies, OKT3[101,102] and anti-T12,[103] and an antilymphoblast monoclonal antibody directed against activated T cells[104] have been tested for their ability to reverse rejection episodes. While OKT3 and anti-T12 are T-cell-specific antibodies, the antiblast antibody that reacts with activated, by not resting, T cells also reacts with many nonlymphoid tissues. Each antibody can reverse rejection episodes, but only OKT3 appears therapeutically superior to conventional high-dose corticosteroids.[102] In this randomized prospective trial, 123 patients receiving conventional azathioprine and corticosteroid immunosuppression and sustaining their first rejection episode were studied. In the experimental group, 63 pa-

tients received 5 mg OKT3/i.v. for a mean period of 14 days during which azathioprine and prednisone dosages were lowered to 25 mg and 0.5 mg/kg per day, respectively. In order to reduce the severity of somewhat unexpected side effects occurring with the first dose of OKT3, a bolus injection of 1 mg/kg of methylprednisone plus 650 mg acetominophen and antihistamines was also given with the first dose of OKT3. Sixty control patients received methylprednisolone, 500 mg/i.v. per day × 3 followed by an increased dose of p.o. prednisone, if needed. In the OKT3 group, resistant rejection episodes were treated with high-dose corticosteroids, while such episodes occurring in the control group were treated with equine antimonocyte globulin.

Overall, the OKT3 group fared better than the conventionally treated control group. OKT3 reversed a remarkable 94% of rejections, while 75% were reversed in the control group ($p = 0.009$). One-year graft survival in the control group was 45%, as compared to 62% in the OKT3 group ($p = 0.029$). Every patient experienced a rejection episode, so overall graft survival was not outstanding. The rate of infections, minor and severe, was equivalent in the two groups. Hence, increased therapeutic efficiency against rejection was not accompanied by a gross increase in opportunistic infection. Nonetheless, a high rate of infection was noted in both groups: 68% of the OKT3 group and 65% of the steroid-treated group. One factor contributing to the high rate of infection was the need for repetitive courses of high-dose antirejection therapy, as rebound rejection episodes occurred in 66% of the OKT3 group and 73% of the steroid-treated group. One-year patient survival was 85% in the OKT3 group and 90% in the control group ($p = 0.47$). Eighty percent of OKT3-treated patients developed antibodies to OKT3, although anaphylactic reactions were rare.

An interesting pattern of adverse side effects was noted in the OKT3 group.[102] The first and, to a lesser extent, second injections of OKT3 were associated with a symptom complex that did not occur with subsequent injections. This typically commenced 45–60 min after the first injection of OKT3 and lasted for several hours. It involved pyrexia (73%), chills (57%), tremor (10%), dyspnea (21%), chest pain and tightness (14%), wheezing (11%), nausea (11%), and vomiting (13%). One patient developed pulmonary edema. Obviously, a pattern of side effects occurring with the initial doses but not occurring with subsequent doses cannot be due to the host immune response against this murine antibody. In the pilot study, four additional cases of pulmonary edema were noted. Most often these bouts of pulmonary edema occurred in patients with hypervolemia; however, OKT3 abruptly precipitated pulmonary edema. It is interesting that this pattern of serious early or more benign late side effects was not noted in the anti-T12 trial[103]; however, it is also

apparent that OKT3 is a far more potent immunosuppressive agent than anti-T12. It is my belief that while both antibodies target essentially the same population of mature T cells, the superior efficacy of OKT3 and curious side effects occurring with its administration can be linked to the specificity of OKT3 for the T3–TCR complex on the T-cell membrane. While the target molecule defined by anti-T12 is not linked to the TCR complex, OKT3 targets the most important set of proteins on the T-cell membrane. The authors attributed, probably correctly, the adverse side effects occurring promptly following OKT3 to an instant release of lymphokines released from T cells after binding by OKT3.

Clearly, the long-term effects of OKT3 *in vitro* are immunosuppressive; however, as noted in the foregoing discussion of the T3–TCR complex, OKT3 can, in the presence of macrophages or interleukin-1, mimic the effects of antigen activation.[39,40] OKT3 is a polyclonal T-cell activator. It is not surprising that the untoward effects noted following administration bear a striking similarity to the side effects noted by Rosenberg and his co-workers[105] in their attempts to treat cancer patients with infusions of activated lymphocytes and interleukin-2. Indeed, the side effects were directly attributed to the infusions of interleukin-2.

Why is OKT3 more effective than anti-T12 in reversing rejection episodes? Both antibodies target essentially the same population of immunocompetent T cells. OKT3 is an IgG2a while anti-T12 is an IgM. Antibodies of these subclasses are excellent in activating mouse complement; however, neither antibody is able to effectively lyse human T cells in the presence of human complement. Despite the inability of these antibodies to support complement-dependent lysis, T cells are cleared from the circulation during therapy. This clearance of T cells from the circulation is almost certainly attributable to the capacity of phagocytes T within the reticuloendothelial system to opsonize, i.e., ingest, antibody-coated cells. Hence, it is unlikely that a difference in the target cell population or antibody class is responsible for the different clinical effects. It is likely that the function of target proteins, i.e., T3 and T12, defined by the antibodies is responsible for the disparate clinical effects. While the T12 protein is not essential to T-cell function, the T3 protein is linked, both physically and functionally, to the TCR for antigen. Anti-T3 monoclonal antibodies cause cocapping (disappearance) of the TCR for antigen and the T3 protein from the membrane both *in vitro*[90–93] and *in vivo*.[106] Hence, during the period of OKT3 therapy, the T cells are literally blindfolded as to the presence of antigen. In brief, T-cell function is initially stimulated by OKT3, leading to an outpouring of lymphokines and symptoms following promptly upon drug administration. Subsequently, T cells are removed from the circulation, and the

T3–TCR complex is modulated from the cell surface by OKT3. Profound immunosuppression ensues.

Unfortunately, OKT3 can be administered for only one course of treatment, as 75–80% of OKT3-treated hosts make antibodies against OKT3.[103,107] Both IgM and IgG antibodies are elaborated. A remarkable portion (~60%) of the anti-OKT3 antibodies recognize the variable, antigen-binding, i.e., idiotypic, portions of OKT3. Indeed, the antiidiotypic antibodies impair OKT3-mediated immunosuppression far more profoundly than antibodies directed against the constant regions of OKT3. It should be possible to determine whether antiidiotypic antibodies against OKT3 cross-react with the idiotypic structures of other anti-T3 monoclonal antibodies (there are many). If the idiotypes of these antibodies differ from OKT3, a second course of anti-T3 therapy may be possible using a second, idiotypically disparate anti-T3 monoclonal antibody.

3.3. Anti-Interleukin-2 Receptor Monoclonal Antibody Therapy

3.3.1. Rationale

An ideal antirejection therapy should be effective in controlling rejection as well as selectively targeting only those T cells that are committed to participate in rejection of the donor graft. Conventional immunosuppressive drugs exact unwanted side effects on nonlymphoid tissues. The introduction of monoclonal antibodies as pharmacologic tools has long been awaited, as therapeutic use of T-cell-specific monoclonal antibodies can obviate these side effects by providing new opportunities for a more targeted form of immunosuppressive therapy. Nonetheless, the pan-T-cell antibodies, used with considerable success in transplantation, react with all T cells, while an ideal therapy would target only those lymphocytes committed to the unwanted immune reaction.

The immune response to a vascularized allograft is a complex T-cell-dependent response. In theory, a perfect therapeutic solution would be obtained by developing antibodies that react with the antigen-combining site of TCR for antigens on the donor graft. This approach has been confounded at least temporarily by the incredible genetic diversity of transplantation antigens and the vast genetic repertoire encoding for the T-cell-antigen receptor.

Our simpler approach is based on the knowledge that activated T cells express a variety of plasma membrane receptors that are absent from the surface of resting T cells, e.g., receptors for interleukin 2 (IL-2),[108–110] insulin,[111,112] and transferrin.[113] *De novo* acquisition of mem-

brane receptors for IL-2 marks a critical event in the course of T-cell activation.[108–110] The induction of IL-2 receptors on T cells is activation dependent.[108–110,114] Interaction of IL-2 with IL-2-receptor-bearing cells initiates a cellular program that is prerequisite for clonal expansion and continued viability of most, if not all, activated T cells.[115,116]

3.3.2. Immunosuppressive Therapy with M7/20 Anti-IL-2 Receptor Antibody

We have characterized M7/20, a rat antimouse IL-2 receptor monoclonal antibody (MAb) which defines a *N*-glycosylated 58-kD glycoprotein expressed on activated, but not resting, T cells, blocks IL-2 mediated growth, and inhibits binding of the IL-2 to its cellular receptor.[117]

We have examined the effect of administration of M7/20, and anti-IL-2 receptor antibody, on allograft rejection in mice.[118,119] Inbred male mice, C57Bl/10, B10.BR, and B10.AKM, were used, as these strains are completely mismatched for the H-2 locus. Vascularized, heterotopic heart or full-thickness tail skin allografts were performed.

3.3.2.1. Effect of M7/20 Treatment on Murine Cardiac Allografts. Untreated B10.AKM recipients of C57BL/10 heart allografts rejected their grafts with a median survival of 8 days (Table III).[118,119] In contrast, intraperitoneal injection with M7/20 MAb at a dose of 5 μg/mouse per day for 10 days caused indefinite survival (>90 days) of four of six grafts, with two rejecting at 20 and 31 days, a highly significant prolongation ($p < 0.01$).

To confirm that these results were related to the specificity of M7/20 for IL-2 receptor bearing cells, a control group of recipients was treated with RA3-2C2, a rat MAb of the same class as M7/20, which binds pre-

Table III. The Effect of M7/20 on Survival of Murine Heart Allografts

Recipient	Donor	Treatment	Allograft survival (days)
B10.AKM	C57B1/10	None	8,8,8,8,16,29
B10.AKM	C57B1/10	M7/20	20,31,>90,>90,>90,>90
B10.AKM	C57B1/10	RA3-2C2[a]	6,9,9,10,>90
C57B1/10	B10.BR	None	9,10,10,10,14,16,20,20
C57B1/10	B10.BR	M7/20[a]	20,27,34,38,>60,>60
C57B1/10	B10.BR	M7/20, day 3[b]	11,15,17,18,47,>60,>60,>60
C57B1/10	B10.BR	M7/20 day 6[c]	7,17,19,27[d],27[d],58,>60,>60

[a] 5 μg i.p. daily for 10 days.
[b] 5 μg i.p. daily beginning day 3.
[c] 5 μg i.p. daily for 10 days beginning day 6.
[d] Died of anesthetic complication with functioning allograft.

B cells but not T cells. The survival times of RA3-2C2-treated hosts were not different from those of the untreated controls, but were significantly shorter in animals treated with M7/20 ($p < 0.50$).

The remarkable effects of M7/20 treatment were not unique to one strain combination. A second set of experiments was performed using C5BL/10 recipients of B10.BR heart grafts. Untreated control recipients rejected their grafts at 10–20 days; treatment with M7/20 prolonged survival to 20, 27, 34, and 38 days, with two grafts still functioning at >60 days ($p < 0.01$) (Table III).

The effect of M7/20 on graft rejection was analyzed histologically in C57B1/10 recipients of B10.BR heart allografts. By 3 days posttransplantation, control grafts were heavily infiltrated by mononuclear cells. Treatment with M7/20 prevented this graft infiltration.[118,119] The experiments demonstrate the utility of M7/20 treatment in preventing graft rejection. The efficacy of M7/20 in reversing established rejection was then examined in C57B1/10 recipients of B10.BR allografts (Table III). In eight animals the onset of treatment was delayed until day 3, by which time rejection was ongoing, and continued through day 12. Five grafts were rejected on days 11, 15, 17, 18, and 47, while three were still functioning at >60 days. When treatment was given on days 6–15, four grafts were rejected at 7, 17, 19, and 58 days, while two were still functioning at >60 days. Two additional grafts were still functioning at 27 days, when the animals succumbed to an anesthetic overdose while being bled. In both delayed-treatment groups overall graft survival was prolonged significantly beyond that of controls ($p < 0.05$).[118,119]

3.3.2.2. Effect of M7/20 Treatment Murine Skin Allograft. Administration of the anti-interleukin-2 receptor monoclonal antibody M7/20, at a dose of 5 μg daily for 10 days, significantly prolonged survival of C57B1/10 mouse strain skin placed onto B10.AKM mouse strain recipients, when compared with controls ($p < 0.01$).[119] Several of these grafts showed no evidence of rejection until 4–5 days after the therapy was discontinued. However, none of the skin grafts survived indefinitely. Nonetheless, prolonged skin graft survival was not observed in the combination B10.BR into C57B1/10.

3.3.3. ART 18 MAb

3.3.3.1. The Effect of ART 18 Anti-IL-2 Receptor Antibody Treatment in Rat Cardiac Allografts. In light of the successful use of M7/20 MAb in mouse allograft models, we have utilized ART 18 MAb, a mouse antirat IL-2 receptor antibody,[119] in an attempt to combat rejection of (LEW × BN) F1 to LEW strain heterotopic cardiac allografts.[120] ART 18 MAb was highly successful at prolonging cardiac graft survival, although per-

manent engraftment was not seen following cessation of therapy when this agent was used in the absence of other immunosuppressives (Table IV). Furthermore, ART 18 plus very-low-dose cyclosporine (1.5 mg/kg per day) therapy yields synergistic prolongation of graft survival (Kupiec-Weglinski *et al.*, unpublished data).

As in the cases of rat antimouse M7/20 MAb, the efficacy of the ART 18 mouse antirat anti-IL-2 receptor MAb therapy in reversing well-established allograft rejection was then tested. Treatment was initiated 5 days after transplantation, at which time the grafts were grossly enlarged and heavily infiltrated with lymphocytes. Interestingly, ART 18 MAb therapy started on day 5 after transplantation and continued for 5 days at a dose of 300 μg/kg per day improved allograft survival to 18 ± 4 days (Table IV, $p < 0.001$), comparable to the effect produced by 10 consecutive injections. The dense cellular infiltrate virtually disappeared after ART 18 MAb treatment. Intermittent ART 18 MAb administration (days 5–9 and 15–19) extended graft survival to 26–28 days, whereas lower doses of mAb were ineffectual in reversing ongoing rejection. To demonstrate that the results of anti-IL-2 receptor MAb treatment were not unique to one strain combination, we treated WF rat recipients of Lewis cardiac grafts with ART 18 MAb (300 μg/kg daily) for 10 days, beginning the day of transplantation. Allograft survival was prolonged to 16 ± 1 days ($p < 0.001$). Thus, ART 18 MAb therapy can be used to prevent or treat acute rejection.

To confirm that these results were related to the specificity of ART 18 MAb for the IL-2 receptor, an additional control group of animals was treated with anti-asialo-GM1 antibody, recognizing a structure on the surface of rat natural killer cells. A single or repeated intravenous

Table IV. The Effect of ART 18 on Survival of Heart Allografts

Donor	Recipient	ART 18 dose (μg/kg per day)[a]	Days of administration	Mean graft survival (days)
(LEW × BN) F1	LEW	None	—	8 ± 1
(LEW × BN) F1	LEW	25	10	13 ± 1
(LEW × BN) F1	LEW	100	10	14 ± 3
(LEW × BN) F1	LEW	300	10	21 ± 1
(LEW × BN) F1	LEW	300	5[b]	14 ± 2
(LEW × BN) F1	LEW	300	5[b]	18 ± 4
WF	LEW	None	—	8 ± 2
WF	LEW	300	10	16 ± 1

[a] Administered intravenously.
[b] i.v. daily for 5 days beginning on day 5.

administration of MAb following transplantation virtually eliminated host NK activity. However, cardiac allograft survival was not modified.

3.3.3.2. ART 18 MAb Therapy Spares Suppressor T cells (Ts). Spleen cells were harvested at day 10 from heart-grafted hosts, after the dose regimen of ART 18 MAb had been completed, and were transferred intravenously (40–50 $\times$ 60^6 cells) into normal recipients that received test cardiac allografts 24 hr later. Such adoptive transfer prolonged donor-specific (Lewis $\times$ BN)F1, but not third-party (WF) test-graft survival (15 $\pm$ 1 days and 8 $\pm$ 1 days, respectively; $p < 0.001$).

In contrast, adoptive transfer of unseparated spleen cells from untreated recipients undergoing acute rejection accelerated donor-specific test-graft rejection in a second-set manner. Thus, potent, antigen-specific suppressor activity, but not alloaggressive immune activity, can be demonstrated in animals maintaining well-functioning cardiac allografts following ART 18 MAb therapy.

3.3.4. The Effect of Anti-Tac on Monkey Kidney Allografts

Anti-Tac, a mouse antihuman anti-interleukin-2 receptor[121] MAb, cross-reacts with the IL-2 receptor expressed on lectin-stimulated monkey T cells.[121] This antibody was evaluated as the sole therapeutic agent in cynomologus (*Macca fascicularis*) recipients of allogeneic renal grafts. Each of the unmodified renal transplant recipients experienced severe cellular rejection within 7 days of engraftment. Rejection was paralleled by a sharp rise in the number of IL-2-receptor-positive T cells in the circulation. Over one-third of circulating helper and cytotoxic T cells expressed the IL-2 receptor at the time of rejection. Each of six monkeys receiving infusions of 2 mg/kg on alternate days, beginning at the time of engraftment, experienced prolonged ($p < 0.05$), but not indefinite, graft survival. Rejection occurred from 14–21 days. The requirement for high doses of the antibody was investigated. Although anti-Tac is potent in activating mouse complement, the antibody does not fix mouse complement. We speculate that anti-Tac therapy functions as an IL-2-receptor-site antagonist, while the antimouse and antirat antibodies destroy IL-2-receptor-bearing T cells, thereby causing more profound immunosuppression than has been noted in the monkey model.

4. Discussion

The precise mechanism by which a vascularized or skin allograft is rejected remains a subject of intense investigation, but the participation of T cells in the process is unquestioned. Our results provide important

evidence that IL-2 receptor-bearing cells are required for allograft rejection. Administration of anti-IL-2 receptor MAbs (M7/20, ART 18, or anti-Tac) significantly prolonged MHC-mismatched vascularized heart allograft survival in mice and rats and renal monkey grafts.[122] Indeed, several mouse grafts survived indefinitely, although the antibody was administered only for the first 10 days posttransplantation. Rejection of the remaining grafts may well reflect inadequate dosage of antibody; no dose–response studies have been performed to date. In addition to preventing rejection, delayed treatment with anti-IL-2 receptor MAb was shown to reverse ongoing rejection in other recipients of heart allografts. Such long-term engraftment following cessation of therapy makes it unlikely that M7/20 antibody in the dosage of 5 mg/day prolongs graft survival by pharmacologic blockade of the IL-2 receptor. Furthermore, exogenous IL-2 does not diminish the beneficial effects of anti-IL-2 receptor MAb therapy in rodents. Whether or not such prolonged graft survival represents deletion of the responding T-cell clones is a subject of current investigation. Successful suppression of delayed-type hypersensitivity reactions with antireceptor antibody has also been achieved.[123] Initial results indicate that complement fixation is required to achieve optimal immunosuppression. Moreover, only antireceptor antibodies that block IL-2 binding cause immunosuppression. Passive transfer experiments clearly prove that anti-IL-2 MAb spares Ts.

Finally, the availability of MAbs directed against the human IL-2 receptor[121] provides an opportunity to extend these principles to clinical transplantation. The presence of IL-2 receptors on all recently activated T cells[108–110,114,124] and their absence from the surface of resting or memory T cells make it possible to target only the relevant responding clones following an allograft, raising the hope of specific immunosuppression. In this regard, anti-Tac, which defines the human IL-2 receptor, is effective in prolonging renal allograft survival in monkeys. The unexpected necessity for high doses of antibody in the monkey model may underscore the importance of complement fixation, as this antibody does not fix human complement. Nonetheless, this antibody will be utilized in order to probe the effects of anti-IL-2 receptor-directed therapy in clinical transplantation.

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16

Drugs and the Kidney

William M. Bennett

1. Introduction

Because of the concurrent medical problems that so frequently occur in patients with renal failure, pharmacokinetic principles need to be emphasized to all physicians prescribing for these complex patients. Fenster reviews this subject with emphasis on cardiac drugs.[1] Benet concisely discusses and defines those kinetic parameters necessary to determine the body's effect on any new compound, with particular attention to the effect of disease states.[2] The necessity for dosage modification when a drug undergoes substantial renal excretion in an unchanged form drug was emphasized by Cutler.[3] He employs a clinical method of dosage adjustment which notes proportional changes from normal in the glomerular filtration rate and then changes the amount of drug given or the interval between doses.[3] In renal failure patients, drugs may produce symptoms that add to the morbidity produced by uremia *per se*. This type of problem, such as drug-induced inhibition of folate metabolism, causes additive neuropathy, which must be anticipated so that irreversible changes can be prevented. The commonly prescribed drugs trimethoprim and triamterene act as folate antagonists by inhibiting the enzyme dihydrofolate reductase.[4] Nephrologists should be particularly

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vigilant for these types of adverse drug reaction which masquerade as worsening manifestations of the uremic syndrome.

2. The Effects of Renal Disease on Pharmacokinetics and Pharmacodynamics

Greenblatt discussed the interpretation of the drug elimination half-life as the major pharmacokinetic parameter used by clinicians. During chronic dosing, the elimination half-life has value in predicting the rate and extent of drug accumulation and washout. Changes in the volume of drug distribution and clearance, particularly in patients with renal disease, may be more important determinants of a drug's clinical behavior than the elimination half-life from plasma or serum.[5] Experimentally, reduction of renal mass alters renal clearance of gentamicin more than would be predicted on the basis of measured decreases in glomerular filtration rate and effective renal plasma flow. These data partially explain discrepancies in predicted versus actual drug excretion when drug dosages are based on glomerular filtration rate alone.[6] Hisaoka and Levy produced experimental acute renal failure in rats by giving uranyl nitrate. Dialyzed serum from these animals, but not controls, caused an increased sensitivity to phenobarbital.[7] These elegant studies support the concept that dialyzable factors in uremic plasma may act to cause the "increased sensitivity" that patients with renal failure exhibit to hypnotic drugs and sedatives.

In patients with renal failure who require individualization of drug therapy, serum drug levels have found widespread application. Perucca *et al.* discuss interpretation of drug levels in acute and chronic disease states where alterations in protein binding, retention of pharmacologically active metabolites, and increased end-organ sensitivity are frequently present. Consideration should be given to the possibility of analytic errors resulting from impairment of assay performance due to interfering metabolites or alteration in serum composition due to uremia.[8]

2.1. Absorption and Distribution of Drugs

Antacids such as those employed for phosphate binding may reduce absorption of drugs commonly prescribed by nephrologists. Particularly affected are phenothiazines, cardiac glycosides, and tetracyclines.[9] Food reduces the bioavailability of the antihypertensive drugs atenolol and captopril. Some relevant drugs have increased, although delayed, ab-

sorption when taken with food. Food may reduce the presystemic clearance of propranolol, metroprolol, labetolol, and hydralazine, resulting in higher plasma concentrations.[9] Changes in drug absorption should be considered by the clinician when a patient appears unusually resistant or sensitive to a given drug dose. This is particularly relevant in a patient with apparently refractory hypertension.[9] The abnormalities in zinc absorption due to chronic renal failure are made worse by aluminum hydroxide and ferrous sulfate. Thus, zinc deficiency should be recognized in patients receiving these drugs so that supplements can be provided.[10]

2.2. Binding to Plasma Proteins

A supplement summarizing the clinical implications of drug–protein binding has been published. Extensive coverage of advances in technology allowing measurement of free-drug levels is given. Widespread use of free-drug concentrations in clinical practice would theoretically avoid interperative errors due to decreased drug–protein binding in uremia.[11] The nephrotic syndrome alters prednisolone binding and results in reduced free-drug clearance relative to creatinine clearance, whether the prednisolone is administered orally or intravenously.[12] This leads to heightened sensitivity to adverse steroid side effects. Uremia, however, does not alter cortisol binding to corticosteroid-binding globulin. Thus, elevated free-cortisol concentrations in uremia are due to true increases of plasma cortisol.[13] Haughey *et al.* reported increased protein binding of disopyramide and elevated concentrations of alpha-1-acid glycoprotein in serum of dialysis and transplant recipients.[4] Other organic bases may have reduced free concentrations in uremia because of increased binding to this 45,000-dalton acute-phase reactant. Alpha-1-acid glycoprotein increases in serum when serum creatinine is above 10 mg/dl.[15]

2.3. Biotransformation

Garattini reviewed the subject of pharmacologically active drug metabolites.[16] These compounds formed in the body may undergo altered elimination in chronic renal failure due to failure of renal excretion. This may cause increased numbers of adverse reactions even if the parent compound is metabolized in a normal fashion. The subject of genetic variability in acetylation and oxidation has received increased attention.[17] Drugs used extensively by nephrologists may be subject to these genetic polymorphisms, which may explain some difficulties in trying to simplify therapy. Cyclosporine, hydralazine, sulfonamides, and beta blockers, all drugs that show large interindividual differences in kinetics and phar-

macodynamics, may be subject to such genetically determined discrepancies in metabolism.

3. Drug Effects on the Kidney

3.1. Tubular Mechanisms of Drug Transport

The proximal tubular secretion of methotrexate in the monkey was studied by Williams *et al.*[18] Penicillin, which is commonly used clinically in oncology patients, blocked methotrexate uptake and stimulated efflux from tubular cells, resulting in inhibition of net secretion. Thus, like probenecid, penicillin shares a common organic acid secretory system with methotrexate.[18] Probenecid decreased total clearance and increased the area under the curve of the nonsteroidal antiinflammatory drug zomepirac and its acylglucoronide metabolite in healthy volunteers. Rather than inhibition of renal organic anion secretion, the usual mechanism proposed for probenecid effects on renal drug clearance, these data suggested that probenecid blocks glucuronidation of parent drugs. This could apply to metabolites of other nonsteroidal drugs, such as naproxen, indomethacin, carprofen, and ketoprofen.[19]

Drugs that are organic cations, such as procainamide and cimetidine, undergo proximal tubular secretion as a major mode of elimination. McKinney published data showing that procainamide transport in isolated rabbit proximal tubular segments was increased by decreasing perfusate pH and blocked by amiloride. Although renal organic base secretion is complex, organic base–proton exchange may enhance the secretion of some drugs.[20] Cimetidine secretion can be inhibited in order of decreasing potency by ranitidine, thiamine, procainamide, guanidine, and choline. Conversely, cimetidine inhibits amiloride and tetraethylammonium secretion. The avian kidney metabolizes cimetidine to cimetidine sulfoxide and hydroxymethylcimetidine.[21] Selectivity for the organic cation transport system was shown by cimetidine in studies using normal humans. An anion, cephalothin, and a zwitterion, cephalexin, were unaffected by cimetidine, while the clearance of ranitidine, a cation, was markedly reduced.[22]

3.2. Changes in Renal Drug Handling with Age

Alterations in renal function with age are one of the major determinants of the increased prevalence of adverse reactions in the elderly. Despite many publications, clinicians still tend to overestimate actual renal function based on "normal" serum creatinine values in elderly

subjects. Since deterioration in renal function often affects drug disposition, parent drugs and active metabolites that are primarily eliminated by the kidney should be prescribed based on measured or calculated creatinine clearance rather than assuming normal renal function based on serum creatinine.[23] Urinary excretion and renal clearance of trimethoprim was reduced by 50% in an elderly population compared to young healthy subjects. Sulfamethoxazole renal clearance was not reduced; however, plasma drug concentrations were three-fold higher in elderly subjects getting therapeutic doses of this commonly prescribed antibiotic combination.[24] At the other end of the spectrum, dosing neonates is difficult because of the technical difficulties of urine collection and plasma creatinine determinations in this age group. Koren *et al.* used gentamicin pharmacokinetic parameters to estimate glomerular filtration rate (GFR) in a neonatal intensive care unit, taking advantage of the fact that aminoglycosides are eliminated almost exclusively by glomerular filtration.[25]

4. Clinical Use of Drugs in Renal Failure

4.1. Assessment of Renal Function

Serum creatinine and creatinine clearance are still used as the standard measure of renal function for purposes of drug-dosing adjustments. Fasting can increase creatinine, as estimated by the Jaffe method. This increase of 0.7 mg/dl in normal subjects fasted for 3 days is probably due to a rise in acetoacetate.[26] Diabetic ketoacidosis obviously causes the same phenomenon.[27] Methyldopa also interferes with creatinine assays, causing "pseudo" renal failure.[28]

Often renal patients requiring drug therapy have unstable renal function. When urinary creatinine is known, the use of the midpoint serum creatinine produced the lowest degree of error when compared to measured values.[29] If only a series of serum creatinines are available, several formulas can estimate creatinine clearance with approximately 15% error.[29] In adolescent boys, the accretion of muscle affects creatinine production. Schwartz and Gauthier validated a formula for children aged 1–20 based on body length which gave excellent results for GFR estimation from serum creatinine:

$$C_{Cr}\ (\text{ml/min/1.73 m}^2) = KL/P_{Cr}$$

where the proportionality constant $K = 0.55$ for girls and 0.7 for boys; L = body length in centimeters.[30] Others could not corroborate the use of similar formulas in an intensive-care unit population.[31]

4.2. Dosing Strategy in Patients with Renal Failure

Burton *et al.* have provided an extensive review of dosing methods advocated in the literature to provide predictable serum concentrations and thus pharmacologic responses to drugs.[32] Using five drugs—gentamicin, digoxin, phenytoin, theophylline, and lidocaine—as prototype drugs that are commonly used, have narrow therapeutic ranges, and exhibit a cross-section of pharmacokinetic variability, the authors conclude that population-based predictive nomograms are too inaccurate to be used except as a starting point for therapy. Individualized pharmacokinetic methods using serum drug concentration feedback and a computerized Bayesian approach were preferred. The latter may be more convenient in that variable numbers of serum drug determinations can be used to determine dose. There are only limited data supporting the actual cost-effectiveness of any of these approaches in reducing clinical toxicity and improving routine clinical care.

4.3. Effects of Extracorporeal Treatment and Peritoneal Dialysis on Drug Treatment

4.3.1. Hemodialysis

Gibson discussed the problems involved in studying drug removal by hemodialysis. Frequently, data are flawed by failure to allow for distribution equilibrium prior to starting the dialysis procedure and to obtain enough postdialysis samples to define drug rebound.[33] Metronidazole and its metabolites do not accumulate in patients with acute renal failure.[34] However, significant amounts of drug may be lost during hemodialysis, necessitating supplemental dosage for critically ill patients.[35] Dialysis patients ingesting vitamin C had elevated serum levels of the vitamin which correlated well with hyperoxalemia. The latter could be a risk factor for vascular disease in patients on chronic hemodialysis.[36]

4.3.2. Poisonings and Overdoses

Extracorporeal therapy for treatment of intoxication was extensively reviewed by Blye *et al.*[37] They conclude that clear-cut indications for dialysis exist only for methanol and ethylene glycol overdoses. Hemoperfusion has not generally improved patient survival, as compared to intensive supportive care.[37] Todd even questions the efficacy and safety of any drug removal measures.[38] There is still some dissent regarding the use of hemoperfusion for barbiturate overdose.[39]

In methanol poisoning, hemodialysis has been used for acidosis, mental or visual abnormalities, or ingestion of more than 30 g.[40] A blood

level of methanol greater than 0.5 g/liter or an increased blood formate level can be used as a dialysis criterion.[41] Dialysis plays little role in acetaminophen[42] or quinine overdoses.[43] However, immediate hemodialysis can be lifesaving in cyanide poisoning. In dogs, dialysis plus thiosulfate promotes cynanide metabolism as well as thiocyanate removal. This increased the dose required to produce death.[44] Oral-activated charcoal increased digoxin clearance in a patient with chronic renal failure. This provides a way to treat patients who have iatrogenic overdoses.[45]

4.3.3. Continuous Arteriovenous Hemofiltration

Continuous arteriovenous hemofiltration is a convenient and safe method for removal of fluid and maintenance of acid–base, electrolyte, and nutritional homeostasis in acute renal failure.[46] This technique consequently is finding increasing applicability in seriously ill patients with multiple-organ failure. Since the membrane used has a molecular weight cutoff of approximately 10,000 daltons, drug removal during the procedure is often significant. Data concerning drug concentrations in ultrafiltrate are shown in Table I. Multiplying this concentration by the

Table I. Drug Removal by Continuous Arteriovenous Hemofiltration[a]

Drug	No. of patients	Arterial plasma concentration (μg/ml)	Ultrafiltrate concentration (μg/ml)	Ultrafiltrate serum ratio
Antibiotics				
Ampicillin	1	7.5	9.0	1.20
Aminoglycosides (gentamicin, tobramycin)	5	4.5 ± 1.3	3.7 ± 1.2	0.82
Cefoperazone	1	278	80	0.29
Cefotaxime	1	600	315	0.53
Clindamycin	1	4.7	4.9	1.04
Metronidazole	2	14.6 ± 18.4	13.2 ± 16.7	0.90
Nafcillin	1	84	35	0.41
Streptomycin	1	32	10.2	0.32
Vancomycin	3	37.2 ± 23	28.2 ± 15.5	0.76
Other drugs				
Digoxin	3	2.3 ± 1.6[b]	2.5 ±2[b]	1.09
Lidocaine	1	4.8	10.2	2.13
Phenytoin	1	8.5	4.6	0.54
Procainamide	1	15	12.6	0.84

[a] Data provided by T. A. Golper.
[b] Concentrations in ng/dl.

volume of filtrate will provide an estimate of the amount of drug that should be replaced. Drugs with low plasma protein binding will be lost to a greater extent.[47,48]

4.3.4. Peritoneal Dialysis

Paton *et al.* reviewed pharmacokinetic considerations in drug therapy of patients undergoing peritoneal dialysis.[49] Peritonitis continues to be a major problem in patients undergoing continuous ambulatory peritoneal dialysis (CAPD). Johnson *et al.* have extensively reviewed antibiotic kinetics in this situation.[50] Cephalosporins are widely used to treat CAPD-associated peritonitis. Since peritoneal clearance is low, oral agents have limited utility. Intravenous administration provides therapeutic serum concentrations, which eventually provide bactericidal concentrations in peritoneal fluid. The optimum way to prescribe cephalosporins is by the direct peritoneal route, since peritoneal-to-drug transport is surprisingly good. As an example, the third-generation cephalosporin cefoperazone has a peritoneal clearance of only 6.9 ml/min. After peritoneal instillation, the bioavailability was 64%, and adequate concentrations were achieved for treatment of peritonitis.[51]

New data on cefuroxime, cefotaxime, and moxalactam are available.[52–54] Vancomycin is an alternative drug for gram-positive peritonitis, with an intraperitoneal loading dose of 10–15 mg/kg followed by 15–30 mg/liter of dialysis fluid in subsequent exchanges.[50] Another way to use vancomycin is a loading dose of 23 mg/kg intravenously, followed by 17 mg/kg every 7 days. Peritoneal dialysate concentrations averaged 2.2 μg/ml with the latter regimen.[55]

With aminoglycosides, parenteral dosage alone will result in inadequate dialysate antibiotic concentrations for the treatment of peritonitis. For gram-negative infections during CAPD, 1 mg/kg of gentamicin or tobramycin given intraperitoneally leads to therapeutic peritoneal fluid concentrations immediately as well as therapeutic serum concentrations after 6 hr. If rapid achievement of therapeutic serum levels is desired, an intravenous loading dose can be given simultaneously.[50] Netilmicin data are now available for CAPD peritonitis.[56]

Penicillins also should be given intraperitoneally, since intravenous loads result in low peritoneal dialysate concentrations.[50] Systemically administered metronidazole is not appreciably dialyzed, although enough drug accumulates in peritoneal fluid to provide adequate coverage for anaerobic peritonitis.[57] Oral trimethoprim–sulfamethoxazole may provide levels sufficient to treat peritonitis.[5]

Fungal peritonitis is usually an indication for catheter removal. A single case of recovery with intraperitoneal 5-fluorocytosine is reported.[59] Ketoconazole in high doses may achieve adequate concentrations in serum and peritoneal fluid for treatment of fungal peritonitis.[60] However, the reliability of achieving these concentrations is poor, and the drug should not be expected to achieve cures in a high percentage of cases.[61,62] Amphotericin given intravenously has limited penetration into peritoneal fluid.[63]

Loss of vitamin D metabolites may occur in CAPD patients, resulting in a need for increased replacement doses.[64] With the increasing use of CAPD, the kinetics of other drugs not related to peritonitis treatment may be altered. Janknegt and Nube have proposed a single formula for estimating CAPD clearance in ml/min.[65] There is little clinically significant removal of tocainide,[66] phenytoin,[67] or atenolol[68] by CAPD.

4.4. Drug Interactions

Although interactions between drugs are traditionally viewed as undesirable, Caranasos *et al.* review beneficial drug interactions.[69] Of importance to nephrologists are the synergistic effects of penicillins and aminoglycoside antibiotics against enterococcal infections.[70] This synergism occurs despite some inactivation of the cationic aminoglycoside by large excesses of anionic penicillins, particularly in patients with renal failure.[71] Experimentally, aminoglycoside–penicillin combinations reduce nephrotoxicity, presenting a strategy that might be exploited clinically.[72,73] Schentag *et al.* reported that complexation of aminoglycosides by ticarcillin or carbenicillin was more effective than hemodialysis in lowering elevated aminoglycoside levels.[74] Cephalosporins do not inactivate aminoglycoside.[75] The combination of the new monolactam imipenem with cilastatin, a renal dehydropetidase I inhibitor, has enabled this valuable antibiotic drug to have better and less expensive therapeutic profiles by avoiding urinary and renal inactivation.[76] For the difficult problem of struvite stones produced by chronic infection with urea-splitting organisms, the addition of the urease inhibitor acetohydroxamic acid to antibiotic regimens is more effective than antibiotics alone.[77]

Enhancing diuresis in patients with refractory edema by the combination of loop diuretics with a thiazide-type agent has become clinically accepted. Care must be taken to maintain electrolyte balance in the face of the massive diuresis that may occur. Thiazides, metolazone, and quinethazone have been added to furosemide, butmetanide, ethacrynic acid, or piretanide for this purpose.[78] Potassium-sparing diuretics have achieved

a major role as an adjunctive measure to prevent diuretic-induced hypokalemia.

4.4.1. Nonsteroidal Antiinflammatory Drug Interactions

Brater, in reviewing the subject of diuretic resistance, discusses the mechanism of nonsteroidal antiinflammatory drug-induced resistance to loop diuretics.[79] Activation of renal prostaglandin synthesis accompanies the natriuresis induced by loop diuretics.[80] Brater concludes that there is no effect on diuretic delivery into the urine, but instead cyclooxygenase inhibitors prevent diuretic-induced increases in prostaglandins and renal blood flow.[79]

Sulindac is less likely to interfere with the antihypertensive and natriuretic properties of diuretics.[80] Serum lithium levels also are unaffected by sulindac, as opposed to other nonsteroidals.[81]

4.4.2. Other Interactions

Drug interactions with cimetidine are comprehensively reviewed by Gerber *et al.*[82] These interactions are particularly relevant for renal failure patients, in whom pharmacokinetics of common drugs are often altered. Drugs interfering with plasma assays during therapeutic drug monitoring can lead to clinical errors.[83] For an example, spironolactone and prednisone both can increase plasma digoxin by 0.4–0.5 ng/ml in concentrations of these drugs used clinically. Whenever the patient's clinical condition does not fit with the measured blood concentration of a drug, interference by another drug should be considered.

The literature regarding pharmacokinetic interactions between digoxin and other drugs is rapidly expanding.[84,85] Antacid gels and binding resins such as cholestyramine may decrease digoxin bioavailability, while some antibiotics may enhance absorption by eliminating intestinal flora. Antiarrhythmic drugs such as quinidine, amiodarone, and verapamil can markedly increase steady-state digoxin levels. Experimentally, digoxin clearance was acutely reduced by 21% by intravenous spironolactone. Renal digoxin clearance was depressed by a mechanism independent of antimineralocorticoid activity.[85] Rifampin may lower steady-state serum digoxin concentrations in patients with severe renal disease. Nephrologists need to be vigilant whenever new medications are added or subtracted from a regimen that contains digoxin.[84] Calcium channel-blocking drugs do not affect renal clearance of digitoxin; however, the extrarenal clearance of this glycoside may be reduced slightly by verapamil and diltiazem.[86,87]

5. Aspects of Specific Drugs in Patients with Renal Disease or Hypertension

Because of the frequency of urinary tract infections in pregnancy, the use of antimicrobial drugs in this setting needs to be considered in view of prenatal risks. The *Medical Letter* summarizes the toxicities of antimicrobials in pregnancy with a useful table.[88] The safety and pharmacokinetics of these drugs are reviewed by Chow and Jewesson.[89] There continues to be a debate about the cost-effectiveness of serum level monitoring of antibiotics. This is a particularly important issue for high-risk patients, such as those with renal failure. Serum level monitoring is indicated for drugs with a low therapeutic index and poor predictability of individual pharmacokinetics. For the antimicrobials used by the nephrologist, this applies to aminoglycosides, vancomycin, and chloramphenicol. Penicillins and cephalosporins can be safely given to renal failure patients, even if levels are high and well above the minimum inhibitory concentrations for infecting organisms. Thus, serum level measurement of these drugs is seldom necessary.[90]

5.1. Aminoglycoside Antibiotics

Three newer aminoglycosides were reviewed by Noone.[91] Sisomicin is a naturally occurring antibiotic produced by *Micromonospora inyoensis* which structurally resembles gentamicin Cla. It is approved by the Food and Drug Administration. Dibekacin and netilmicin are semisynthetic aminoglycosides. All three drugs exhibit synergy with β-lactam antibiotics. Pharmacokinetics are similar to those of other aminoglycosides with elimination half-lives of 2–2.5 hr. Elimination of these aminoglycosides is proportionally reduced with increasing renal failure. Although netilmicin may be less nephrotoxic and ototoxic than other available aminoglycosides,[91] at present it is difficult to determine any clear-cut clinical advantage of this or the other new aminoglycosides. Aminoglycoside serum levels determined by enzyme-multiplied immunoassay technique were reported to be falsely elevated in patients with jaundice.[92] However, recent data did not confirm this finding.[93] Holloway *et al.*, using decision analysis, found gentamicin to be more cost-effective than tobramycin unless nephrotoxicity is severe enough to prolong hospitalization over 3 days. In their study, although the incidence of nephrotoxicity was 26% for gentamicin and 12% for tobramycin, the combined drug and nephrotoxicity costs were $127 for tobramycin and $72 for gentamicin.[94] Burton *et al.* used a Bayesian method that generates a

feedback loop from initial dosing and measured drug concentrations in order to reestimate an individual's pharmacokinetic parameters for aminoglycosides. This method was superior to routine physician aminoglycoside dosing in achieving desired peak and trough levels.[95] Experimentally, low-dose dopamine increases renal clearance of aminoglycosides, presumably by increasing renal blood flow and GFR.[96] This may have relevance in critically ill patients receiving aminoglycosides.

5.2. Other Antimicrobial Drugs

A variety of new cephalosporins have become available for clinical use. Dosage adjustments for renal insufficiency and dialysis are based on pharmacokinetic studies in patients with varying degrees of renal failure. Some recent data are summarized in Table II.[97]

Baron *et al.* reported the sodium content of the various available β-lactam antibiotics. The maximum recommended daily dose of carbenicillin would provide 142 mmoles, while ticarcillin provides 93 mmoles. Mezlocillin, piperacillin, and azlocillin yield only 30–35 mmoles of sodium per daily dose.[98]

The combination of amoxicillin and potassium clavulanate, an inhibitor of bacterial β-lactamases, may find widespread use in the treatment of complex urinary tract infections.[99] Similarly, ticarcillin can be combined with clavulanic acid. Both components have renal excretion, so that dosage adjustment is necessary in renal failure. Ticarcillin and clavulanic acid have significant hemodialysis clearance.[100] The dosage of

Table II. Adjustments of New Cephalosporins in Severe Renal Failure

Drug	Elimination half-life (hr)		Dosage (GRF < 10 ml/min)	Effect of hemodialysis
	Normal	Uremia		
Cefonicid	4.5	65	1 g/24 hr	Not dialyzed
Cefotetan	5.1	10.1	1 g/24 hr	?
Cefotiam	1.1	13	Reduce dose by 25%	?
Ceftazidime	1.6–2.3	15–25	0.5–1.5 g/ 36–48 hr	Reduction in plasma concentration by 88%
Ceftizoxime	1.7–2.1	19–35	0.5–1.0 g/ 24–48 hr	Not dialyzed
Ceftriaxone	6.9	Variable (6–40 mg)	Limit daily dose to 2 g daily	Not dialyzed

mecillinam, an amidino penicillin, needs to be reduced to 25% of normal in patients with a GFR < 10 ml/min. Hemodialysis increases drug clearance by 100%, necessitating supplemental doses following the procedure.[101]

Although erythromycin had been thought to need little adjustment in renal failure, a recent report of ototoxicity necessitates a reevaluation. Because of enhanced oral bioavailability, daily oral doses should not exceed 1.5 g.[102] Chloramphenicol serum concentrations may be higher after intravenous administration in uremic patients, although elimination half-life is unchanged. Dosage modification is not required.[103] Likewise, the presence of acute renal failure does not overtly disturb metronidazole pharmacokinetics.[104,105] A nomogram for vancomycin initial and maintenance dosing in renal failure was developed by Matzke *et al.*[106] The nomogram was based on the relationship between serum clearance and creatinine clearance in 56 patients with varying degrees of renal dysfunction. Delayed neutropenia as part of a hypersensitivity reaction to vancomycin can be seen in dialysis patients.[107] Guidelines for treatment of systemic fungal infections with amphotericin B, flucytosine, ketoconazole, and miconazole recently have been concisely summarized.[108]

5.3. Cardiovascular Drugs

5.3.1. Cardiac Glycosides

Cardiac glycosides need careful adjustment in renal failure to avoid digitalis intoxication. Serum levels have been extensively used to monitor therapy. However, interpretive problems may arise in renal failure patients. The new antiarrhythmic drug amiodarone may double serum digoxin levels and produce clinical toxicity in patients who have been previously stable on long-term glycoside therapy.[109] Total-body clearance of digoxin is reduced by amiodarone because of reduction in renal and nonrenal digoxin clearance. Amiodarone also reduced the volume of digoxin distribution by 11%. Douste-Blazy *et al.* demonstrated marked increases in urine digoxin excretion produced by amiodarone and suggested that the drug displaces digoxin from tissue-binding sites.[110] Conversely, the digoxin dose necessary for maintenance of therapeutic serum levels is reduced by rifampin; serum digoxin concentrations may fall to ineffective levels if rifampin is added to a stable cardiac patient's regimen.[111] The serum of patients with uremia not taking cardiac glycosides may have digoxinlike activity, with Na, K-ATPase inhibiting properties as measured by radioimmunoassay. This can cause confusion in interpretation of serum digoxin levels in uremic patients.

5.3.2. Diuretics

Many new diuretics are available for the treatment of renal edema and hypertension. Xipamide is a sulfonamide diuretic used for treatment of hypertension and edema. The pharmacodynamics and potency of the drug resemble those of furosemide, although its mechanism of action is similar to that of hydrochlorothiazide. Xipamide induces a saluresis even when creatinine clearance is less than 30 ml/min.[112] Indipamide, another new sulfonamide diuretic, has an antihypertensive effect at low doses which do not necessarily produce saliuresis. It is hypocalciuric, like the thiazides.[113] Piretanide is a new high-ceiling loop diuretic. Differences from furosemide and bumetanide which confer clinical advantage are not apparent from clinical experience to date.[114] A dose of 6 mg of oral piretanide or 2 mg intravenously is equivalent to 40 mg of furosemide. In chronic renal failure more than 90% of the drug is eliminated by nonrenal routes.[114]

5.3.3. Antihypertensive Drugs

Clonidine hydrochloride is well absorbed through the skin, which has led to the weekly application of drug-containing patches. This delivery system provides smooth, continuous antihypertensive action. A drug-containing film of defined surface area delivers the drug via a microporous membrane interposed between the drug reservoir and the adhesive. Transdermal patches are renewed weekly. Better compliance and relatively constant plasma concentrations seem to represent major advantages.[115] Tricyclic antidepressants may antagonize the hypotensive effect of clonidine over a period of 1–2 weeks, although occasionally the blood pressure rises more quickly.[116]

The clinical pharmacokinetics of labetalol were reviewed. This compound is one of a new class of antihypertensive drugs with both α- and β-adrenergic-blocking properties. The plasma half-life is 3–3.5 hr, and the drug is eliminated primarily by hepatic metabolism. Kinetics are unaltered by renal disease.[117]

Captopril clearance is markedly reduced in chronic renal failure.[118] Hemodialysis may remove 35% of an administered dose, since hemodialysis clearance is 120 ml/min.[118] Likewise, enalaprilat, the active converting enzyme-inhibiting moeity of enalapril, is accumulated in renal failure. With GFR less than 30 ml/min, smaller doses of enalapril will be required.[119] Bernstein and O'Conner summarized the growing literature on antiadrenergic antihypertensive drug effects on renal function. Peripheral alpha antagonists preserve renal hemodynamics, while central alpha agonists have variable effects. Clonidine preserves both renal blood

flow and glomerular filtration rate. Beta blockers, particularly propranolol, may reduce renal blood flow by 10–20%. However, beta-blocker-induced reductions in glomerular filtration rate are seldom of clinical importance.[120]

5.3.4. Antiarrhythmics

Flecainide provides sustained suppression of ventricular arrhythmias with twice-daily oral doses. Half-life in patients with normal renal function is 13–16 hr, but is extended with chronic renal failure. The dosage should be reduced for patients with severe chronic renal insufficiency. Hemodialysis is ineffective in the removal of unchanged drug but does provide substantial removal of conjugated metabolites.[121] Lorcainide has a relatively long half-life, allowing 12-hr dosing intervals. Norlorcainide, a slowly eliminated metabolite, contributes to the drug's antiarrhythmic effect. Renal dysfunction has little effect on lorcainide kinetics.[122]

Calcium entry blockers are not greatly affected by the presence of renal failure, although total-body verapamil clearance is reduced.[123] Felodipine, a structural analog of nifedipine, causes natriuresis by inhibiting distal tubular and collecting-duct sodium reabsorption.[124] Since other potent vasodilators, such as minoxidil and hydralazine, cause sodium and water retention, the natriuretic properties of the vasodilating calcium entry blockers might present a therapeutic advantage. Amiodarone has highly unpredictable kinetics owing to its lipophilic properties. Large amounts accumulate in adipose tissue and skeletal muscle. Plasma half-life ranges from 3.2 to 80 hr. Total-body clearance is very low, and renal dysfunction is not a variable affecting drug disposition.[125] Cimetidine competitively inhibits renal procainamide clearance by 36%, probably by interfering with active tubular secretion. Plasma half-life increases, while systemic clearance decreases.[126]

5.4. Miscellaneous Drugs

5.4.1. Psychotropic, Hypnotic, and Analgesic Drugs

Patients with renal failure are more sensitive to usual doses of codeine, presumably owing to newly described changes in distribution and elimination.[127] The dependence of morphine clearance on renal function has been emphasized.[128] Although total-body clearance and terminal elimination half-life of unchanged morphine are unaffected by renal failure, an active metabolite, morphine-3-glucuronide, accumulates.[129] Others have found marked elevations of plasma morphine and increased

elimination half-life in renal failure.[130] The half-life of chlorpheniramine is markedly prolonged by renal failure.[131]

Benzodiazepines are commonly prescribed for uremic patients. Therapeutic doses of diazepam to uremic patients produced lower total serum concentrations of diazepam and its pharmacologically active metabolite desmethyldiazepam in patients with renal failure compared to patients with normal renal function receiving similar doses.[132] This is due to decreased binding of both parent drug and metabolite to plasma proteins. Unbound (free) levels in renal patients were normal.[132] Lorazepam kinetics were likewise unaffected by renal failure, and only 8% of intact drug was removed by a standard hemodialysis.[133] Triazolam pharmacology is reviewed. This short-acting benzodiazepine is useful for insomnia in renal failure patients.[134]

Levy presents a lucid discussion of the use of psychotropics in patients with renal failure.[135] Amitriptyline hydrochloride and its unconjugated metabolites have reduced plasma concentrations in uremic patients, while there is accumulation of inert conjugated metabolites. This may diminish the drug's efficacy in renal failure.[97,136] Accumulated metabolites may cause side effects.[137] Peritoneal dialysis does not remove clinically relevant amounts of amitriptyline.[138] Doxepin and its active metabolite desmethyldoxepin are not removed by hemodialysis.[139] Second-generation antidepressants were reviewed.[140] Fatal necrotizing vasculitis with renal failure has been reported with nomifensine.[141]

5.4.2. Rheumatologic Drugs

Auranofin is an orally active gold compound for treatment of rheumatoid arthritis. Gastrointestinal reactions are the most common adverse reaction. Proteinuria occurs in about 0.5% of patients.[142] The pharmacokinetics of ibuprofen have been studied.[143] Age does not alter drug disposition.[143] However, indobufen, an inhibitor of platelet aggregation, has a reduced elimination rate in renal failure.[144] Salicylate pharmacokinetics are unaltered by renal failure.[145]

5.4.3. Anesthetics

The two new nondepolarizing muscle relaxants atracurium and vecuronium can be given safely to renal failure patients, since they do not depend on normal renal function for excretion.[146]

5.4.4. Immunosuppressive Drugs

Azathioprine does not alter the bioavailability or elimination of prednisolone in doses used for renal transplantation.[147] Likewise, hemodi-

alysis has no effect on prednisone kinetics.[148] Total prednisolone clearance was increased by anticonvulsant drugs in transplant patients.[149] Immunosuppressive activity, as determined by the percent inhibition of mixed lymphocyte reaction, decreased by one-third after phenytoin dosing.[150]

5.4.5. Gastrointestinal Drugs

Both cimetidine and ranitidine are eliminated largely unchanged by the kidneys. The half-life of ranitine is slightly longer (2.1–3.1 hr) than that of cimetidine (1.7–2.1 hr). Renal disease causes an increase in ranitidine plasma concentrations because of reduced clearance and increased bioavailability.[151] Although serum levels of cimetidine are decreased by hemodialysis, there is a rapid rebound of serum concentrations owing to released drug sequestered in body tissues.[152]

6. Nephrotoxicity of Therapeutic Agents

6.1. Cyclosporine

Nephrotoxicity continues to be a major problem with the expanding use of cyclosporine[153] (see also Section 4). The clinical manifestations of this problem are difficult to separate from allograft rejection in renal transplant recipients.[154] Although rises in serum creatinine may be reversible with reduction in drug dosages, the consequences of acute episodes of renal dysfunction on subsequent long-term allograft function are of concern. In cardiac transplant recipients, chronic tubulointerstitial fibrosis and glomerulosclerosis with progressive renal dysfunction have been noted despite good preservation of cardiac function.[155] Prolonged initial oligoanuria has been related to high initial doses of cyclosporine. Renal biopsies from these patients may reveal diffuse interstitial fibrosis.[156]

Although experimentally there are prominent morphologic changes in renal proximal tubular cells, cell necrosis is unusual.[157] Furthermore, tubular functions such as proximal tubular sodium and lithium reabsorption are usually well preserved.[158] Murray *et al.* demonstrated cyclosporine-induced renal vasoconstriction which could be averted by denervation or α-adrenergic blockade.[159] *De novo* appearance of hypertension has been noted, possibly because of drug-induced sodium retention.[160] It is unclear whether hypertension is secondary to renal hemodynamic changes or is due to other mechanisms.

Monitoring of cyclosporine blood levels is advocated to avoid toxicity because of the marked interindividual variation in absorption and he-

patic metabolism.[161] Although elevated levels in general correlate with nephrotoxicity, it is clear that reversible renal dysfunction may occur even when cyclosporine concentrations are well within accepted "therapeutic" limits.[154] Drugs that inhibit hepatic P-450 mixed-function oxidase-mediated metabolism of cyclosporine, such as ketoconazole, erythromycin, and cimetidine, may increase levels and enhance toxicity.[162] Conversely, drugs that induce mixed-function oxidases, such as phenytoin and rifampicin, reduce levels and necessitate larger doses.[162]

6.2. Lithium

There continues to be great interest in the long-term effects of lithium on the kidney, in view of this agent's unique place in the therapeutic armamentarium of the psychiatrist. In long-term animal studies, Kling *et al.* reported a distinctive distal tubular lesion as well as increased tritiated thymidine uptake by nuclei of distal tubules.[163] They hypothesized that these changes, which are similar to those reported in human biopsy materia,[164,165] predispose the kidney to injury from otherwise insignificant insults. Data from an experimental model of lithium nephrotoxicity in rabbits suggest that intratubular cast formation and intratubular obstruction may be important in producing tubulointerstitial fibrosis.[3] In rats treated with lithium shortly after birth, tubulointerstitial nephropathy is irreversible even if the drug is withdrawn.[166]

Data from long-term experience in patients continue to accumulate. In 101 unselected patients on lithium without clinical intoxication, 8.9% had creatinine clearances lower than predicted for age.[167] In a series with some biopsies available, 9% had a moderate decline in GFR, and 75% had decreased concentrating ability. Sclerotic glomeruli and the focal distribution of interstitial fibrosis were increased compared to age-matched controls.[168] In a large experience of 153 manic-depressive patients treated with lithium for more than 5 years, GFR was slightly decreased, but not until 17 years did the regression line of renal function versus time reach the lower limit of confidence compared to normals.[169] Urinary-concentrating ability was reduced throughout the study period and did not change with time. Dose regimen was not a factor.[169] Chronic histologic changes in the kidney correlate with patient age rather than duration of lithium treatment. Age-related decreases in GFR may reduce drug clearance, requiring dosage reduction.[170] A single case of malignant hypertension was reported in a patient who had chronic interstitial nephritis due to lithium.[171] Lithium in overdose situations may produce acute renal failure due to direct tubular damage.[172] More commonly, nephrogenic diabetes insipidus and distal renal tubular acidosis are reported. Lithium must penetrate renal cells by entry through the apical

membrane, after which it acts on vasopressin-activated adenyl cyclase and probably impedes the assembly of microtubules.[173] Recent studies in rats with nephrogenic diabetes insipidus show urea depletion of renal papillary tissue as well as a decreased ability of medullary collecting tubule and papillary collecting duct to generate cyclic AMP in response to arginine vasopressin.[174] Amiloride, in doses of 5 mg b.i.d., mitigated lithium-induced polyuria by inhibiting collecting tubule water transport, perhaps by limiting drug entry into the cell. Plasma lithium and fractional lithium clearance were unaffected by amiloride.[175]

6.3. Cisplatin

The uses and toxicities of cisplatin were extensively reviewed.[176] Finley *et al.* summarized the literature concerning vigorous hydration, diuretics, and hypertonic saline as preventive maneuvers. Nephrotoxicity is cumulative and dose-related[177] but is not enhanced by age or pretreatment renal function.[178,179] Drug concentrations in renal cortex correlate with nephrotoxicity.[180] Magnesium wasting, renal concentrating, and acidifying defects should be anticipated.[181,182] Cisplatin increased the magnitude of the negative potential difference in rat late distal tubule. This effect, which could enhance cation loss, was blocked by amiloride.[183] When cisplatin was administered within a body cavity in high doses, intravenous thiosulfate protected against nephrotoxicity.[184] Derivatives of cisplatin with less nephrotoxic potential are undergoing preclinical studies.[185] In rats, cisplatin metabolites are more nephrotoxic but have less effective antitumor activity than the parent compound.[186] Free-platinum clearance which exceeds GFR can be reduced by probenicid in normal volunteers.[187] Nephrotoxicity may be enhanced by probenecid, however.[188] Experimentally, inhibitors of organic cation transport reduce nephrotoxicity.[189] Cisplatin is a competitive inhibitor of organic cation transport in renal membrane vesicles.[190] Nephrotoxicity probably relates to intracellular biotransformation of the drug to ultimately produce cell necrosis. Renal dysfunction precedes frank tubular necrosis, suggesting that tubular obstruction or fluid backleak is not involved in the initiation of renal failure.[191]

6.4. Aminoglycoside Antibiotics

Despite good control of serum peak and trough levels, nephrotoxicity is reported in a large percentage of patients receiving high-dose, prolonged therapy for serious infections.[192] The uptake of the drug into the proximal tubule of the human kidney produces a lysosomal phospholipidosis, which is similar to that of gentamicin and tobramycin. Ami-

kacin produces less cortical uptake and less inhibition of lysosomal phospholipase A 1.[193] Risk factors for clinical nephrotoxicity were determined from case records of 214 patients by Moore *et al.* Using an elaborate statistical approach, an equation was developed to discriminate between patients with and without nephrotoxicity. Patients with liver disease, female sex, and better initial renal function more often became toxic.[194] Other workers failed to validate the utility of this approach since patients with low risk-factor scores developed renal dysfunction while others at high risk failed to do so.[195] Another analysis of aminoglycoside therapy with amikacin associated the intuitively obvious duration of therapy and area under the curve with nephrotoxicity.[196] Despite prolonged periods of subtherapeutic serum concentrations, treatment of complicated urinary tract infections was successful with single daily doses of aminoglycosides with minimal toxicity.[197,198] Reexamination of dosing strategy for aminoglycosides toward higher but less frequent dosing may achieve the dual purpose of equal efficacy with reduced nephrotoxicity.

A prospective study of aminoglycoside use revealed that more than one-third of patients treated developed hypomagnesemia complicated by hypokalemia and hypocalcemia, presumably due to toxic tubular effects.[199,200] It appears prudent to monitor serum levels during therapy. Toxic tubular effects of aminoglycosides, as indicated by enzymuria and β_2 microglobulinuria, probably occur in virtually all patients treated, making these tests unsuitable for use clinically, since nephrotoxicity has conventionally been defined based on decreases in GFR.[201,202] The long-term use of aminoglycosides in animals suggests that chronic tubulointerstitial nephropathy may occur despite relative preservation of GFR.[203] Thus, aminoglycosides should be considered as inevitable toxins, the clinical importance of which requires a decision relative to their value for treatment of infection. There is little evidence that even excellent control of serum levels will modify this toxicity.[204] Differences in renal handling and reabsorption of various aminoglycosides probably account for the known differences in nephrotoxic potential of the various congeners and the wide interindividual differences in critically ill septic patients.[205]

6.5. Radiographic Contrast Media

Mission and Cutler reviewed the published literature on radiocontrast-induced acute renal failure and concluded that clinically apparent renal failure is extremely rare without preexistant renal insufficiency, particularly in the diabetic.[206] A recent prospective study could not confirm these risk factors, although 31% of 120 patients undergoing angiography developed renal dysfunction.[207] Gomes *et al.*, using a control

group of patients undergoing CT scan without contrast, could not find an excess incidence of risk factors and emphasize that 1.5–3.5% of patients may require dialysis.[208] Newer nonionic agents seem to be less toxic in animal studies, but proof of clinical advantage is not yet available.[209–211] Nicot *et al.* reported transient nonselective proteinuria and enzymuria in a prospective study before and after arteriography in 27 patients, none of whom had clinical renal failure.[212] Increased urinary alkaline phosphatase was the most sensitive brush border enzyme marker detected.[213]

The pathogenesis of nephrotoxicity remains obscure. Although acute tubular obstruction by contrast Tamm–Horsfall glycoprotein complexes has been proposed, *in vitro*[214] and *in vivo*[215] studies produce no support for this hypothesis. Experimentally, ischemia and decreased cortical perfusion have been reported, although glomerular and tubular damage is evident in these animal models.[216] Infusion of calcium entry blockers and a calcium chelator-ameliorated contrast agent induced decreases in renal blood flow and GFR in dogs.[217] Recent evidence suggests direct toxic damage to proximal tubular cells by radiocontrast media.[218]

6.6. Nonsteroidal Antiinflammatory Drugs

Multiple recent authoritative reviews and editorials are available on the effects of nonsteroidal antiinflammatory drugs (NSAID) on prostaglandins and renal functions.[219–226] Functional renal failure due to inhibition of prostaglandins in clinical situations where renal blood flow is prostaglandin dependent continues to be commonly seen. This is particularly noteworthy in elderly patients on potassium-sparing diuretics.[227–229] Experimentally, sepsis has been identified as a risk factor.[230] Acute pyelonephritis can precipitate acute renal failure.[231] Sulfinpyrazone in normals, despite decreases in urinary prostaglandin E metabolites, did not decrease GFR, emphasizing the requirement that renal hemodynamics be prostaglandin dependent before adverse effects of prostaglandin inhibitors become clinically manifest. In addition, sulfinpyrazone competes with creatinine and PAH for tubular secretion, leading to lower clearances of these two compounds.[232] Perhaps only with massive overdose, as reported by Bennett *et al.*, would NSAIDs reduce renal function in otherwise normal individuals.[233]

Sulindac has been reported to differ from other NSAIDs in having a sparing effect on renal prostaglandin synthetase.[234,235] This has been attributed to the capacity of renal oxidative enzymes to convert the active sulfide metabolite back to its inactive producing form, sulfindac sulfoxide.[234] This may produce less adverse effect on blood pressure in

patients being treated for essential hypertension, as compared with indomethacin.[236] The capacity to oxidize the sulfide metabolite back to the inactive sulfoxide could be overcome by giving sulindac disulfide intravenously to chronic bile duct-ligated dogs.[237] Other workers have demonstrated that the putative renal-sparing effects of sulindac are only quantitative and correlate with a less potent inhibition by sulindac of cyclooxygenase-related functions, such as platelet aggregation and thromboxane synthesis.[238,239] This drug, as well as other NSAIDs, may enhance a renal ischemic stress.[240]

Interstitial nephritis mediated by immunologic mechanisms, while less common than hemodynamically induced renal dysfunction, has been associated with new congeners of varying chemical structures.[241–244] This suggests that inhibition of prostaglandins modifies important immune responses irrespective of the structure of the offending agent. Cytotoxic T cells in the renal infiltrate may also be important in the pathogenesis of glomerular morphologic changes.[245] Focal cortical necrosis following anaphylactoid shock was reported following zomepirac given for toothache pain.[246]

Hyperkalemia in elderly subjects given nonsteroidal drugs due to hyporeninemic hypoaldosteronism should be recognized so that fatalities can be avoided. This complication may be observed in the absence of overt renal failure.[247,248] Koopmans *et al.* reported that NSAIDs did not affect the antihypertensive action of thiazide diuretics,[249] while inhibition of cyclooxygenase did not impair excretion of a water load in normals.[250] Mechanisms other than cyclooxygenase inhibition were suggested for indomethacin reduction of sodium and water excretion.[250] Microscopic hematuria, papillary necrosis, as well as chronic renal failure are underemphasized consequences of NSAID use.[251–253]

Analgesic-associated nephropathy continues as an important cause of chronic renal failure in many parts of the world, including some regions of the United States.[254,255] An NIH consensus conference on the subject recommended more research on individual susceptibility, the nature of toxic metabolites, and epidemiologic factors predisposing to abuse.[256] Some have argued that since combination analgesic mixtures were virtually eliminated in the United States, this preventable renal disease has decreased in importance.[257] It is disturbing, however, that the use of acetaminophen alone, which has markedly increased in recent years, can produce analgesic nephropathy.[258] Since papillary necrosis may be clinically silent unless specifically sought, the prevalence of the disease in its milder form may be underestimated. Ultrasound can noninvasively detect calcified renal papillae surrounding the central sinus in a garland pattern suggesting the diagnosis.[259] Various animal models using analgesic and environmental chemicals may be helpful in understanding the pathogenesis of renal papillary necrosis.[260,261] The activa-

tion of prostaglandin H synthase to metabolize protoxins and procarcinogens in the inner medulla may produce oxygen-free-radical intermediates and tissue damage.[262] Secondary factors resulting from the direct toxic effects, such as acute inflammation and infection, may interact to produce evolution of the papillary necrosis to chronic renal failure.[263]

6.7. Miscellaneous Drugs

Cush and Goldings reviewed drug-induced lupus erythematosus. Renal involvement is present in 13% of patients with hydralazine lupus but is virtually nonexistent when the disease is caused by procainamide.[264] Hydralazine-induced lupus was present in 6.7% of patients in a longitudinal study even when restricted doses of less than 200 mg/day were used.[265] Rapidly progressive glomerulonephritis may occur.[268] The spectrum of renal biopsy findings in drug-induced nephropathies is presented by Jao *et al.* using illustrative cases.[267]

Drugs other than NSAIDs used to treat rheumatoid arthritis produce adverse renal reactions. Penicillamine produces various glomerulopathies, including membranous glomerulopathy and rapidly progressive glomerulonephritis with crescents. Recently IgM nephropathy and nephrotic syndrome have been reported.[268] The spectrum of pathologic glomerular lesions associated with gold treatment now includes minimal-change nephrotic syndrome and focal–segmental glomerulonephritis.[269] Experimentally, gold may release tubular basement membrane and renal epithelial antigens, which leads to antibody formation and tubulointerstitial nephritis or immune complex glomerulonephritis.[270] The new oral gold coordination complex, Auranofin, produced mild proteinuria in 0.8%, moderate proteinuria (1–3.5 g) in 0.9%, and nephrotic syndrome in 0.5% of 1800 patients. All proteinuria was reversible, and the drug could be reintroduced safely in most patients.[271] Thus, this drug is safer than other gold salts.

Renal hemodynamics can be adversely affected by angiotensin-converting enzyme inhibition initially in severe normotensive congestive heart failure. When blood pressure falls, during chronic therapy there is usually a sustained increase in renal plasma flow and maintenance of glomerular filtration rate.[272] Renal dysfunction can rarely occur in chronic renal failure patients, even in the absence of bilateral renal artery stenosis.[273,274] When renal artery stenosis is present, captopril can produce permanent anuria due to thrombosis induced by the abrupt fall in arterial pressure.[275] Sodium depletion potentiates the hemodynamic effects of the drug.[276]

Acute renal failure has been reported after overdoses with amoxapine[277] and amphetamine.[278] The former was due to rhabdom-

yolysis and the latter to interstitial nephritis. Intravenous vitamin C produced permanent renal failure in a patient with preexisting renal insufficiency.[279] A controlled study of the renal effects of enflurane and halothane in patients with abnormal renal function showed no adverse reaction to either agent.[280] Mazze *et al.* reviewed the subject of fluorinated anesthetic nephrotoxicity.[281] Ethylenediaminetetraacetic acid (EDTA) has been used widely for the unproven indication of treatment of arteriosclerosis despite its well-known nephrotoxicity. A recent case of acute renal failure due to EDTA emphasizes this hazard, which is made more tragic by the doubtful value of this therapy.[282]

Thrombotic microangiopathy with renal failure is being increasingly reported with antineoplastic chemotherapy.[283] Mitomycin seems particularly likely to produce hemolytic–uremic syndrome.[284] The barbiturate thiopental induced hemolytic anemia and acute renal failure due to antidrug antibodies.[285]

Acute interstitial nephritis continues as an unusual complication of therapy with a diverse group of drugs. Eosinophiluria expressed as a percentage of total urinary white cells is a predictor of acute interstitial nephritis when the value exceeds 5%. The simple finding of eosinophiluria does not indicate interstitial nephritis since values less than 5% are found with other conditions, most commonly upper-urinary-tract infections.[286] Immunologic mechanisms in tubulointerstitial nephritis were reviewed by Darwish and Vaziri.[287] Dicloxacillin has been implicated as a cause of renal failure in postoperative patients undergoing prophylactic treatment for joint replacement.[288] The diagnostic agent pentagastrin was associated with acute interstitial nephritis requiring temporary hemodialysis.[289] Triamterene may cause acute interstitial nephritis alone and with thiazides or nonsteroidal drugs, particularly in the elderly.[290]

Preventative measures for amphotericin B nephrotoxicity were succinctly reviewed by Warda and Barriere.[291] Recent studies have shown that experimentally, nephrotoxicity can be prevented by ouabain, which limits the amount of oxygen consumed in medullary thick ascending limb transport activity made necessary by the polyene antibiotic damage to cell membranes.[292] Renal magnesium wasting may complicate amphotericin treatment and be partially responsible for refractory hypokalemia.[293]

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