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The Physiologic Determinants of Glomerular Ultrafiltration

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I. Glossary

A_{II}	Angiotensin II.
ADH	Antidiuretic hormone.
Ach	Acetycholine.
$\bar{AP}$	Mean arterial pressure, mm Hg.
AVP	Arginine vasopressin.
BK	Bradykinin.
C	Protein concentration, g/100 ml.
FF	Whole kidney filtration fraction.
G	Gentamicin.
GFR	Glomerular filtration rate, ml/min.
k	Effective hydraulic permeability, nl/ (sec·mm Hg·cm^2).
K_f	Ultrafiltration coefficient, nl/ (sec·mm Hg).
NE	Norepinephrine.
NSN	Nephrotoxic serum nephritis.
P	Hydraulic pressure, mm Hg.
PAN	Puromycin aminonucleoside
PGE_1	Prostaglandin E_1
P_{UF}	Net ultrafiltration pressure, mm Hg.
ΔP	Transmembrane hydraulic pressure difference, $P_{GC} - P_T$, mm Hg.
Q_A	Initial glomerular capillary plasma flow rate, nl/min.
Π	Colloid osmotic pressure, mm Hg.
$\Delta\Pi$	Transmembrane osmotic pressure difference, $\Pi_{GC} - \Pi_T$, mm Hg.
R	Resistance to blood flow, dynes·s·cm^{-5} · 10^{10}
RBF / RPF	Renal blood/plasma flow rate, ml/min.
S	Surface area available for ultrafiltration, cm^2
SNFF	Single nephron filtration fraction.
SNGFR	Single nephron glomerular filtration rate, nl/min.

Superscripts

$\bar{}$	Mean Value.

Subscripts

A	Afferent arteriole.
E	Efferent arteriole.
GC	Glomerular capillary.
T	Bowman's space or beginning of proximal tubule.

II. Determinants of Glomerular Ultrafiltration in the Normal Animal

The net force determining the local rate of ultrafiltration of fluid across the glomerular capillary wall (P_{UF}), as across capillaries generally, is given by the difference between the transcapillary hydraulic pressure (ΔP), which favors filtration, and the corresponding difference in colloid osmotic pressure ($\Delta\Pi$), which opposes it. At any point along the glomerular capillary network, the local rate of ultrafiltration is equal to the product of the local effective hydraulic permeability of the capillary wall (k) and the local net driving pressure (P_{UF}). The rate of ultrafiltration for the entire capillary network of a single glomerulus is given by:

$$\begin{aligned} SNGFR &= K_f \cdot \bar{P}_{UF} \\ &= K_f (\overline{\Delta P} - \overline{\Delta \Pi}) \\ &= k \cdot S \cdot [(\bar{P}_{GC} - P_T) - (\bar{\Pi}_{GC} - \Pi_T) \end{aligned} \quad (1)$$

where SNGFR, the single nephron glomerular filtration rate, is the product of the ultrafiltration coefficient, K_f, and the net driving pressure *averaged* over the length of the capillary network, $\bar{P}_{UF}$. K_f is the product of k and the surface area available for filtration, S, and $\bar{P}_{UF}$ is the difference between the *mean* transcapillary hydraulic and oncotic pressure differences, $\overline{\Delta P}$ and $\overline{\Delta \Pi}$, respectively. The mean transcapillary hydraulic pressure difference, $\overline{\Delta P}$, is equal to the difference between mean hydraulic pressure within the glomerular capillary ($\bar{P}_{GC}$)[1] and that on the urinary side of the filtering capillary, i.e., in Bowman's space. Since pressures measured simultaneously in Bowman's space and in superficial proximal tubule segments (P_T) of the same nephron are essentially identical in magnitude (*Brenner* et al., 1971), the latter is routinely measured and its value employed in Equation 1. The mean transmural oncotic pressure difference ($\overline{\Delta \Pi}$) is equal to the difference between mean oncotic pressure of the plasma within the glomerular capillary ($\bar{\Pi}_{GC}$), and that of the filtrate in Bowman's space (Π_T). Because the composition of this filtrate is very nearly that of an ideal ultrafiltrate of plasma, the total protein concentration is extremely low (*Gaizutis* et al., 1972; *Eisenbach* et al., 1975) and Π_T may be regarded as negligible. Therefore, $\overline{\Delta \Pi}$ is essentially equal to $\bar{\Pi}_{GC}$.

A. The Mean Net Ultrafiltration Pressure

1. Transcapillary Hydraulic Pressure Difference

Recent technologic advances in micropuncture methodology and the discovery of a unique strain of Wistar rats, the so-called Munich-Wistar strain, which possess glomeruli on the renal cortical surface, thereby accessible to micropuncture, have allowed direct measurement of mean glomerular capillary hydraulic pressure, $\bar{P}_{GC}$. The magnitude of this quantity must be known in order to asses the hemodynamic determinants of the ultrafiltration process. Direct measurements of $\bar{P}_{GC}$ in these rats were made possible by application of the servo-null pressure measuring technique of *Wiederhielm* et al. (1964). This system allows the use of micropipettes with average tip diameters of 2 - 4 μ, thus permitting continuous, on-line

[1] Values for $\bar{P}_{GC}$ represent time averages. Peak-to-valley amplitudes of single glomerular capillary pressure pulses average approximately 10 mm Hg and generally bracket these time-averaged values equally during systole and diastole. (*Brenner* et al., 1971).

measurements of $\bar{P}_{GC}$ in glomerular capillaries with diameters of 8 μ or less. Utilizing these techniques, *Brenner* et al. (1971) reported the first direct measurements of $\bar{P}_{GC}$ in mammals, obtaining average values of ~45 mm Hg in these rats under conditions of normal hydropenia. These initial observations have since been reproduced repeatedly in this same laboratory (*Brenner* et al., 1972a; *Robertson* et al., 1972; *Daugharty* et al., 1974; *Deen* et al., 1974a; *Maddox* et al., 1975; *Myers* et al., 1975a, 1975b; *Baylis* et al., 1976b) as well as in laboratories elsewhere (*Blantz* et al., 1972, 1974, 1975; *Arendshorst* and *Gottschalk,* 1974; *Blantz,* 1974, 1975; *Israelit* and *Seldin,* 1974; *Ott* and *Knox,* 1975; *Andreucci* et al., 1976).

This value of $\bar{P}_{GC}$, approximately 40% of mean aortic pressure, is considerably lower than most earlier indirect estimates obtained using the stop-flow method. In this stop-flow technique, it is assumed that when flow of fluid along the tubule is interrupted by insertion of an oil block, pressure within the tubule will rise and eventually attain a value sufficient to prevent continued filtration. The value of P_T so measured is termed the stop-flow pressure. According to Equation 1, when the sum of this measure of P_T and Π_{GC} (both are quantities which oppose the formation of ultrafiltrate) becomes equal to the hydraulic pressure within the glomerular capillary, $\bar{P}_{GC}$, filtration will cease[2], the net force favoring filtration having been reduced to zero. Hydraulic pressure within the glomerulus has, therefore, been estimated indirectly as the sum of the stop-flow pressure, P_T, and the intracapillary colloid osmotic pressure which, in the absence of filtration, is assumed to equal Π_A. Values for $\bar{P}_{GC}$ obtained in this way, however, have varied from ~ 35-90 mm Hg under control hydropenic conditions (*Jaenike*, 1969; *Gertz* et al., 1966). For a variety of reasons beyond the scope of this review, the act of stopping flow in the tubule may of itself elevate the value of $\bar{P}_{GC}$; thus, the validity of this technique has been questioned (*Wright* and *Giebisch,* 1972).

As further support for the conclusion that the relatively low values of $\bar{P}_{GC}$ obtained by direct measurement in the Munich-Wistar rat are representative of rats generally, *Brenner* et al. (unpublished observations) have obtained similarly relatively low values in a number of adult rats of the commonly studied Sprague-Dawley strain, in which occasional superficial glomeruli have also been encountered. *Källskog* and co-workers (1975a), however, have reported much higher values for $\bar{P}_{GC}$, averaging approximately 63 mm Hg in surface glomeruli in hydropenic rats of a Sprague-Dawley strain bred in Sweden. Certain pathologic features of the

[2] The assumption that filtration may cease completely following sufficient elevation in P_T has been questioned by several investigators since it is probable that reabsorption of previously formed filtrate by renal tubules will result in "replacement" filtration (*Wright* and *Giebisch,* 1972).

kidneys of these rats have been noted, however, by *Brenner* and *Troy* (unpublished observations) including the findings of bilateral ureteral and pelvic dilation in each of 16 consecutively studied rats supplied to these investigators by this same Swedish group. In view of these abnormalities which went unrecognized by *Källskog* et al. (1975a), concern arises as to the physiologic significance of the relatively high values for $\bar{P}_{GC}$ originally reported for this Swedish strain since, as will be discussed in a subsequent section of this review, values for $\bar{P}_{GC}$ have been shown to increase when ureteral pressure is elevated. Indeed, the often very high values for $\bar{P}_{GC}$ inferred from stop-flow measurements, discussed above, may also reflect this same response to elevated ureteral or tubule pressure and must, therefore, be interpreted with caution. Although studies involving direct measurements of $\bar{P}_{GC}$ are usually conducted in the Munich-Wistar rat, there is reason to believe that the value of $\bar{P}_{GC}$ is similar in other mammals as well. Thus, in studies in the squirrel monkey, a small primate also possessing accessible surface glomeruli, *Maddox* et al. (1974) reported similarly low values for $\bar{P}_{GC}$, again averaging ~45 mm Hg, or some 40% of the mean aortic pressure under conditions of normal hydration.

Given these direct measurements of $\bar{P}_{GC}$, together with measurements of the other pertinent pressures, it becomes possible to evaluate the net driving force for ultrafiltration as a function of distance along the glomerular capillary network. The value of $\bar{P}_{GC}$ may be taken to be the same at afferent and efferent ends of the network since the axial pressure drop along the network, due to flow, appears to be extremely small[3]. P_T averages ~ 10 mm Hg under normal hydropenic conditions is both rat and monkey. Therefore, the length average value of the glomerular transcapillary hydraulic pressure difference, $\overline{\Delta P}$, is approximately 35 mm Hg in both rat and monkey (Fig. 1).

[3] Some axial pressure drop must exist in order for blood to flow through the capillary. That this pressure drop is small is supported by the finding that the sum of the pressure opposing filtration at the efferent end of the glomerular capillary (as inferred from the sum of efferent arteriolar oncotic pressure, Π_E, plus proximal tubule hydraulic pressure, P_T) reaches a value that, on average, balances P_{GC}. Since P_{GC} is measured at random sites along the glomerular capillary, the finding that $\Pi_E + P_T = P_{GC}$ indicates that P_{GC} changes little along the capillary network. The sensitivity of the methods used to determine Π_E, P_T and P_{GC} makes it likely that this pressure drop is no greater than 2 - 3 mm Hg. Thus, the measured value of P_{GC} is essentially the same as the value of P_{GC} averaged over the length of the glomerular capillary ($\bar{P}_{GC}$).

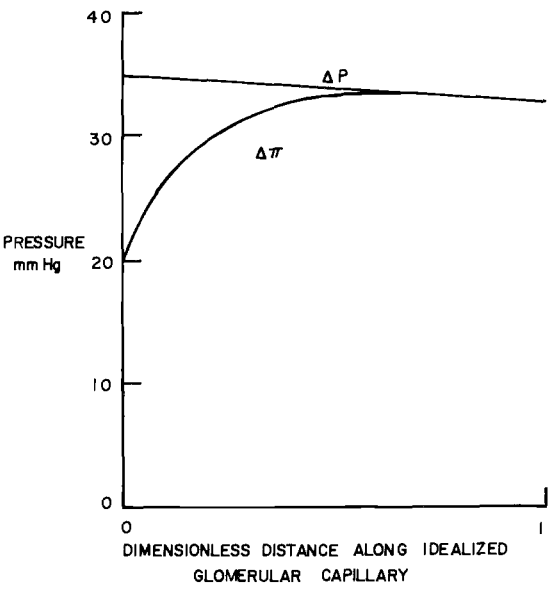

Fig. 1. Hydraulic and colloid osmotic pressure profiles along an idealized glomerular capillary in the rat. $\Delta P = P_{GC} - P_T$ and $\Delta\Pi = \Pi_{GC} - \Pi_T$, where P_{GC} and P_T are the hydraulic pressures in glomerular capillary and Bowman's space, respectively, and Π_{GC} and Π_T are the corresponding colloid osmotic pressures

2. *Transcapillary Oncotic Pressure Difference*

Measurements of total plasma protein concentration (C) at afferent and efferent ends of the glomerular network, C_A and C_E, respectively, reveal that C rises as blood flows along the glomerular network, a consequence of the fact that the filtrate is essentially protein-free. In practice, the value for C_A is not measured directly, but is taken to be equal to the value of C measured in systemic (usually femoral) arterial blood plasma. The value of C_E is determined from measurements on blood plasma obtained directly from surface efferent arterioles (*Brenner* et al. 1972b). Values for C_A and C_E in hydropenia average 5 - 6 g%, and 8 - 9 g%, respectively. Oncotic pressure, Π, calculated from these measured values of C_A and C_E[4], therefore increases from a minimum value of ~20 mm Hg at the afferent end (Π_A) of the capillary network to approximately 35 mm Hg by the efferent end (Π_E) (Fig. 1). The local net ultrafiltration pressure, P_{UF}, the difference between ΔP and $\Delta\Pi$ at any given point along the glomerular capillary, therefore declines from a maximum of ~ 15 mm Hg at the afferent end of the network to zero by the efferent end, as shown in Figure 1. In other words, under conditions of normal hydropenia, $\Delta\Pi$ rises to a value by

[4] $\Delta\Pi$ increases in a nonlinear fashion with increases in C according to the equation: $\Delta\Pi = a_1 C + a_2 C^2$
For protein concentrations in the range of $4<C<10$ g/100 ml and a normal albumin to globulin concentration ratio of unity, a_1 = 1.63 mm Hg/ (g/100 ml) + a_2 = 0.294 mm Hg/ (g(100 ml)^2.

the efferent end of the capillary network which, on average, equals and opposes ΔP, and thus prevents further net filtration (*Brenner* et al., 1971, 1972a; *Robertson* et al., 1972; *Blantz*, 1974, 1975; *Daugharty* et al., 1974; *Deen* et al., 1974a; *Maddox* et al., 1975; *Myers* et al., 1975a, 1975b; *Baylis* et al., 1976b). This equality of ΔP and $\Delta\Pi$ is referred to as *filtration pressure equilibrium,* a term first introduced by *Smith* and coworkers in 1940.

In view of the relative constancy of the value of ΔP with distance along the length of the glomerular capillary, the decline in the local value of P_{UF} from an afferent (maximum) value of ~ 15 mm Hg to the efferent (minimum) value of zero is almost entirely the consequence of the progressive increase in $\Delta\Pi$. It is of importance to appreciate that the profile of $\Delta\Pi$ along the glomerular capillary is more complicated than its hydraulic counterpart, as shown by Figure 1, where the rate of change of Π along the glomerular capillary is seen to be highly nonlinear. This nonlinearity occurs because the local rate of ultrafiltration is proportional to the local net driving pressure, $\Delta P - \Delta\Pi$, so that formation of ultrafiltrate will occur most rapidly at the afferent end of the capillary network. Thus, glomerular capillary protein concentration, and hence $\Delta\Pi$, will increase most rapidly near the afferent end of the capillary. An additional effect contributing to the exponential increase in oncotic pressure with distance along the glomerulus relates to the fact that Π increases in a nonlinear manner with increases in C, as described by the expression given in Footnote 4.

Since measurements of Π can only be performed on samples of systemic blood (which are taken as representative of blood at the afferent end of the glomerular capillary) and blood from efferent arterioles, the exact profile of the change in Π with distance along the capillary cannot be determined by direct measurement. The $\Delta\Pi$ curve in Figure 1 is but one of an infinite number of profiles consistent with the measurements of Π_A and Π_E obtained in hydropenia. This point is emphasized further in Figure 2 which shows several possible $\Delta\Pi$ profiles along an idealized glomerular capillary for a given value of ΔP. For values of Π_A and Π_E representative of normal hydropenia in the rat, a multiplicity of $\Delta\Pi$ curves, including example curves A and B, satisfy the conditions of filtration pressure equilibrium, namely, that the *local* net driving pressure, P_{UF}, declines from the same maximum value at the afferent end of the capillary to zero by the efferent end. The *mean* net ultrafiltration pressure. $\bar{P}_{UF}$, equal to the area between the ΔP and $\Delta\Pi$ curves in Figure 1. represents the *local* net driving force (P_{UF}) integrated over the entire length of the glomerular capillary network. In view of the uncertainty in determining the exact oncotic pressure profile in an animal at filtration pressure equilibrium (i.e., when $\Pi_E \equiv \overline{\Delta P}$), it is not possible to ascertain an exact or unique value for $\bar{P}_{UF}$. Only a maximum value for $\bar{P}_{UF}$ may be

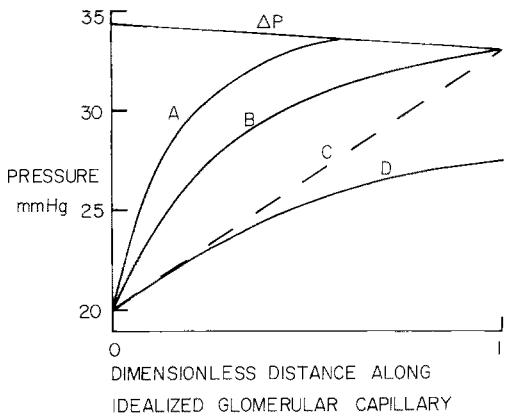

Fig. 2. Several possible profiles of the transcapillary colloid osmotic pressure difference ($\Delta\Pi$) under hypothetical conditions of filtration pressure equilibrium (*curves A, B,* and *C)* and disequilibrium (*curve D*) for a given transcapillary hydraulic pressure difference, ΔP, along an idealized glomerular capillary

obtained and only then by assuming: (1) a linear rate of rise of $\Delta\Pi$ along the glomerular capillary, as indicated by the dashed line (curve C) in Figure 2, and (2) that $\Delta\Pi$ only becomes equal to ΔP at the efferent-most point along the glomerular capillary. The extent to which these assumptions overestimate the true value of $\bar{P}_{UF}$ will, therefore, depend upon: (1) the degree of departure of the true $\Delta\Pi$ curve from linearity, and (2) the exact site at which filtration pressure equilibrium is actually reached along the glomerular network. For example, if curve A in Figure 2 is taken as the true $\Delta\Pi$ profile, corresponding to given values for Π_A and Π_E, and curve C is the assumed linear $\Delta\Pi$ profile, the latter will obviously greatly overestimate the true value of P_{UF}, even though curves A and C both employ the same values for Π_A and Π_E. Alternatively, if curve B is taken to represent the true $\Delta\Pi$ profile, again corresponding to the given values of Π_A and Π_E, but with filtration equilibrium being attained at the efferent-most point of the capillary, the difference between true and maximum values for $\bar{P}_{UF}$ will be less, as shown. Given these uncertainties in calculating an exact value for $\bar{P}_{UF}$ under conditions of filtration pressure equilibrium, it is likewise impossible to calculate an unique value for the glomerular ultrafiltration coefficient, K_f. This can readily be seen by rearranging Equation 1, thus:

$$K_f = \frac{SNGFR}{\bar{P}_{UF}} \tag{2}$$

For any given measured value of SNGFR, a unique K_f value requires a unique value for $\bar{P}_{UF}$, which, as we have discussed, is an inexact quantity under conditions of filtration pressure equilibrium. Nevertheless, a minimum value of K_f can be adduced, using a maximum value of $\bar{P}_{UF}$ in Equation 2, obtained by employing the assumptions which give rise to curve C in Figure 2.

Because of this uncertainty in the determination of $\Delta\Pi$ profiles under conditions of normal hydropenia (i.e., when filtration pressure equilibrium obtains), a mathematical model of glomerular ultrafiltration was developed by *Deen* et al. (1972), in an attempt to gain further insight into the filtration process and also to identify experimental conditions which would allow a precise determination of the $\Delta\Pi$ profile, and hence $\bar{P}_{UF}$ and K_f. In this model, conservation of mass and the Starling hypothesis are used to derive a differential equation giving the rate of change of protein concentration with distance along an idealized glomerular capillary network. The numerical solution of this equation can be used to compute $\Delta\Pi$ profiles for given values of C_A, K_f, $\overline{\Delta P}$, and the initial glomerular plasma flow rate, Q_A. Details of the computations and assumptions employed in this model are given in detail elsewhere (*Deen* et al., 1972). Development of the model led to the prediction that with sufficient increases in the value of Q_A (and in the absence of significant changes in any of the other determinants of ultrafiltration) the rate of rise of C, and thus Π, along the capillary network would be sufficiently reduced to prevent attainment of filtration pressure equilibrium. In other words, with sufficient elevation in Q_A, the value of Π attained by the efferent-most point of the glomerular capillary (Π_A) would be expected to be significantly less than the corresponding value of ΔP, as depicted by example curve D in Figure 2. The advantage of circumstances in which filtration pressure equilibrium in *not* obtained (i.e., $\Pi_E < \overline{\Delta P}$) is that only one $\Delta\Pi$ curve can connect the measured values of Π_A and Π_E, given the constraints imposed by conservation of mass and the Starling hypothesis. Thus, when disequilibrium obtains, a unique $\Delta\Pi$ profile can be computed from measured values of C_A, C_E, $\overline{\Delta P}$, and Q_A.

B. The Ultrafiltration Coefficient

This approach of increasing Q_A to values which ensure disequilibrium has been employed experimentally in the Munich-Wistar rat by *Deen* et al. (1973b) to enable calculation of unique $\Delta\Pi$ profiles and thus, unique values for $\bar{P}_{UF}$ and K_f. In this study, infusion of isoncotic rat plasma intravenously, in volumes equal to 5% of body weight, served to increase Q_A to some 200 nl/min, thereby resulting in disequilibrium. Calculated values of K_f in this study were found to average ~0.08 nl/ (s·mm Hg). Furthermore, K_f, was found to remain essentially unchanged within a twofold range of changes in Q_A, suggesting that K_f is unaffected by variations in Q_A per se (*Deen* et al., 1973b). This insensitivity of K_f to changes in Q_A has also been found to hold under conditions of primary

glomerular injury, where K_f is less than normal (*Chang* et al., 1976). Furthermore, in recent studies in which Q_A was increased to disequilibrium levels without plasma volume expansion, i.e., during isovolemic reduction in hematocrit, values for K_f were similar, averaging $\sim$ 0.07 nl/ (s·mm Hg) (*Myers* et al., 1975b). Because K_f appears to be insensitive to variations in Q_A, these values for K_f, obtained in studies in normal animals in which Q_A had been elevated, are likely to reflect an upper limit for K_f in the normal hydropenic Munich-Wistar rat, where Q_A averages $\sim$ 75 nl/min, and the existence of filtration pressure equilibrium prevents calculation of unique values for K_f. This view is further supported by the finding, using the mathematical model and typical values for pressures and flows measured during normal hydropenia, that these same K_f values ($\sim$ 0.07 - 0.08 nl/ (s·mm Hg)) yield calculated values for SNGFR virtually identical to those measured in hydropenia. Taking this value of K_f, together with measured values for SNGFR, it follows from Equation 2 that during hydropenia the mean net ultrafiltration pressure, $\bar{P}_{UF}$, averages approximately 4 - 6 mm Hg in the normal Munich-Wistar rat and rises to $\sim$ 8 - 12 mm Hg with plasma volume expansion, thereby accounting for the elevated values of SNGFR measured under plasma loaded conditions (*Brenner* et al., 1972 a; *Deen* et al., 1973b; *Baylis* et al., 1976c; *Blantz* et al., 1976).

Since $K_f = k \cdot S$ (Eq. 1), the effective hydraulic permeability (k) of the glomerular capillary wall can be calculated from K_f and available estimates of the filtration surface area (S). Using the value of $S = 0.0019\ cm^2$ obtained for the rat glomerulus by *Kirkman* and *Stowell* (1942) and the value for K_f = 0.08 nl/ (s·mm Hg) for the normal Munich-Wistar rat (*Deen* et al., 1973b), the calculated value of effective hydraulic permeability of the glomerular capillary wall (k) = 42.1 nl/ (s·mm Hg·cm^2). Employing a more recent estimate of $S = 0.0028\ cm^2$ obtained by *Shea* and *Morrison* (1975) for the Sprague-Dawley rat and the same value for K_f of 0.08 nl/ (s·mm Hg), the calculated value of k = 28.6 nl/ (s·mm Hg·cm^2). Taking either of these values for k, it is apparent that the effective hydraulic permeability of the glomerulus for the rat is approximately one to two orders of magnitude greater than that reported for capillaries in other tissues in a variety of species, including frog mesentery (*Landis,* 1927, 1928; *Brown* and *Landis,* 1947), rat skeletal muscle (*Smaje* et al., 1970), rat peritubular capillaries (*Deen* et al., 1973a), and rabbit omentum (*Zweifach* and *Intaglietta,* 1968; *Lee* et al., 1971). This very high hydraulic permeability, therefore, allows glomerular ultrafiltration to proceed rapidly despite a mean driving pressure that is normally only $\sim$5 mm Hg in the normal hydropenic rat.

C. The Single Nephron Filtration Fraction

The single nephron filtration fraction, SNFF, the fraction of the initial glomerular plasma flow rate that undergoes filtration, may be expressed as:

$$SNFF = \frac{SNGFR}{Q_A} \tag{3}$$

SNFF may be calculated experimentally from measured values of C_A and C_E., thus:

$$SNFF = 1 - \frac{C_A}{C_E} \cong 1 - \frac{\Pi_A}{\Pi_E} \tag{4}$$

which, at filtration pressure equilibrium, can be represented as:

$$\cong 1 - \frac{\Pi_A}{\overline{\Delta P}}$$

Thus, since at filtration pressure equilibrium the value of SNFF is independent of Q_A and K_f, SNFF will ultimately be determined by C_A and $\overline{\Delta P}$, as given above in Equation 4. The value of $\overline{\Delta P}$ limits the final value to which C_E (and therefore Π_E) rises by the efferent end of the capillary. The dependence of filtration fraction on C_A can also be depicted in the manner shown in Figure 3. The solid curves in this figure show Π_E as a function of SNFF, each curve corresponding to a different assumed value of C_A (ranging from 5 - 7 g/100 ml). The horizontal dashed line corresponds to the approximate mean value of $\overline{\Delta P}$ measured in the normal hydropenic rat. Since the average value of C_A in the rat is $\sim$ 5.5 g/100 ml, and since the rat is normally at filtration pressure equilibrium (where $\Pi_E \cong$

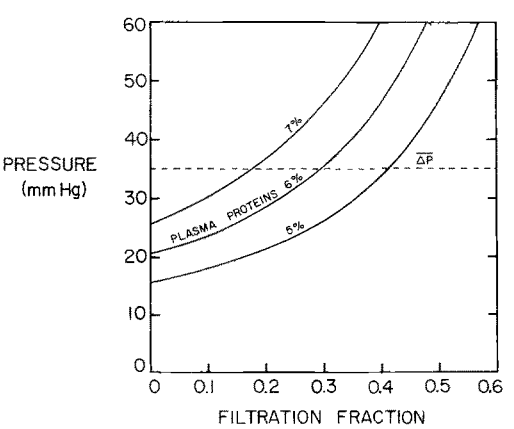

Fig. 3. The dependence of filtration fraction on systemic plasma protein concentration (C_A), assuming filtration pressure equilibrium and a value for the mean glomerular transcapillary hydraulic pressure difference ($\overline{\Delta P}$) of 35 mm Hg. The *curved lines* denote values for Π_E computed from C_A for given values of filtration fraction

$\overline{\Delta P}$), the appropriate C_A curve and dashed line ($\overline{\Delta P}$) intersect at a value of SNFF of ~0.33, the approximate value for the rat observed experimentally (*Brenner* et al., 1971, 1972a; *Robertson* et al., 1972; *Blantz*, 1974, 1975; *Blantz* et al., 1974, 1975; *Daugharty* et al., 1974; *Deen* et al., 1974a; *Maddox* et al., 1975; *Myers* et al., 1975a, 1975b; *Baylis* et al., 1976b). Furthermore, in observations in the squirrel monkey, $\overline{\Delta P}$ has also been found to equal ~35 mm Hg, but in this primate mean values of C_A are higher (~7 g/100 ml) and SNFF lower (~0.2) than in the rat, (*Maddox* et al., 1974), in accord with Figure 3. Since C_A and filtration fraction (determined from whole kidney clearance data) in man are similar to values in the monkey, it follows that if filtration pressure equilibrium exists in man, $\overline{\Delta P}$ will also be some 35 mm Hg.

III. Effects of Selective Variations in the Various Determinants of SNGFR

As shown in Equation 1, changes in SNGFR may be evoked by alterations in either the ultrafiltration coefficient, K_f, or in the net driving force for ultrafiltration, $\bar{P}_{UF}$, which, in turn, is determined by $\overline{\Delta P}$, Π_A, Q_A, and K_f. Using the mathematical model of *Deen* et al. (1972), it is possible to estimate the theoretical effects of selective perturbations in these four determinants of ultrafiltration, and these are discussed below.

A. Selective Variations in Initial Glomerular Plasma Flow Rate

It is evident from Figure 2 that for an animal at filtration pressure equilibrium, changes in Q_A in the absence of changes in C_A and $\overline{\Delta P}$ will not affect the efferent protein concentration, C_E, and thus Π_E, since at equilibrium Π_E will be determined solely by the value of $\overline{\Delta P}$. Under these circumstances, SNFF will remain constant and SNGFR will vary in direct proportion to the changes in Q_A. Large increases in Q_A, however, tend to produce disequilibrium, and, therefore, progressively lower values of C_E and thus Π_E, since Π_E no longer rises to a value high enough to equal and oppose $\overline{\Delta P}$. Thus, for given values of K_f, $\overline{\Delta P}$, and C_A at disequilibrium, it follows from Equation 3 that SNFF must decrease with increasing Q_A.

The theoretical relationship between SNFF and Q_A is shown in Figure 4A, calculated assuming values of K_f, C_A, and $\overline{\Delta P}$ representative of the normal hydropenic Munich-Wistar rat. As shown, SNFF is predicted to remain at a constant value of 0.33 for values of Q_A up to ~100 nl/min, or roughly one-third greater than the normal hydropenic value of ~75 nl/min. Further increases in Q_A, however, lead to progressively lower values of

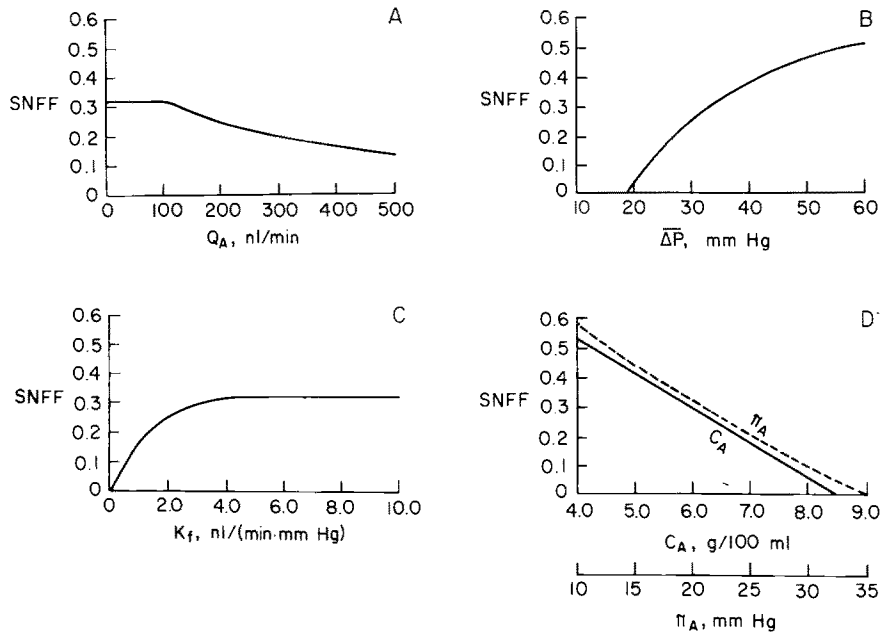

Fig. 4 A - D. The predicted effects on SNFF of selective perturbations in the four determinants of ultrafiltration, Q_A, $\overline{\Delta P}$, K_f, and C_A (or Π_A). Unless specified otherwise, Q_A = 75 nl/min, $\overline{\Delta P}$ = 35 mm Hg, K_f = 0.08 nl/ (sec·mm Hg), and C_A = 5.7 g/100 ml (Π_A = 19 mm Hg)

SNFF. A relationship between Q_A and SNFF quantitatively similar to that derived theoretically (Fig. 4A) has been found experimentally in the rat. In the absence of experimental measurements of $\overline{\Delta P}$ and K_f in other mammals, including man, it is not yet possible to quantify the relationship between SNFF and Q_A in the same detail for other species as that shown for the Munich rat in Figure 4A.

The theoretical dependence of SNGFR on Q_A is shown in Figure 5A. It follows from Equation 3 that as long as SNFF remains constant, SNGFR will vary linearly with Q_A, for values of Q_A up to ~100 nl/min, as shown. For higher values of Q_A, where disequilibrium obtains and SNFF declines, SNGFR is predicted to increase less than in proportion to increases in Q_A, hence the departure of the solid curve from the dashed line, the latter denoting a constant filtration fraction of 0.33. Even at disequilibrium, however, SNGFR will be expected to be plasma-flow dependent, but to a lesser extent than when equilibrium obtains. Experimental observations in the Munich-Wistar rat made in the authors' laboratory fully substantiate this predicted relationship between Q_A and SNGFR. Figure 6 depicts mean experimental data from a variety of studies, together with the same theoretical curve relating SNGFR and Q_A

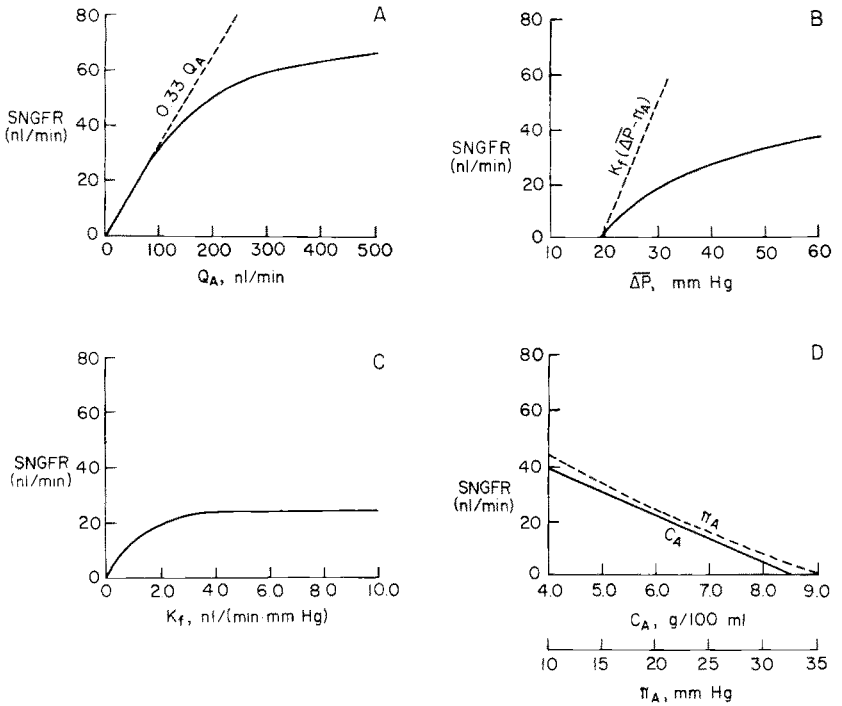

Fig. 5 A - D. The predicted effects on SNGFR of selective perturbations in the four determinants of ultrafiltration, $Q_A \cdot \overline{\Delta P}$, K_f, and C_A (or Π_A), for the same conditions as in Fig. 4. The *dashed lines* in A and B are given by SNGFR = 0.33 Q_A and SNGFR = K_f ($\overline{\Delta P}$ - Π_A), respectively

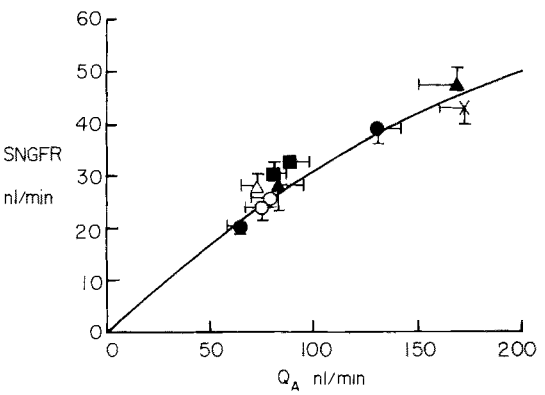

Fig. 6. The observed relationship between SNGFR and Q_A, derived from mean data (±1 SE) obtained in experimental studies in the Munich-Wistar rat where mean values of C_A and $\overline{\Delta P}$ and calculated values for K_f were similar to those observed in normal hydropenia. The *closed squares* denote control hydropenic data obtained by *Myers* et al. (1975b), while the *open circles* refer to data obtained during progressive aortic constriction (*Robertson* et al., 1972). The *closed circles,cross* (X), and *closed triangles* refer to experimental observations before and during isoncotic plasma-volume expansion (*Brenner* et al., 1972a;*Deen* et al., 1973b): (*Baylis* et al., 1976c). Finally, the *open triangle* refers to data obtained in the unilaterally nephrectomized rat (*Deen* et al., 1974a). Also shown is the theoretical relationship between SNGFR and Q_A (*solid curve)* reproduced from Fig. 5A

employed in Figure 5A. Reductions in Q_A below the normal hydropenic level of ~75 nl/min were achieved by either isovolemic elevation in hematocrit (*Myers* et al., 1975b) or progressive aortic constriction (*Robertson* et al., 1972). Over the range of values of Q_A from subnormal to normal, animals remained at filtration pressure equilibrium, and SNGFR was found to vary in proportion to variations in Q_A, as predicted, indicating a high degree of *plasma-flow dependence* of SNGFR. Further, when Q_A was elevated experimentally to levels above $\sim$ 75 nl/min, the observed dependence of SNGFR on Q_A is seen to conform to theoretical predictions. The maneuvers by which Q_A was elevated to supernormal values include various degrees of plasma volume expansion, at times accompanied by carotid occlusion or partial aortic constriction (*Deen* et al., 1973b); *Baylis* et al., 1976c). Elevation in Q_A was also achieved, in the absence of plasma volume expansion, by isovolemic reduction in hematocrit (*Myers* et al., 1975b). Finally, markedly elevated values for Q_A were also induced by unilateral nephrectomy (*Deen* et al., 1974a).

The mechanism whereby selective alterations in Q_A affects SNGFR involves Q_A-dependent variations in the mean net ulfrafiltration pressure, $\bar{P}_{UF}$. Selective decreases in Q_A displace the point at which filtration pressure equilibrium is reached towards the afferent end of the glomerular capillary, as from curve B to curve A in Figure 2, thus reducing the area between ΔP and $\Delta\Pi$ curves and hence, $\bar{P}_{UF}$. Conversely, increases in Q_A displace the $\Delta\Pi$ profile toward the efferent end of the capillary, causing elevation in P_{UF} and hence, SNGFR.

1. Plasma-Flow Dependence of (SN) GFR as an Index of the Presence or Absence of Filtration Pressure Equilibrium

The degree of dependence of SNGFR on Q_A (or whole kidney GFR on renal plasma flow) has been used as an index for assessing whether filtration pressure equilibrium obtains in animals such as man and dog, where direct measurements of $\bar{P}_{GC}$, and, therefore, $\overline{\Delta P}$ are not at present available. It has been argued that the normally hydrated dog is not at filtration equilibrium since large increases in total renal plasma flow, RPF, induced by infusion of vasodilator drugs failed to change total GFR or SNGFR appreciably (*Knox* et al., 1975). An assumption fundamental to this conclusion, however, is that vasodilator drugs selectively increase renal plasma flow and do *not* significantly alter the other determinants of GFR.

It is important to recognize that even in the Munich-Wistar rat, an animal known normally to be at filtration pressure equilibrium, increases in Q_A evoked by infusion of vasodilator hormones such as acetylcholine,

prostaglandin E_1, or bradykinin similarly fail to increase SNGFR appreciably (*Baylis* et al., 1976b). Of importance, this failure of SNGFR to rise with Q_A in the rat was found to be the result of an offsetting effect of marked vasodilator-induced reductions in K_f. In view of these reductions in K_f, the assumptions relating to the conclusions cited above for the dog given vasodilators may not be valid. Nevertheless, other experimental maneuvers designed to elevate RPF suggest that differences may indeed exist between rat and dog regarding the plasma-flow dependence of (SN)GFR. For example, volume expansion with colloid-free solutions produces plasma-flow-dependent increases in SNGFR in the rat (*Brenner* et al., 1972a), whereas in the dog, infusion of an equivalent volume of fluid fails to significantly affect GFR, producing marked increases in RPF (*Davis* et al., 1969). Similarly, hyperoncotic albumin infusions in the Munich rat result in marked increases in both Q_A and SNGFR (*Blantz* et al., 1974), while similar infusions in the dog, although increasing RPF, have little effect on GFR (*Knox* et al., 1975). On the other hand, certain perhaps more physiologic perturbations in the dog, such as protein feeding (*Reinhardt* et al., 1975; *O'Connor* and *Summerill,* 1976), developmental maturation of the kidney (*Horster* and *Valtin,* 1971; *Kleinman* and *Reuter,* 1974), and acute renal denervation (*Berne,* 1952; *Surtshin* et al., 1952; *Kamm* and *Levinsky,* 1965), are associated with a marked parallelism between renal blood flow and GFR. At the present time, therefore, it remains uncertain whether filtration pressure equilibrium does or does not obtain normally in dog and man, where reliable measurements of $\overline{\Delta P}$ are not yet possible.

B. Selective Variations in the Glomerular Transcapillary Hydraulic Pressure Difference

The predicted effects of selective variations in the glomerular transcapillary hydraulic pressure difference ($\overline{\Delta P}$) on both SNFF and SNGFR are shown in Figures 4B and 5B. Ultrafiltration of fluid across the walls of the glomerular capillary network occurs only when the local value of ΔP exceeds $\Delta\Pi$. Therefore, for values of $\overline{\Delta P}$ less than $\sim$ 20 mm Hg (the normal value for Π_A), SNFF and SNGFR are zero since even at the afferent end of the glomerular capillary, no driving force for ultrafiltration exists. As $\overline{\Delta P}$ increases above this threshold value, however, SNFF and SNGFR are shown to increase. The predicted rate of increase in SNFF and SNGFR is less for larger values of $\overline{\Delta P}$. These nonlinear relationships result from the fact that as $\overline{\Delta P}$ is increased, the resulting increase in the rate of ultrafiltration leads to a concurrent, but smaller, increase in $\overline{\Delta\Pi}$. This accompanying rise in $\overline{\Delta\Pi}$ tends to partially offset the increment in

$\overline{\Delta P}$. Were this increase not to occur, $\bar{P}_{UF}$ and, therefore, SNGFR would rise in a linear fashion with increases in $\overline{\Delta P}$, as given by the dashed line in Figure 4B, the slope of which is K_f.

In animals at filtration pressure equilibrium, the value of SNFF is determined solely by the value of $\overline{\Delta P}$ and C_A, since $\overline{\Delta P}$ determines the level to which Π_E may rise (Eq. 4). Once disequilibrium occurs, however, and $\overline{\Delta P}$ is no longer the sole determinant of Π_E, SNFF tends to vary inversely with Q_A and directly with the ultrafiltration coefficient, K_f in addition to being determined in part by C_A and $\overline{\Delta P}$.

It has proved difficult to evaluate the effects of selective variations in $\overline{\Delta P}$ on SNFF and SNGFR experimentally, since maneuvers designed to alter $\overline{\Delta P}$, such as aortic constriction or carotid occlusion, also produce marked variations in Q_A (*Deen* et al., 1973b). Further, since $\overline{\Delta P}$ seems to be quite effectively autoregulated under most experimental situations, changes of more than ±5 mm Hg rarely occur. However, Figure 7 shows data derived from several different experimental groups in which values for C_A and K_f were essentially constant, while $\overline{\Delta P}$ varied significantly. The relationship between SNGFR and $\overline{\Delta P}$ is shown for two levels of Q_A:(1) $Q_A \cong 60$ nl/min, and (2) $Q_A \cong 135$ nl/min (*Brenner* et al., 1972a; *Robertson* et al., 1972; *Deen* et al., 1974a; *Myers* et al., 1975b); also included is the theoretical curve (Fig. 5B) calculated for $Q_A \cong 80$ nl/min. It is apparent from Figure 7 that the experimental relationship between SNGFR and $\overline{\Delta P}$ concurs with theoretical predictions described above in that lowering $\overline{\Delta P}$ results in lowered SNGFR, while elevations in $\overline{\Delta P}$ are associated with relatively small increases in SNGFR. As also predicted by theory and as shown in Figure 7 for experimental data, changes in $\overline{\Delta P}$ evoke less profound alterations in SNGFR when compared to the effects of proportionally similar variations in Q_A. Because of this, and the near constancy of $\overline{\Delta P}$ under many experimental situations, due to its effective autoregulation (discussed below), variation in $\overline{\Delta P}$ will usually play a less important role in determining SNGFR than will changes in Q_A.

C. Selective Variations in the Ultrafiltration Coefficient

The theoretical relationship between K_f and both SNFF and SNGFR are shown in Figures 4C and 5C, assuming values of Q_A, C_A, and $\overline{\Delta P}$ representative of the normal hydropenic rat. If K_f is greater than $\sim$ 0.06 nl/(s·mm Hg), SNFF remains constant at $\sim$ 0.33 and SNGFR varies directly with $\bar{P}_{UF}$ (Eq. 1); thus, both SNFF and SNGFR are predicted to be independent of K_f in an animal at filtration pressure equilibrium. Therefore, so long as K_f achieves a value large enough to yield filtration pressure equilibrium, further increases in K_f will fail to affect the values of

SNFF and SNGFR, as shown in Figures 4C and 5C. According to these predictions, given an estimated value of 0.08 nl/ (s·mm Hg) for the normal Munich-Wistar rat, it is noteworthy that a selective reduction in K_f of at least 50% would be required to evoke even a 20% reduction in SNGFR.

A reduction in K_f to a value below ~0.05 nl/ (s·mm Hg) will lead to disequilibrium, causing SNFF to fall (Fig. 4C). In the absence of any compensating changes in the other determinants of ultrafiltration, SNGFR will also fall in proportion to the fall in K_f. It is, therefore, unlikely that variation in the absolute value of K_f will have an important influence on SNGFR except under conditions in which K_f or its determinants, capillary hydraulic permeability, and/or surface area are reduced markedly. Profound reductions in K_f associated with filtration pressure disequilibrium, have been demonstrated following induction of experimental glomerulonephritis in the rat (*Maddox* et al., 1975) and in studies of acute renal failure by *Blantz* (1975) and *Cox* and co-workers (1974). Moreover, similar appreciable reductions in K_f have been found by *Baylis* et al. (1976c) to attend experimental reductions in serum protein concentration, as well as the infusion of a variety of vasoactive substances (*Baylis*

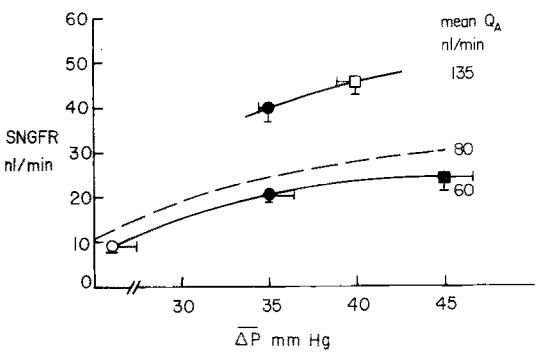

Fig. 7. The observed relationship between SNGFR and $\overline{\Delta P}$ for two levels of Q_A: (1) $Q_A \cong 60$ nl/min, and (2) $Q_A \cong 135$ nl/min. Mean data (±1 SE) are shown from several experimental studies in the Munich-Wistar rat, again with mean values for C_A and K_f being representative of those obtained during normal hydropenia. The *closed square* denotes data obtained during isovolemic elevation in hematocrit, where the mean value for $Q_A = 49 \pm 7$ nl/min (*Myers* et al., 1975b), and the *open circle* refers to observations made during aortic constriction, where the mean value for $Q_A = 51 \pm 9$ nl/min. The *closed circles* refer to data obtained before and during isoncotic plasma volume expansion where Q_A averaged 69±11 nl/min and 131±13 nl/min, respectively (*Brenner* et al., 1972a), while the *open square* denotes data obtained in the uninephrectomized rat, where the mean value of $Q_A = 136 \pm 14$ nl/min (*Deen* et al., 1974a). Also shown is the theoretical relationship between SNGFR and $\overline{\Delta P}$, for a value of $Q_A = 80$ nl/min (*dashed line*) from Fig. 5B. It can be seen that irrespective of the precise value of Q_A, the relationship between $\overline{\Delta P}$ and SNGFR observed experimentally conforms to the slope predicted by theory

et al., 1976b; *Blantz* et al., 1976) (see below). Because these experimentally observed reductions in K_f are invariably attended by marked changes in one or more of the other determinants of ultrafiltration, it is not possible to experimentally isolate the relationship between K_f and SNGFR. However, analysis of data from several studies in which K_f was reduced, using the mathematical model of *Deen* et al. (1972), has substantiated the predictions given in Figures 4C and 5C.

D. Selective Variations in Afferent Protein Concentration

The theoretical relationship between C_A (or Π_A) and both SNFF and SNGFR are shown in Figures 4D and 5D, again assuming values for K_f, $\overline{\Delta P}$, and Q_A representative of the normal hydropenic rat. It is apparent that both SNFF and SNGFR are predicted to vary reciprocally with C_A. As C_A (and thus Π_A) is reduced, the oncotic force opposing ultrafiltration is reduced and, according to Equation 1, in the absence of any other changes, the value of $\bar{P}_{UF}$ will be increased. The effect of reducing Π_A alone on SNFF and SNGFR is, therefore, predicted to be similar to the effect of selectively increasing $\overline{\Delta P}$, in that both perturbations increase $\bar{P}_{UF}$. As can be seen in Figures 4D and 5D, as Π_A approaches $\overline{\Delta P}$ ($\sim$ 35 mm Hg for the rat), SNFF and SNGFR both approach zero, since $\bar{P}_{UF}$ also approaches zero.

A recent study by *Baylis* et al. (1977) was carried out to investigate the effect of reductions in C_A (and thus Π_A) on the determinants of SNGFR in the Munich-Wistar rat. C_A was reduced by a variety of maneuvers, including colloid-free Ringer loading, dextrose-saline loading, and exchange transfusion with blood containing a low plasma protein concentration. In these experiments, SNGFR failed to rise *as predicted*, despite large measured increases in $\bar{P}_{UF}$. As shown in Figure 8, reductions in C_A from normal hydropenic values of 5-6 g% to values of 3-4 g% were attended by marked falls in K_f, which served to offset the effect of raising $\bar{P}_{UF}$ (the latter by lowering C_A). *Blantz* (1974) made similar observations on the Munich-Wistar rat where infusion of mannitol produced dilution of plasma proteins and marked reductions in the measured value for K_f. Moroever, *Blantz* et al (1974) showed that elevation in C_A, induced by infusion of hyperoncotic albumin, was again paralleled by increases in K_f.

There are many other, albeit less direct, observations also supporting this dependence of K_f on C_A. For example, in several studies in the isolated perfused kidney of dog and rat, reduction in perfusate total protein concentration (and thus Π_A) from normal to low values was not attended by the expected rise in GFR (*Nizet*, 1968; *Bowman* and *Maack*, 1974; *Little* and *Cohen*, 1974). Furthermore, *Klahr* and *Alleyne* (1973) have

pointed out that in chronic protein malnutrition in man, low values for C_A are more commonly associated with reduced, rather than elevated values of GFR. In the intact dog, *Vereerstraeten* and *Toussaint* (1969) selectively and acutely reduced C_A with little concomittant change in plasma volume or renal blood flow and failed to observe systematic elevations in GFR, in spite of significant falls in C_A. Finally, a relationship between C_A and K_f might explain the observations of *Arendshorst* and *Gottschalk* (1974), in which hydropenic Munich-Wistar rats were found to be at filtration pressure disequilibrium and to have low values for both C_A and K_f.

At present, the mechanism by which reduction in C_A (or Π_A) evokes this fall in K_f is unclear. However, in the study by *Baylis* et al. (1977) the low C_A-dependent reduction in K_f evoked by Ringer loading was found to be acutely reversible upon restoration of C_A to or toward normal values by hyperoncotic plasma infusion. Whether this dependence of K_f on C_A reflects a specific effect of plasma proteins or an effect of

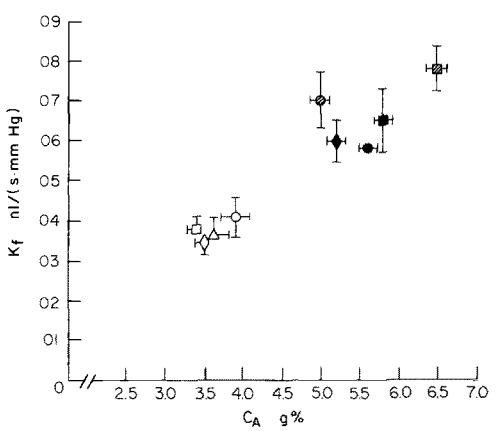

Fig. 8. Summary of the relationship between K_f and C_A. All *open symbols* denote animals in which C_A was reduced acutely while *solid and cross hatched symbols* denote data obtained in normoproteinemic rats. The *open square* denotes data obtained in Ringer-loaded animals while the *solid square* denotes data obtained in isoncotic plasma-loaded control rats. The *open triangle* refers to hypotonic dextrose-saline loaded animals. The *open and closed circles* refer to data obtained in exchange transfusion studies, in which exchange blood contained low and normal protein concentrations, respectively. The *open and solid diamonds* refer to animals in which C_A was initially reduced by bicarbonate-Ringer loading, and then selectively restored toward normal values by infusion of concentrated plasma proteins, respectively. The *cross-hatched circle and square* denote normoproteinemic data previously obtained in this laboratory. The *cross hatched square* refers to data obtained during isoncotic plasma loading (*Deen* et al., 1973b), while the *cross-hatched circle* refers to a group of animals in which isovolemic reduction in hematocrit led to filtration pressure disequilibrium without volume expansion (*Myers* et al., 1975b). Data are shown as mean ± 1 SE. Reproduced from *Baylis* et al. (1977) with permission of the publisher

colloid osmotic pressure, generally, on K_f is at present unclear. Recent work by *Nizet* (1968) on the isolated dog kidney is of interest in this regard. He observed that when perfusate oncotic pressure was raised from low to normal values with albumin, the predicted fall in GFR failed to occur. However, when dextran was used to alter perfusate oncotic pressure over a similar range, the expected fall in GFR was found. These observations suggest that the dependence of K_f on perfusate oncotic pressure may be specific for plasma proteins, rather than a property of colloids generally. However, since glomerular pressures and flows were not measured in these experiments of *Nizet* (1968), it remains a possibility that reduced values for $\overline{\Delta P}$ during perfusion with dextran might have accounted for the reduction in GFR observed during dextran perfusion.

Whether a similar direct relationship between C_A and K_f exists when C_A is raised to values exceeding normal is at present unknown. As discussed previously, however, once filtration pressure equilibrium is reached, increases in K_f will have little effect on SNGFR, serving only to displace the $\Delta\Pi$ profile toward the afferent end of the glomerular capillary. Therefore, in the event that high values of C_A lead to high values of K_f, the effect will probably be of little physiologic significance, in comparison to the low C_A-dependent reductions in K_f already discussed for animals normally at filtration pressure equilibrium (i.e., when $C_A \sim 5$ - 6 g%).

IV. Autoregulation of SNGFR

It is well known that the kidney exhibits an ability to maintain relative constancy of blood flow in spite of marked variations in mean arterial pressure ($\overline{AP}$) above a value of $\sim$80 mm Hg. This phenomenon, termed autoregulation of blood flow, has been demonstrated in both denervated and isolated kidney preparations (*Forster* and *Maes,* 1947; *Waugh,* 1964) and, therefore, appears to be a property intrinsic to the renal vasculature and independent of extrarenal or humoral elements. Furthermore, many investigators have demonstrated that in addition to blood flow, GFR is also autoregulated in the kidneys of man, dog, and the rat (*Forster* and *Maes,* 1947; *Selkurt* et al., 1949; *Gomez,* 1951; *Shipley* and *Study,* 1951; *Smith,* 1951; *Thurau,* 1964; *Robertson* et al., 1972).

Use of the Munich-Wistar rat preparation affords an opportunity to directly investigate the effects of alterations in $\overline{AP}$ on pressures, flows, and resistances in single preglomerular, glomerular, and postglomerular vessels. Figure 9A summarizes the effects in the normal hydropenic rat of graded reductions in $\overline{AP}$ on Q_A and SNGFR (upper panel), glomerular

transcapillary pressure differences (middle panel), and resistances of single afferent and efferent arterioles (lower panel) (*Robertson* et al., 1972). As can be seen, when $\overline{AP}$ was reduced from 120 mm Hg to 80 mm Hg, only modest falls in Q_A and SNGFR were noted. Further reduction in $\overline{AP}$ to 60 mm Hg resulted in steeper declines in both Q_A and SNGFR, indicating that the autoregulatory ability of the kidney was attenuated during further hypotension. Over the range of values of $\overline{AP} \sim 120$ - 80 mm Hg (the so-called autoregulatory range of $\overline{AP}$), SNFF fell slightly, indicating that the decline in SNGFR was almost entirely due to the nearly proportional decline in Q_A, as shown. When $\overline{AP}$ was reduced below ~ 80 mm Hg, however, SNFF fell notably. Therefore, the reduction in SNGFR observed with values of $\overline{AP} < 80$ mm Hg was proportionally greater than the accompanying reduction in Q_A. Despite the declines in $\overline{AP}$ from 120 to 80 mm Hg, values for $\Delta\overline{P}$ ($\overline{P}_{GC} - P_T$) declined from mean values of 36 to 30 mm Hg (middle panel, Fig. 9A). The further reduction in $\overline{AP}$ from 80 to 60 mm Hg resulted in a further fall in $\Delta\overline{P}$ (from 30 to 26 mm Hg). Because P_T remained essentially constant over the range of $\overline{AP}$ studied,

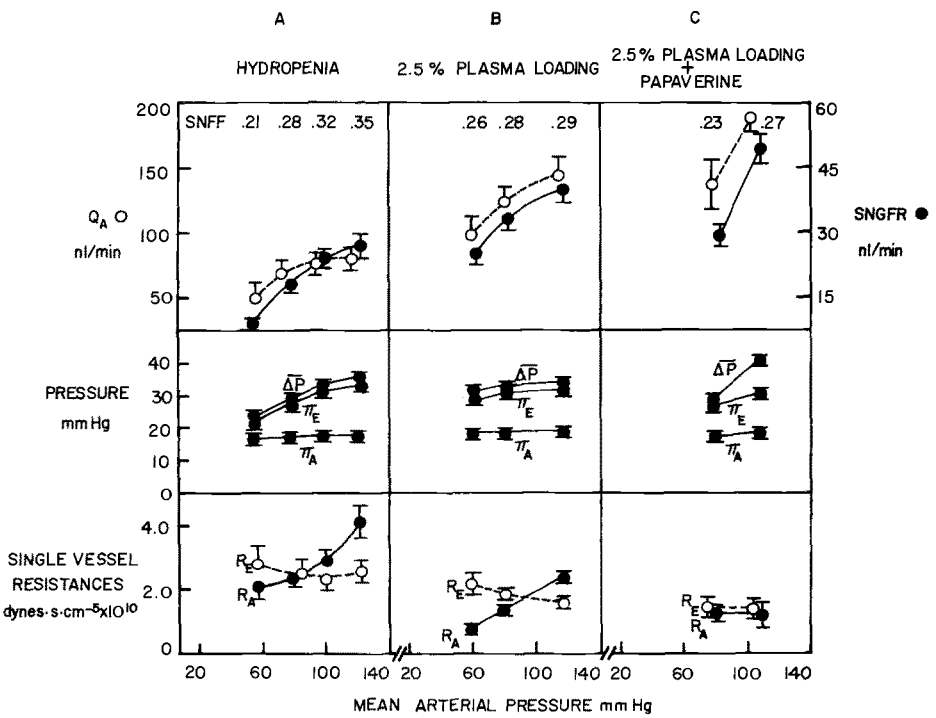

Fig. 9 A - C. Summary of the effects of reducing mean arterial pressure ($\overline{AP}$) on the measured determinants of glomerular ultrafiltration in normal hydropenic Munich-Wistar rats (A), and mildly plasma-loaded Munich-Wistar rats before (B) and during (C) the administration of papaverine. Data are shown as mean ± SE

the reductions in $\overline{\Delta P}$ were due almost entirely to falls in $\bar{P}_{GC}$. Constancy of Π_A was maintained throughout, while Π_E fell with $\overline{\Delta P}$, so that filtration pressure equilibrium was maintained at each level of $\overline{AP}$ studied. The falls in $\overline{\Delta P}$ were due to decreases in R_A and little change in R_E, as shown in the lower panel of Figure 9A. In this same study, the effect of induction of a mild degree of plasma volume expansion on the autoregulatory capability of the rat kidney was investigated. Figure 9B summarizes the effects of variation in $\overline{AP}$ over a range of values from 115 to 60 mm Hg. In these animals, the declines in SNGFR and Q_A with decreasing $\overline{AP}$ were less than was seen during hydropenia. Also, values for SNFF were essentially constant over the entire range of $\overline{AP}$ studied; thus, declines in SNGFR were paralled by the falls in Q_A. In these plasma-loaded rats, reduction in $\overline{AP}$ had little effect on $\bar{P}_{GC}$ or P_T; thus, $\overline{\Delta P}$ remained almost constant, as shown in the middle panel of Figure 9B. Values for Π_A and Π_E were similarly unaffected by changes in $\overline{AP}$, and the close equality between Π_E and $\overline{\Delta P}$ indicates that filtration pressure equilibrium was maintained throughout the arterial pressure range examined in these plasma-loaded rats.

This remarkable constancy of $\overline{\Delta P}$ was maintained by a marked fall in R_A and simultaneous increase in R_E[5] (bottom panel, Fig. 9B). The resistance changes seen to occur in afferent and efferent arteriolar vessels were far more profound during plasma loading than were seen to occur in the hydropenic rat (bottom panel, Fig. 9A), accounting for the enhanced autoregulatory ability exhibited by the plasma-loaded rat.

The results of this investigation on the autoregulatory ability of the renal circulation of the rat (*Robertson* et al., 1972) are compatible with the findings reviewed by *Gore* and *Bohlen* (1975) for capillaries in the mesentery of the dog and cat, where near constancy of capillary hydraulic pressure is also known to be maintained over a wide range of perfusion pressure. In further analogy with the renal cortical microcirculation, *Johnson* and *Hanson* (1962) have suggested that this near constancy of mesenteric capillary hydraulic pressure is the consequence of a simultaneous decrease in precapillary resistance (equivalent to R_A for the

[5] The values for afferent and efferent arteriolar resistances (R_A and R_E, respectively) are calculated as follows:

$$R_A = \frac{(\overline{AP} - \bar{P}_{GC}}{GBF} \cdot (7.962 \cdot 10^{10})$$

$$R_E = \frac{(\bar{P}_{GC} - P_C}{EGBF} \cdot (7.962 \cdot 10^{10})$$

Where GBF and EGBF are afferent and efferent glomerular blood flows, respectively, and P_C is the peritubular capillary hydraulic pressure. The factor $7.962 \cdot 10^{10}$ gives values of resistance in units of dyne·s·cm^{-5}.

renal microcirculation) and elevation in postcapillary resistance (equivalent to R_E). The probability that these alterations in R_A and R_E were mediated, at least in part, by changes in arteriolar diameter is suggested by the findings illustrated in Figure 9C. During administration of papaverine, a potent smooth muscle relaxant, the autoregulatory responses of R_A and R_E to reductions in $\overline{AP}$ in the plasma-loaded rat were essentially abolished (*Deen* et al., 1974b). As a result, $\overline{\Delta P}$ no longer remained constant, but instead declined in proportion to the reduction in $\overline{AP}$. Under these circumstances, SNGFR fell precipitously with decreasing $\overline{AP}$, proportionally more than the fall in Q_A; hence, the decline in SNFF, as shown. In other words, following papaverine, the decline in SNGFR appears to have resulted from reduction in two of the determinants of ultrafiltration, Q_A and $\overline{\Delta P}$. The reduction in $\overline{\Delta P}$ served to reduce SNGFR more than in proportion to the fall in Q_A alone.

These studies in the Munich-Wistar rat indicate that autoregulation of GFR is mainly the consequence of autoregulation of renal plasma flow, since GFR is highly plasma-flow dependent. Perfect autoregulation of renal plasma flow will tend to ensure perfect autoregulation of GFR. Assuming filtration equilibrium and constancy of Π_A, the degree to which $\overline{\Delta P}$ declines in response to reductions in $\overline{AP}$ will determine whether filtration fraction remains constant or declines. At equilibrium, near constancy of filtration fraction will result if $\overline{\Delta P}$ remains relatively constant, as in the plasma-loaded rat, since SNGFR will change only in proportion to the change in Q_A. Reductions in filtration fraction will occur, however, when $\overline{\Delta P}$ declines moderately, as in the normal hydropenic rat, and to a greater extent in the papaverine-treated rat, since SNGFR will fall more than in proportion to the reduction in Q_A.

It should be noted that in a recent study by *Källskog* et al. (1975b) on the Sprague-Dawley rat, no autoregulation of blood flow was found in the outer cortical portions of the kidney, in spite of pronounced autoregulation of inner cortical and whole kidney blood flow. These observations differ from those of *Robertson* et al. (1972) and *Deen* et al. (1974b) (discussed above) where superficial cortical nephrons showed marked autoregulation of both blood flow and SNGFR. They are also at variance with earlier observations from the same Swedish group (*Wolgast*, 1968; *Grängsjö* and *Wolgast*, 1972) and others (*Aukland*, 1966; *Løyning*, 1971) where no differences were found between cortical and medullary regions of the kidney in regard to autoregulatory ability. The reasons for these disparate observations of *Källskog* et al. (1975b) are at present unclear, although it is possible that certain pathologic features of this Swedish Sprague-Dawley rat strain (discussed above) mignt be responsible.

V. Effects of Various Hormones and Vasoactive Drugs on the Determinants of SNGFR

The actions of a variety of hormones and drugs on GFR and SNGFR have been investigated by many workers in an effort to define their effect on the factors controlling the rate of glomerular filtration. Inferences regarding the precise mode of action of some commonly studied substances, such as acetylcholine (Ach), prostaglandin E_1 (PGE_1), bradykinin (BK), angiotensin II (AII), norepinephrine (NE), and epinephrine have usually been based on estimates of their effects on inulin and PAH clearances (i.e. whole kidney GFR, renal plasma flow, filtration fraction) and $\overline{\Delta P}$. The usual interpretation of the results of such studies has been based on the view that the filtration fraction (FF) and GFR are primarily dependent on the mean transcapillary hydraulic pressure difference, $\overline{\Delta P}$, thought to be varied by changes in afferent and/or efferent arteriolar tone. For example, afferent arteriolar constriction and thus a selective *increase* in afferent resistance, R_A, is usually assumed to lead to reduction in renal blood flow with a resultant fall in $\overline{\Delta P}$. As a consequence of this fall in $\overline{\Delta P}$, FF and GFR will also decline. Alternatively, if efferent arteriolar resistance, R_E is selectively *increased,* it will usually be assumed that $\overline{\Delta P}$ will rise even though renal blood flow declines, thereby increasing filtration fraction, with little, if any, decline in GFR. Selective *decreases* in R_A or R_E are viewed as having effects opposite to those described above. These assumptions have led to predictions regarding the site of action on renal resistance vessels of various vasoactive drugs, based solely on observations of the effects on $\overline{\Delta P}$, whole kidney GFR, renal plasma flow, and filtration fraction evoked by such drugs.

In view of the evidence reviewed above, however, indicating that ΔP is considerably lower than previously believed, and that GFR is highly plasma-flow dependent, the possible hemodynamic responses to changes in R_A and R_E become more difficult to predict. For example, as shown in Figure 10 (lower panel), an increase in R_A might elicit at least four possible combinations of responses. Whether renal blood flow remains unchanged or declines moderately or markedly cannot be predicted from simple hydrodynamic considerations. Similarly, the magnitude or direction of changes in $\overline{\Delta P}$ and filtration fraction cannot be anticipated, despite the likelihood that GFR will decrease in all four cases. The situation for a selective increase in R_E is likewise complicated in that given the expected decline in RBF, GFR could conceivably decrease, increase, or remain unchanged, depending on the extent to which $\overline{\Delta P}$, and hence FF, increase. Possible effects of reductions in R_A or R_E are shown in the upper panel of Figure 10 and appear to be equally complex. From the foregoing argument it becomes apparent that it is not possible to

HEMODYNAMIC RESPONSES TO SELECTIVE CHANGES IN RENAL ARTERIOLAR RESISTANCES

RESISTANCE CHANGE	RBF	$\overline{\Delta P}$	FF	GFR
R_A ↓	↑	0	0	↑
	0	↑	↑	↑
	↑	↑	↑	↑
	↑↑	↓	↓	↑
R_E ↓	↑	↓	↓	0 or ↑
	↑	↓↓	↓↓	↓
R_A ↑	↓	0	0	↓
	0	↓	↓	↓
	↓	↓	↓	↓
	↓↓	↑	↑	↓
R_E ↑	↓	↑	↑	0 or ↓
	↓	↑↑	↑↑	↑

Fig. 10. Possible effects of selective variation in R_A and R_E on renal blood flow (RBF), mean transcapillary hydraulic pressure difference ($\overline{\Delta P}$), filtration fraction (FF), and GFR

predict the mechanism by which vasoactive and other substances influence GFR in the absence of direct measurements of the glomerular pressures and flows governing ultrafiltration. This shortcoming has been remedied with the availability of the Munich-Wistar rat, in which the effects of many drugs on renal resistances can now be readily examined directly.

A. Vasodilator Substances

It is well established that dilation of the kidney vasculature of the dog with potent vasodilators such as PGE_1, Ach, or BK produces significant elevation in renal blood flow rate in spite of which total GFR and SNGFR fail to change significantly (*Johnston* et al., 1967; *Willis* et al., 1969; *Baer* et al., 1970). As discussed above, this apparent lack of plasma-flow dependent behavior of (SN)GFR has been taken as evidence against the existence of filtration pressure equilibrium in this species (*Knox* et al., 1975). An alternative explanation is that the constancy of SNGFR, in the face of vasodilator-induced increases in Q_A, is due to a compensatory and offsetting effect of one or more of the other determinants of SNGFR, such as a possible reduction in $\overline{\Delta P}$ and/or K_f. *Baylis* et al. (1976b) recently

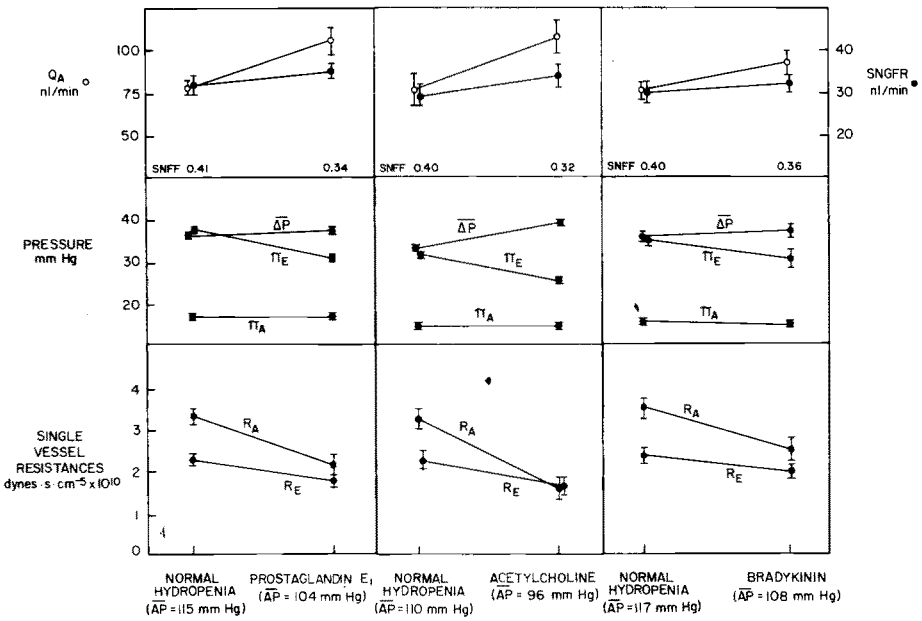

Fig. 11. Summary of effects of intra-arterial infusion of prostaglandin E_1 (0.4 - 0.8 μg/kg/min), acetylcholine (2 - 3 μg/min), and bradykinin (1 μg/kg/min) on the measured determinants of glomerular ultrafiltration in the Munich-Wistar rat. Data are shown as means ±1 SE

investigated the actions of mildly vasodepressor doses of PGE_1, Ach, and BK on the determinants of SNGFR in the Munich-Wistar rat. As shown in Figure 11 (top panels), the characteristic increase in Q_A and near constancy of SNGFR was observed to occur with all three drugs. Thus, the gross renal response to drug-induced vasodilation is similar in these rats to that observed in dogs. These increases in Q_A were due to marked falls in the values of afferent and efferent arteriolar resistances, R_A and R_E (bottom panels, Fig. 11). Because of the proportionally greater fall in R_A than R_E, vasodilator-induced increases in Q_A were not attended by falls in $\overline{P}_{GC}$ or $\overline{\Delta P}$ (middle panels, Fig. 11). Since C_A, and therefore Π_A, remained unaffected by the drugs, as shown in the middle panels (Fig. 11), the only remaining explanation for the observed near constancy of SNGFR is that the increase in Q_A must have been opposed by a fall in K_f. In the study by *Baylis* et al. (1976b), filtration pressure equilibrium failed to obtain during administration of PGE_1, Ach, or BK, (due to marked falls in Π_E, as shown in Fig. 11), thus permitting calculation of unique values for K_f during infusion of the drugs. As shown in Figure 12, PGE_1, Ach, and BK were found to produce significant reductions in K_f in comparison to the mean control values for K_f of 0.07 - 0.08 nl/(s·mm Hg) obtained during either isoncotic plasma leading (*Deen* et al. 1973b) or isovolemic

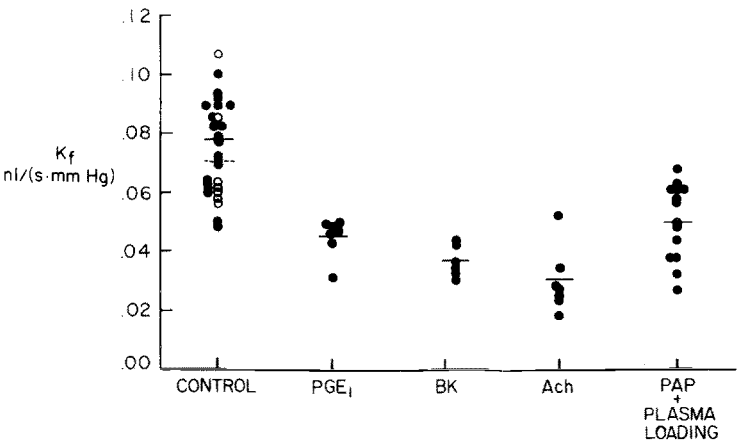

Fig. 12. Summary of individual and mean values for K_f calculated for a variety of conditions which produce filtration pressure disequilibrium. The *first column* shows control data obtained during isoncotic plasma volume expansion, *closed circles* (*Deen* et al., 1973b) and isovolemic reduction in hematrocrit, *open circles* (*Myers* et al., 1975b). *Other columns* show the effects of administering PGE_1, Ach, BK (*Baylis* et al., 1976c), and papaverine (PAP), (*Deen* et al., 1974b) on the calculated value for K_f. Reproduced from *Baylis* et al. (1976b) with permission of the publisher

reduction in hematocrit (*Myers* et al., 1975b). In addition to the vasodilators studied by *Baylis* et al. (1976b), an earlier study by *Deen* et al. (1974b) reported reductions in K_f following administration of the vasodilator drug, papaverine (Fig. 12). This observation of vasodilator drug-induced reductions in K_f in the Munich-Wistar rat provides an attractive explanation for the apparent failure of (SN)GFR to change despite marked drug-induced increases in glomerular (or total renal) plasma flow reported for both the dog and rat.

B. Vasoconstrictor Substances

The vasoconstrictor hormones, including norepinephrine (NE) and angiotension II (AII), are known to be capable of producing marked reductions in renal plasma flow, but generally little change in GFR (*Smythe* et al., 1952; *Zimmerman* et al., 1964; *Gagnon* et al., 1970; *Regoli* and *Gauthier,* 1971; *Bonjour* and *Malvin,* 1974). In order to assess the precise mode of action of these vasoconstrictors, *Myers* et al. (1975a) investigated the effects of NE and AII on preglomerular, glomerular, and postglomerular pressures and flows in the Munich-Wistar rat. As shown in Figures 13A and 13C, pressor doses of NE and AII resulted in relative stability of SNGFR despite moderate (NE) and marked (AII) falls in Q_A. These falls

in Q_A were associated with significant increases in R_A and R_E, due to constriction of both afferent and efferent arterioles. The value of $\bar{P}_{GC}$ rose markedly with these drugs, and since P_T remained essentially unchanged, $\overline{\Delta P}$ also increased markedly (middle panels, Fig. 13 A and 13C). Values for Π_A remained unchanged during infusion of the vasoconstrictor hormones while Π_E rose significantly, to values essentially equal to the value of $\overline{\Delta P}$. Therefore, filtration pressure equilibrium obtained both before and during infusion of both drugs. Given this persistance of filtration pressure equilibrium during NE and AII infusion, SNGFR is independent of the value of K_f, as discussed above. SNGFR is, therefore, determined solely by $\bar{P}_{UF}$, which in turn is determined by Q_A, $\overline{\Delta P}$, and Π_A. Since Π_A remained unchanged in these studies, the observed constancy of SNGFR resulted from offsetting effects of (1) reduction in Q_A, and (2) elevation of $\overline{\Delta P}$. These observations have since been confirmed in the Munich-Wistar rat for a similar dose of AII (*Bohrer* et al., 1976). Of interest is the extent to which the rise in $\overline{\Delta P}$ observed with NE and AII is dependent on the simultaneous rise in mean aortic pressure, which regularly occurs with the doses of AII and NE employed in these studies. To examine this question, experiments with identical doses of NE and AII were repeated by *Myers* et al. (1975a) in separate groups of Munich-Wistar rats in which constriction of the aorta prevented the rise in $\overline{AP}$ and thus in renal perfusion pressure. The results are summarized in Figures 13B and 13D. It can be seen that when the aorta is constricted during infusion of both hormones, the increase in R_A is strikingly attenuated with AII and abolished with NE. Changes in R_E, SNGFR, Q_A, SNFF, $\overline{\Delta P}$, and Π_E, however, are similar to those seen when the rise in $\overline{AP}$ was permitted. These observations suggest that the increase in R_A which occurs when $\overline{AP}$ rises is the result, not of a pharmacologic effect of the vasoconstrictors per se, but is secondary to the rise in renal perfusion pressure, perhaps the consequence of a myogenic reflex. In other words, it appears that the direct pharmacologic actions of both NE and AII are confined to the efferent arteriole, a site of action suggested by *Richards* and *Plant* (1922) for the hormone, epinephrine, more than 50 years ago. This raises the possibility that α-adrenergic receptors are localized to this postglomerular vessel.

Because filtration pressure equilibrium obtained during NE and AII infusion into hydropenic rats, assessment of possible effects on these vasoconstrictor drugs on the unique value for K_f was not possible in the studies by *Myers* et al. (1975a) and *Bohrer* et al. (1976) discussed above. *Blantz* et al. (1976), however, have investigated the effects of both subpressor and pressor doses of native and synthetic AII in the plasma-loaded Munich-Wistar rat. In these studies, filtration pressure disequilibrium obtained before and during AII infusion, thus enabling calculation of

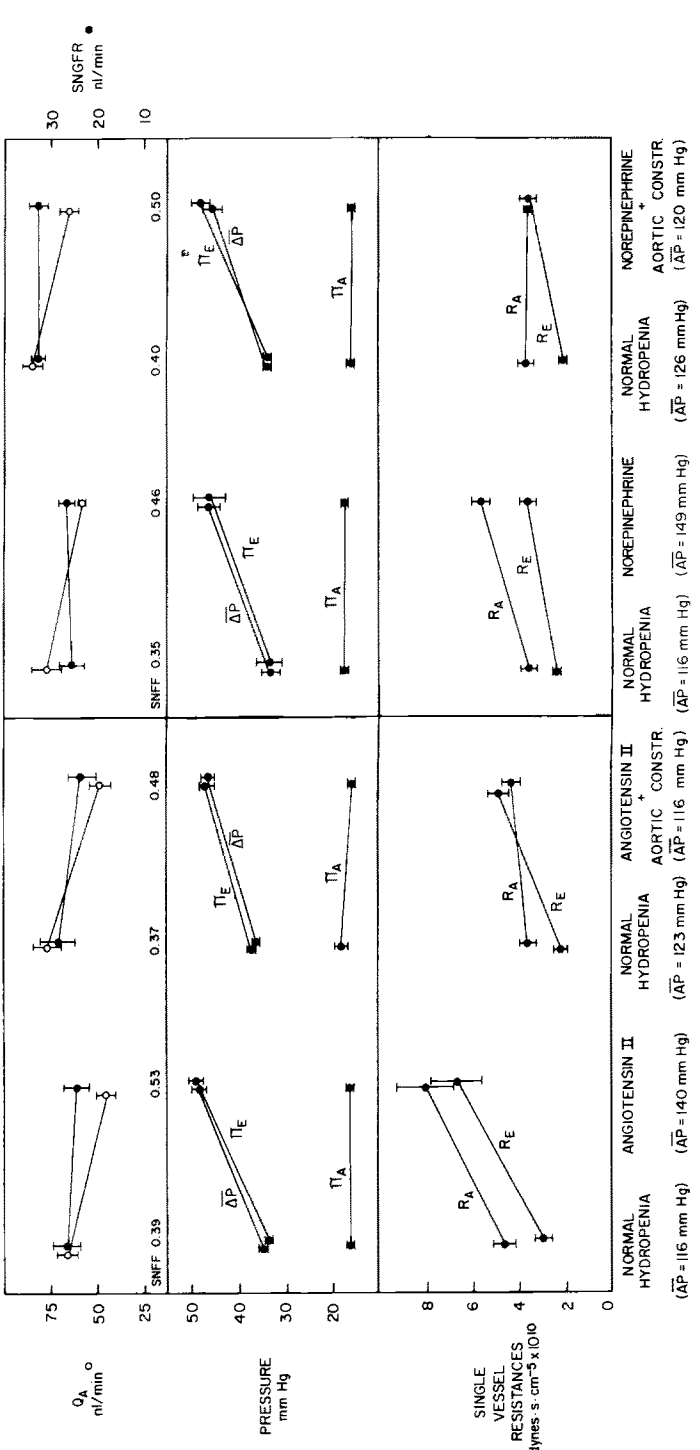

Fig. 13. Summary of effects of intravenous infusions of norepinephrine (2 - 4 μg/kg/min) and angiotension II amide (0.2 - 0.6 μg/kg/min) on the measured determinants of glomerular ultrafiltration in the Munich-Wistar rat. *Panels A* and *C* represent results obtained when renal perfusion pressure ($\overline{AP}$) was allowed to increase; results obtained during prevention of this increase in $\overline{AP}$ are summarized in *panels B* and *D*.

unique values for K_f both before and during drug infusion. These workers found significant reductions in K_f, to $\sim$ 50% of control values, during infusion of AII. Whether this effect of AII in reducing K_f obtains during hydropenia is unclear; the observed near constancy of SNGFR in the studies of *Myers* et al. (1975a) and *Bohrer* et al (1976) appeared to be adequately explained by the offsetting effects of reduction in Q_A and elevation in $\overline{\Delta P}$. As discussed above, however, SNGFR is insensitive to reductions in K_f when filtration pressure equilibrium obtains (Fig. 5C). Thus, an AII-evoked reduction in K_f of the magnitude described by *Blantz* et al. (1976) might well have been masked by the offsetting effect of reduced Q_A in preventing production of filtration pressure disequilibrium.

C. Antidiuretic Peptides

The antidiuretic hormone, ADH, is known to exert an influence on GFR in amphibia, reptiles, and birds (*Sawyer,* 1951; *Burgess* et al., 1933; *Dantzler,* 1967; *Ames* et al., 1971; *Braun* and *Dantzler,* 1974). It is generally believed, however, that the action of ADH in mammals is limited to the distal portion of the nephron, where its action in regulating osmotic water flow is well established, (*Handler* and *Orloff,* 1973; *Jamison,* 1976). Nevertheless, there are certain observations which suggest that ADH may also exert in action on glomerular dynamics in mammalia. For example, *Davis* and *Schnermann* (1971) have suggested that ADH influences SNGFR in the rat, and *Imbert* et al. (1974) have detected an ADH-sensitive adenyl cyclase enzyme system in the isolated rabbit glomerulus.

A systematic investigation of the possible glomerular actions of ADH in the mammal was recently undertaken by *Ichikawa* and *Brenner* (1977). Experiments were performed in Munich-Wistar rats made chronically water diuretic (in order to suppress endogenous production of ADH). A summary of the effects on glomerular dynamics of infusion of synthetic arginine vasopressin (AVP, structurally identical to native rat ADH) is shown in Figure 14. During infusion of AVP, which raised urine osmolality from 151 ± 15 to 315 ± 32 mosmol, it is apparent that the mean value for $\overline{\Delta P}$ rose significantly, and since mean values for $\bar{P}_{GC}$ remained unchanged, this increase in $\overline{\Delta P}$ was due solely to the fall in P_T. The latter was regularly observed to occur upon transition from water diuresis to antidiuresis, an effect induced by the hydro-osmotic action of AVP on the distal nephron. Thus, in spite of significant increases in $\overline{\Delta P}$ during infusion of AVP, mean values for SNGFR failed to rise. Filtration pressure disequilibrium obtained during (but not before) AVP infusion, as indicated by the inequality between $\overline{\Delta P}$ and Π_E (middle panel). Calcula-

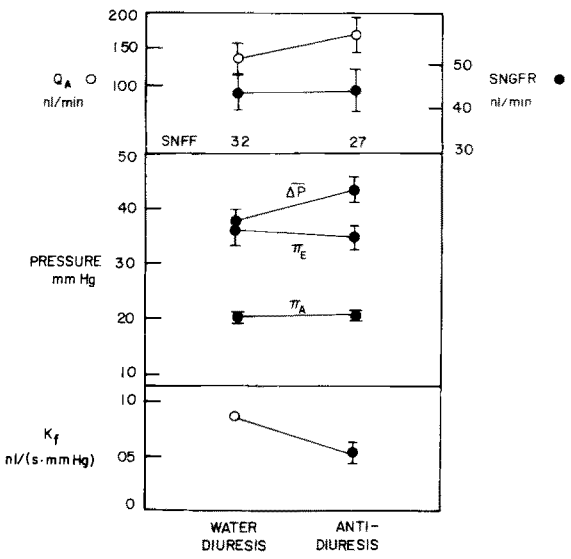

Fig. 14. Summary of effects of intraarterial infusion of synthetic arginine vasopressin (3.6 mU/kg/h) on the measured determinants of glomerular ultrafiltration in the initially water diuretic Munich-Wistar rat

tion of unique values for K_f was, therefore, possible only after AVP, and as shown in Figure 14 (bottom panel), mean values of K_f observed during AVP infusion were significantly lower than the mean of the minimum values estimated prior to AVP infusion. The constancy of SNGFR observed during AVP infusion was, therefore, due to the offsetting effects of an increase in $\overline{\Delta P}$ and reductions in K_f. This previously unsuspected glomerular action of AVP was reproduced with other antidiuretic peptides in this same study including Pitressin, synthetic dVDAVP (an antidiuretic agent known to possess no pressor activity) (*Manning* et al., 1973; *Sawyer* et al., 1974), and dibutyryl cyclic AMP, a substance known to mimic the hydro-osmotic action of ADH in vivo and in vitro (*Levine,* 1968; *Orloff* and *Handler,* 1962). In addition, stimulation of endogenous ADH release by acute mild arterial hemorrhage also resulted in significant reductions in K_f in the rat.

Thus, from the study by *Ichikawa* and *Brenner* (1977), it appears that ADH acts at the level of the glomerulus as well as the distal nephron. It remains to be determined, however, whether the actions of ADH on glomerular dynamics are of physiologic significance in regulating GFR.

VI. Control of SNGFR in the Neonate

During maturation in the young mammal, the rate of glomerular filtration per gram kidney weight rises markedly (*McCrory,* 1972). The mechanism of this increase in GFR with age has not been defined with cer-

tainty although a number of explanations have been advanced. For example *Spitzer* and *Edelmann* (1971) claimed that the increase in SNGFR with age observed in the developing guinea pig was due to an increase in $\overline{\Delta P}$, itself due to overall increases in $\overline{P}_{GC}$. Their estimates of $\overline{P}_{GC}$, however, were obtained indirectly using the stop-flow technique, and as discussed earlier, such estimates of $\overline{P}_{GC}$ may be unreliable. Also *Tucker* and *Blantz* (1976) reported no such increase in the directly measured value of $\overline{\Delta P}$ in the developing Munich-Wistar rat. An alternative possibility which would account for the maturational increase in GFR is that concomitant increases in renal blood flow may be responsible. Indeed, increases in whole kidney blood flow rate have been reported to occur in the developing piglet, puppy, lamb, rat, and human (*Gruskin* et al., 1970; *Horster* and *Valtin,* 1971; *Kleiman* and *Reuter,* 1974; *Aperia* et al., 1974; *Aperia* and *Herin,* 1975; *Guignard* et al., 1975). This suggests that the maturational increase in GFR may reflect a high degree of plasma flow dependence of GFR in the developing mammal. A recent micropuncture study by *Aperia* and *Herin* (1975) has shown that increases in SNGFR with age, in the young rat, are paralleled by increases in single nephron plasma flow rate (Q_A), the latter estimated using microspheres. A similar conclusion has also been reported by *Guignard* et al. (1975) for the newborn human where mean values of GFR and RPF (estimated from inulin and PAH clearances, respectively) were shown to increase in parallel, approximately doubling during the first 14 days of life. The study by *Tucker* and *Blantz* (1976) cited above also demonstrated some increase in Q_A with growth in the developing Munich-Wistar rat, using micropuncture techniques. In this study, however, rats were initially volume expanded with isoncotic rat plasma, thus artificially elevating Q_A in order to produce the condition of filtration pressure disequilibrium to enable calculation of unique values for K_f. According to these workers, the observed increases in SNGFR, with age, correlated best with increases in K_f, which they attributed to a probable increase in the glomerular capillary surface area available for filtration. *Aperia* and *Herin* (1975) have pointed out, however, that although kidney mass increases with age, glomerular density actually falls, at least in the rat. In addition, glomerular volume of the puppy has been demonstrated to increase at a much slower rate than that of the rest of the kidney during maturation (*Horster* et al., 1971). As a corollary to the above discussion, if filtration pressure equilibrium is achieved early in postnatal life, increases in K_f, associated with a growth in glomerular volume as suggested by *Tucker* and *Blantz* (1976), would not be expected to affect SNGFR, for the reasons discussed previously in connection with Figure 5C. Clearly, additional studies are required to assess the factors controlling development of GFR in the normally hydrated neonate. Nevertheless, given the findings of *Aperia* and *Herin*

(1975) in the young Sprague-Dawley rat and the well-documented plasma-flow dependence of SNGFR in the adult Munich-Wistar rat (discussed above), it seems likely that maturational increases in SNGFR are the consequence, at least in part, of these increases in Q_A.

VII. Effects of Increased Ureteral Pressure on SNGFR

Increases in ureteral pressure due to partial or complete ureteral obstruction are frequently associated with reductions in GFR in man and experimental animals (*Papadopoulou* et al., 1969; *Jaenike,* 1970; *Wilson,* 1972; *Howards* and *Wright*, 1976). In addition, ureteral obstruction has been shown to evoke increases in the pressure of fluid proximal to the obstruction, usually measured as P_T (*Gottschalk* and *Mylle,* 1956), and the increase in P_T appears to be proportional to the fall in GFR (*Jaenike,* 1970). This observation is in accord with the earlier discussion of the determinants of (SN)GFR, since marked increases in P_T might be expected to lower the value of $\overline{\Delta P}$, thus reducing the transcapillary hydraulic pressure difference favoring ultrafiltration. That the effect of ureteral obstruction on the determinants of GFR, at least in the dog, is not limited solely to elevations in P_T, is suggested by observations in which increases in ureteral pressure are associated with *increases* in renal blood flow, an effect which would of itself tend to increase GFR (*Nash* and *Selkurt,* 1964; *Carlson* and *Sparks,* 1970). *Blantz* et al. (1975) have investigated the effects of elevation in ureteral pressure on glomerular dynamics in the Munich-Wistar rat. These workers found that in the hydropenic rat elevation of ureteral pressure resulted in increases in both $\bar{P}_{GC}$ and P_T, and hence little net fall in $\overline{\Delta P}$. Values for SNGFR fell significantly in this study, while Q_A remained essentially unchanged. The only remaining explantation for the observed fall in SNGFR (since values for Π_A remained essentially unchanged) is that K_f fell as the result of the increased ureteral pressure. Indeed, *Blantz* et al. (1975) were able to calculate unique values for K_f during elevated ureteral pressure in hydropenic rats, since filtration pressure disequilibrium obtained in these animals and K_f was found to be significantly lower during ureteral obstruction than values reported as representative of normal hydropenia, both by *Blantz* and co-workers (*Blantz,* 1974, 1975; *Blantz* et al., 1974, 1975, 1976) and by *Brenner* and co-workers (*Deen* et al., 1973b; *Myers* et al., 1975b). Observations in a plasma-expanded group of rats in the study by *Blantz* et al. (1975) again showed marked increases in P_T following increased ureteral pressure, although in this situation $\bar{P}_{GC}$ failed to change significantly, with the result that $\overline{\Delta P}$ fell. Again values for SNGFR were

reduced while Q_A failed to change during increased ureteral pressure. Filtration pressure disequilibrium obtained, but in these plasma-loaded rats, values for K_f were found to be normal, the reduction in SNGFR in this group of animals being attributed by *Blantz* and co-workers to the fall in $\overline{\Delta P}$. It appears, therefore, that the mechanisms by which SNGFR are reduced during increased ureteral pressure, and possibly ureteral obstruction, depend at least in part on the prior state of hydration of the animal.

VIII. The Determinants of SNGFR in Response to Renal Injury

There are many pathologic conditions afflicting the kidney in which GFR is affected, and although the clinical implications of kidney disease are well-documented, there is at present little information available concerning the mechanisms responsible for these changes in GFR. Some new insights have been obtained in recent micropuncture studies, however, and these are discussed below.

A. Response to Reduced Renal Mass

Loss of functional renal mass, whether induced by surgical excision or a primary nonglomerular disease such as pyelonephritis, is usually associated with an increase in the rate of filtration in surviving nephrons (*Bank* and *Aynedjian,* 1966; *Hayslett* et al., 1968, 1969; *Arrizurieta* et al., 1969; *Schultz* et al., 1970; *Gottschalk,* 1971; *Allison* et al., 1973; *Weinman* et al., 1973). In an effort to determine the mechanism of this increase in GFR, several micropuncture investigations have recently been reported in animals subjected to prior surgical ablation of varying fractions of total renal mass. In one study, *Deen* et al. (1974a) examined the determinants of SNGFR in chronically uninephrectomized Munich-Wistar rats, studied 2 - 4 weeks following the initial surgery. The characteristic response to uninephrectomy of compensatory increases in both total renal mass and total GFR of the remaining kidney of approximately 50% was observed in these studies. Results of single nephron measurements in uninephrectomized rats and a separate group of normal hydropenic control rats are shown in Figure 15. As with whole kidney GFR, both SNGFR and Q_A were uniformly and proportionally higher in uninephrectomized animals than in controls (top panel). SNFF was essentially the same in both groups. Although $\overline{AP}$ was almost identical in the two groups, $\overline{\Delta P}$ was significantly greater after uninephrectomy, averaging 40 mm Hg, compared to 34 mm Hg in controls (middle panel). This ele-

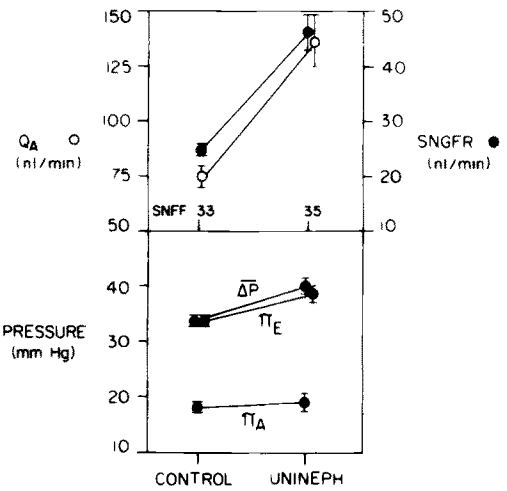

Fig. 15. Summary of the effects of uninephrectomy on the measured determinants of glomerular ultrafiltration in the Munich-Wistar rat. Data (mean ±SE) were obtained in control rats (*left*) and rats which had been uninephrectomized 2 - 4 weeks prior to study (*right*), as reported in detail elsewhere (*Deen* et al., 1974a). Reproduced from *Brenner* et al., Phys. Revs. **56**, 502-534, 1976 and *Maddox* and *Brenner*, Ann. Rev. Med. 28, 91-102, 1977, with permission of the publishers

vation of $\overline{\Delta P}$ was entirely due to increases in $\bar{P}_{GC}$ since values for P_T were similar in both groups. Π_A was very nearly the same in both groups, and Π_E rose in the uninephrectomized animals to $\cong\overline{\Delta P}$, thus filtration pressure equilibrium obtained in both control and uninephrectomized rats.

Of interest is whether or not compensatory renal growth following uninephrectomy resulted in increases in the surface area available for filtration (S) and thus a change in K_f. Since filtration equilibrium was found by *Deen* et al. (1974a) to obtain after uninephrectomy, only minimum values of K_f could be determined. In order to obtain an unique estimate of K_f in uninephrectomized rats, disequilibrium was produced by further increases in Q_A, induced by intravenous infusion of isoncotic plasma, equivalent to $\sim$2% of body weight. Under these conditions, K_f was found to average $\sim$0.075 nl/ (s·mm Hg) a value similar to that found previously for plasma-loaded rats with intact kidneys (*Deen* et al., 1973b; *Baylis* et al., 1976c), suggesting that, on average, the values of K_f were unaffected by uninephrectomy. Indeed, in view of the persistence of filtration pressure equilibrium in nonplasma-loaded uninephrectomized rats, even if variations in K_f had occurred, these would not have been expected to have much effect on SNGFR for reasons discussed above. Since C_A (and thus Π_A) was unchanged following uninephrectomy, the increase in the mean net ultrafiltration pressure, $\bar{P}_{UF}$, and resulting increase in SNGFR must have been the consequence solely of the observed increases in Q_A and $\overline{\Delta P}$. Using the mathematical model of glomerular ultrafiltration developed by *Deen* et al. (1972), it was estimated that approximately 70% of the compensatory increase in SNGFR was due to the rise in Q_A, the remainder being due to the increase in $\overline{\Delta P}$.

In accord with these findings, *Kaufman* et al. (1974) observed that a 50% reduction in renal mass increased SNGFR essentially in proportion to Q_A, since SNFF did not differ significantly from that in nonnephrectomized control rats. In the same study, 75% ablation of renal mass resulted in even more marked increases in SNGFR and Q_A but was now associated with a decrease in SNFF. Assuming that there was no substantial fall in $\overline{\Delta P}$ or increase in Π_A following 75% ablation, the further increase in Q_A to remaining nephrons probably produced filtration disequilibrium, as was observed in plasma-loaded rats previously subjected to a 50% ablation of renal mass (*Deen* et al., 1974a). Thus, it appears likely that following either 50% or 75% ablation of renal mass, the compensatory increases in SNGFR are governed, at least in the rat, by the extent to which Q_A increases in surviving glomeruli.

B. Response to Primary Glomerular Injury

In experimental or clinical conditions which primarily affect glomerular capillary structure and function, a reduction in total GFR is frequently observed, attended by parallel *reductions* in SNGFR, as discussed by *Allison* et al. (1974). This is a contrast to the situation discussed above, where reduction in functioning renal mass by surgical ablation is associated with an *increase* in SNGFR in surviving nephrons, thereby tending to minimize the reduction in total GFR. Relatively little is known about the determinants of glomerular ultrafiltration in response to primary glomerular injury despite the wealth of information now available concerning the morphologic and immunologic aspects of these disorders. Several recent micropuncture studies, however, have served to enhance our knowledge of certain specific lesions and these are discussed below.

1. Nephrotoxic Serum Nephritis

Maddox et al. (1975) have recently examined the determinants of glomerular ultrafiltration in Munich-Wistar rats 1 - 2 weeks following induction of a mild to moderate form of nephrotoxic serum nephritis (NSN). Both light and electron microscopy revealed that the proliferative response associated with this glomerular injury caused partial or complete obliteration of some capillary loops, while other capillaries remained essentially normal. Despite this morphologic evidence of glomerular injury, mean values of GFR, SNGFR, and Q_A were, on average, not significantly different between groups of NSN and normal hydropenic control rats (top panel, Fig. 16). In NSN, $\overline{\Delta P}$ was elevated considerably, due solely to increased values for $\overline{P}_{GC}$ (middle panel). Despite this profound

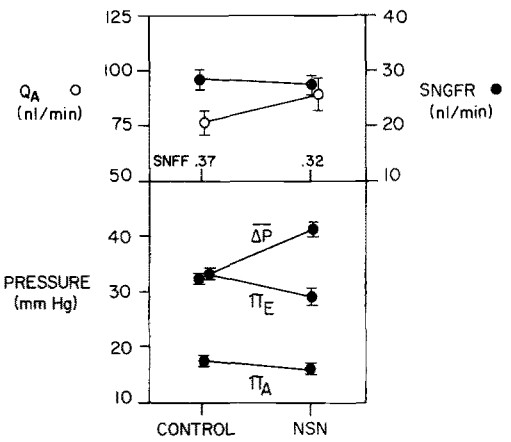

Fig. 16. Summary of the effects of nephrotoxic serum nephritis (NSN) on determinants of glomerular ultrafiltration in the Munich-Wistar rat. Data obtained from normal control rats (*left*) and rats with NSN (*right*), reported in detail by *Maddox* et al. (1975). Reproduced from *Brenner* et al., Phys. Revs. **56**, 502-534, 1976 and *Maddox* and *Brenner*, Ann. Rev. Med. **28**, 91-102, 1977, with permission of the publishers

increase in $\overline{\Delta P}$ and essentially normal values for Q_A and Π_A, the values for SNGFR failed to rise in NSN rats. Values for Π_E were moderately reduced compared to normal hydropenia, and a marked *inequality* between Π_E and $\overline{\Delta P}$ was seen to obtain in NSN rats (middle panel, Fig. 16). The existence of filtration pressure disequilibrium which followed induction of NSN, therefore, permitted calculation of unique values for K_f in this group, which were found to average $\sim$0.03 nl/(s·mm Hg) or approximately one-third of the value previously reported for normal Munich-Wistar rats (*Deen* et al., 1973b; *Myers* et al., 1975b). The constancy of SNGFR observed in NSN rats in this study was, therefore, due to the offsetting effects of elevation in $\overline{\Delta P}$ and reduction in K_f. Similar reductions in K_f were noted by *Allison* et al. (1974) in studies of more severe NSN as well as Heymann's nephritis in the rat. It is likely that these reductions in K_f noted in the studies by *Maddox* et al. (1975) and *Allison* et al. (1974) were, at least in part, the result of a reduction in the number of patent capillaries in each glomerulus, i.e., a reduction in S. Whether a decrease in effective hydraulic permeability (k) also contributes to the fall in K_f cannot be ascertained from these studies. This latter possibility is suggested, however, by the recent findings of *Blantz* and *Wilson* (1975) who observed a fall in K_f (similar to that noted above) 1 h following intravenous injection of nephrotoxic serum. At this early interval following induction of NSN, the number of patent capillaries in each glomerulus was not found to be reduced appreciably, so that a fall in k, rather than a reduction in S, was postulated to account for the observed marked fall in K_f during early NSN.

In the study by *Maddox* et al. (1975), in which NSN had been induced some 1 - 2 weeks prior to study, SNGFR and total kidney GFR remained at normal levels despite a pronounced reduction in K_f, because of the compensating effect of an increase in $\overline{\Delta P}$. If $\overline{\Delta P}$ remains elevated in

more severe, chronic forms of glomerulonephritis, as suggested by the findings of *Allison* et al. (1974), then the progressive deterioration in filtration rate characteristically noted in more advanced stages of glomerulonephritis must result from further reductions in K_f and/or declines in Q_A.

2. Effect of Puromycin Aminonucleoside

Puromycin aminonucleoside (PAN) has been used clinically for chemotherapy of malignancy and also experimentally to produce a model of nephrotic syndrome and chronic renal failure (*Maher,* 1976). Electron microscopy has revealed that PAN nephrosis is associated with widespread fusion of the glomerular epithelial foot processes (*Venkatachalam* et al., 1969; *Ryan* and *Karnovsky,* 1975). A preliminary report by *Baylis* et al. (1976a) deals with the action of this drug on the determinants of SNGFR and the etiology of the observed proteinuria in the Munich-Wistar rat. PAN was administered for 6 - 7 days in a s.c. dose of 1.7 mg/kg body weight. In comparison to control hydropenic rats, marked falls in SNGFR (of $\sim$ 40%) were observed following PAN administration, and Q_A fell less than in proportion to the fall in SNGFR (by $\sim$ 25%). Values for $\overline{\Delta P}$ were unaffected by the drug while both Π_A and Π_E fell significantly. Filtration pressure disequilibrium obtained in most animals following PAN and calculation of K_f showed a significant fall in comparison to normal hydropenic values. The marked fall in SNGFR observed in PAN-induced nephrosis, therefore, appears to be due to the cumulative effects of reductions in K_f and Q_A.

3. Effect of Gentamicin

There have been frequent reports of nephrotoxic effects following administration of gentamicin (G) (an aminoglycoside antibiotic), in both man and experimental animals (*Wilfert* et al., 1971; *Kahn* and *Stein,* 1972; *Kosek* et al., 1974; *Luft* et al., 1975). Electron microscopic investigations on the renal ultrastructural changes induced by G have mainly been confined to the tubule. There is some ultrastructural evidence of glomerular injury, however, in that *Kosek* et al. (1974) have shown a dose-related formation of lysosomal cytosegresomes in glomerular (as well as proximal and distal tubular) epithelium in the rat. A preliminary study in the Munic-Wistar rat by *Baylis* et al. (1977) has investigated the effects of both low (4 mg/kg/day) and high (40 mg/kg/day) i.p. doses of G, administered for 10 days. The lower dose is equivalent to that given to man. Values for SNGFR were decreased, compared to normal hydropenia, by $\sim$ 30 - 35%, a reduction which was paralled by whole kidney GFR. Q_A fell less markedly, by $\sim$ 10 - 15% and SNFF was slightly lower

than the value observed in the hydropenic control group. In the low dose group, values for $\overline{\Delta P}$ and Π_A were unaffected by G, with Π_E being significantly reduced, resulting in production of disequilibrium in most of the animals studied. The calculated mean value for K_f was markedly reduced, to about 50% of the *minimum* value observed in normal hydropenia. Thus, reduction in SNGFR and whole kidney GFR by the low dose of G was mainly due to the reduction in K_f and to a lesser extent, the fall in Q_A, not unlike that occuring with PAN.

With the higher dose of G ($\sim$ 10 times the normal therapeutic dose in man), slight falls in $\overline{\Delta P}$ were also observed, due entirely to increases in P_T. Since absolute proximal reabsorption was also seen to decline significantly with the high dose of G in this study, and in view of the previous reports that G induces proximal tubular lesions (*Kosek* et al., 1971; *Luft* et al., 1975), it is possible that the rise in P_T observed with high doses of the drug was due to an additional effect on proximal fluid reabsorption. Values for Π_E were again markedly reduced with G and calculated values for K_f were also significantly lower than those considered normal for the Munich-Wistar rat. Thus, with the high dose of G, reduction in SNGFR was again mainly the result of falls in K_f, but to a lesser extent to smaller falls in Q_A and $\overline{\Delta P}$ as well.

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Models for the Ontogenetic Development of Higher Organisms

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Contents

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I. Introduction

The organization of development in a higher organism is one of the major unsolved problems in biology. The lack of knowledge about the basic mechanisms by which development is controlled is especially surprising in view of the large amount of detailed knowledge about how a particular organism will react after a particular experimental manipulation. A full understanding of development cannot be derived from biochemical investigations alone. Even an ideal biochemical analyzer which could measure the concentration of any relevant substance at any time and at any location would be limited to measuring changes in the local concentrations which can then be correlated with developmental events. We would still have no insight into why these changes have occurred, what the driving force is, what the cause is, and what the effect is – these questions would remain in the dark.

As in any other branch of science, attempts have been made to explain the known details through the invention of hypothetic mechanisms – models – which account for the observations as well as possible. A criterion of a good model is that several seemingly unrelated observations appear as the expression of a single underlying mechanism. If the assumed mechanism is simple, fewer parameters will be involved. There is then less danger that a wrong mechanism will fit the observations through the choice of convenient values for the parameters.

To compare the experimental results to a hypothetic model, it is invaluably helpful to have the model in a precise mathematical form. Only then will discrepancies between the intuitively reasonable and the actual properties of the model, between the model and the experimental results, be detected. Mathematically formulated models, which are usually called theories, have been a common tool in physics for a long time but still lack an adequate place in developmental biology. The superior power of a mathematically formulated model versus a verbally formulated model may be recalled by citing a well-known example from physics. The orbits of the inner planets were so precisely described by Newton's theory that deviations found in the orbits of outer planets compelled the assumption of additional planets – which were subsequently observed. It is evident that such a prediction would never have been possible by a nonmathematical model or by any arbitrarily precise observations alone.

Our hope toward understanding the process of development is based on the assumption that a separation into easier-to-understand submechanisms is possible. Such mechanisms are gene regulation, change in cell shape, cell movement, cell recognition by surface properties, etc. In addition, there must exist a signaling and a signal-receiving mechanism which

enables the cell to develop in a manner appropriate to its position. The necessity of such a signal system is easily demonstrated by the fact of regeneration. The removal of a part of an organism can cause a signal to the remaining parts initiating a development through a completely different pathway than normally would be followed. This, of course, requires some kind of communication. This present article will deal mainly with the formation of such signal systems.

Intensive research in developmental biology has yielded evidence for some general principles, such as: the gradient (*Boveri*, 1901; *Morgan*, 1904; *Child*, 1929), the organizer (*Spemann*, 1938; *Child*, 1946), the embryonic field (*Weiss*, 1939), inhibitory fields (*Schoute*, 1913), and embryonic induction (*Spemann*, 1938; *Saxen* and *Toivonen*, 1962), all of which overlap one another to a greater or lesser degree. The experimental facts significant for the understanding of such a signal system have been repeatedly reviewed in recent years (*Sondhi*, 1963; *Wolpert*, 1969, 1971; *Robertson* and *Cohen*, 1971, *Cooke*, 1975b). There are also excellent review articles available for particular developmental systems, such as insects (*Counce* and *Waddington*, 1972; *Sander*, 1976, *Lawrence*, 1970), the sea urchin (*Czihak*, 1975), planarians (*Chandebois*, 1975), and hydroids (*Webster*, 1971).

Attempts toward a theoretic understanding led *Wolpert* (1969, 1971) to the concept of positional information. *Goodwin* and *Cohen* (1969) attempted to describe developmental organization by traveling waves and their phase shift (see also *Robertson* and *Cohen*, 1971).

In this article, I will restrict myself to a type of model in which it is assumed that a development-controlling prepattern is generated by the mutual interaction of a few substances which may act as substrates, activators, or inhibitors either in their own, or in each other's production and breakdown reactions. This type of model has been proposed by *Turing* (1952) and further elaborated by *Gmitro* and *Scriven* (1966), *Prigogine* and *Nicolis* (1971), *Martinez* (1972), and *Edelstein* (1972).

By considering known principles of development – the fields of inhibition and the possibility of local induction – we have shown that among many possible pattern-forming interactions the choice of only a few biologically reasonable reactions is possible (*Gierer* and *Meinhardt*, 1972, 1974; *Meinhardt* and *Gierer*, 1974). The interactions can be given in the mathematically precise form of coupled differential equations which can lead directly to molecular interpretations. The properties of these systems will be illustrated by comparing their behavior (numerical solutions of the differential equations) with biologic observations. A detailed theoretic description will be given for early insect development and for the formation of net structures, such as the vascular system of leaves. The discussion will be centered more on the generation of the signal, the prepattern.

The response of the cell could consist in the switching on and off of genes, but this will be discussed only marginally.

II. Generation of Structures by Autocatalysis and Lateral Inhibition

One of the most fascinating aspects of development is the generation of structures from a more or less homogeneous egg. This was felt to be so miraculous that a long argument arose as to whether the laws of physics were sufficient for an explanation of development. *Driesch,* for example (see *Herbst,* 1942) – who made such great discoveries, i.e., that the cell nuclei in a developing sea urchin egg are totipotent and therefore the interaction of cytoplasm with the nuclei is decisive for the developmental pathway – believed in a vital power which was assumed to be unexplainable by physical laws.

However, a look at inorganic nature reveals that formation of pattern is not peculiar to living objects. Pattern formation is the rule also in the nonliving world. Formation of galaxies, stars, clouds, rain drops, lightning, river systems, mountains, crystals, all forms of erosion – all these testify to the generation of ordered structures. It is instructive to look for common principles in the generation of these structures. If a small deviation from a homogeneous distribution has a strong positive feedback on itself, the deviation will increase. For example, erosion proceeds faster at the location of some random initial injury. A large sand dune may result from a stone in a desert which produces a wind shelter and may thus locally accelerate the deposition of sand; this deposition increases the probability of further sand deposition, and so on.

As well as the strong positive feedback – autocatalysis – another element is required for pattern formation: lateral inhibition. Once an autocatalytic center has arisen, a suppression of the autocatalysis in the neighborhood must occur otherwise the reaction would spread like a grassfire. The strong short-range positive feedback must therefore be supplemented by a longer-range negative feedback.

A biologic example of the expression of autocatalysis and of lateral inhibition may be seen in the appearance of additional heads in hydra. A small piece of tissue which was derived from near the head and grafted into the body column can activate the adjacent tissue into forming a normal-sized head. After a while, the implanted tissue can even be removed, and the induced head formation will still proceed. On the other hand, the existing head has an inhibitory influence on the induction of a secondary head. This inhibition is highest in the head area but is detectable throughout the animal (for review see *Webster,* 1971).

Table 1. Pattern-forming reactions

Some interactions are listed which lead, according to the general criterion (*Gierer* and *Meinhardt*, 1972, 1974), to a patterned distribution of substances. The activator A (x) is assumed to be autocatalytic. The autocatalysis is compensated for by the antagonistic action of either the inhibitor H(x) or by the depletion of the substances S(x). The necessary lateral inhibition is achieved by a higher diffusion range for either H or S. $\rho(x)$ and $\rho'(x)$ are sources of the activator and inhibitor, e.g., enzymes, messengers, particular cell types. The local activator and inhibitor concentration decide to what extent the sources are active. A graded source distribution will orient the emerging activator distribution, but a randomly oriented activator pattern can also be formed if the source distribution is homogeneous. Eq. 1 means: the change of A per time unit ($\dot{A}$ or $\delta A/\delta t$) is proportional to an autocatalytic term (A^2), the autocatalysis is slowed down with increasing inhibitor concentration ($1/H$). The activator decays in a first-order reaction ($-\mu A$), diffuses ($D_a \Delta A$), and a small basic activator production ρ_o remains, even if no activator is present. The change of H per time unit ($\dot{H}$, Eq. 1c) is a function of the cross-catalytic influence of the activator (A^2), of the decay ($-\nu H$), and of the diffusion ($D_h \Delta H$). A (small) activator-independent inhibitor production ρ_1 can suppress the pattern formation if the activator concentration is low. The necessity of the quadratic term in the autocatalysis is intuitively reasonable, since the autocatalysis has to overcome the normal decay ($-\mu A$). This signifies that the autocatalysis must be a cooperative reaction with at least two activator molecules involved. A further requirement was that a very small area – too small for the different diffusion rates of the activator and the inhibitor to come into play-should show a stable and unique equilibrium concentration. Frequently, developing structures are growing structures and this requirement insures that pattern formation starts from defined initial conditions if a critical extension has been exceeded by growth.

Eq. 1: Activator-inhibitor model (Fig. 1); the saturation introduced in Eq. 1b leads to size regulation (Fig. 3). Eq. 2: Conversion model: the decay of the activator ($-\mu A$) leads to an inactive molecule fragment which acts as inhibitor, since it can still compete for the binding site of the activator. Eq. 3: Depletion model: the inhibition of the activator production is obtained from a depletion of a substrate S which is produced everywhere with the rate c_o and consumed mainly for the activator production.

Coupled nonlinear equations of this type are not analytically solvable; one has to use a computer. For this, the space has been divided into individual "cells" i, the differential equation is rewritten as a differentiation equation. Eq. 4 is the discretized form of Eq. 1a and c which is mostly used in this work. Such an equation allows us to compute the concentration change (dA, dH) in a given time unit. These changes have to be added to the existing concentrations, and the development in time is obtained by the repetitions of such iterations

$$\dot{A} = c\rho A^2/H - \mu A + D_a \Delta A + \rho_o \rho \tag{1a}$$

$$\dot{A} = c\rho A^2/((1+\kappa A^2)H) - \mu A + D_a \Delta A + \rho_o \rho \tag{1b}$$

$$\dot{H} = c\rho' A^2 - \mu A + D_h \Delta A + \rho_1 \tag{1c}$$

$$\dot{A} = c\rho A^2/H^2 - \mu A + D_a \Delta A + \rho_o \rho \tag{2a}$$

$$\dot{H} = \mu A - \nu H + D_h \Delta H + \rho_1 \tag{2b}$$

$$\dot{A} = c\rho A^2 S - \mu A + D_a \Delta A + \rho_o \rho \tag{3a}$$

$$\dot{S} = c_o - c\rho A^2 S - \nu S + D_s \Delta S \tag{3b}$$

$$dA_i = c\rho_i A_i^2/H_i - \mu A_i + D_a (A_{i+1} + A_{i-1} - 2A_i) + \rho_o \rho_i \tag{4a}$$

$$dH_i = c\rho'_i A_i^2 - \nu H_i + D_h (H_{i+1} + H_{i-1} - 2H_i) + \rho_1 \tag{4b}$$

The theory (*Gierer* and *Meinhardt*, 1972, 1974; *Meinhardt* and *Gierer*, 1974) developed on the basis of these properties – short-range autocatalysis, long-range inhibition – provides a criterion as to which interactions will lead to a formation of pattern. The mathematical formulations of some simple pattern-forming interactions meeting this criterion are listed in Table 1. As demonstrated below, a strongly patterned distribution can arise in an almost homogeneous tissue. The resulting pattern can be monotonic or periodically varying over a region. The extension of an area with a high substance concentration can be proportional to the total size. The pattern can be stable or oscillating in time, the location of a high concentration can be directed by minor internal or external asymmetries. An active center which has been removed can "regenerate." The existence of all these properties has been postulated for many developmental systems.

A. The Basic Principle

Let us assume a substance – to be called the activator A – which stimulates its own production (autocatalysis) and the production of its antagonist – the inhibitor H. To carry out the necessary long-range inhibition, the inhibitor must diffuse more rapidly. In an extended field of cells a homogeneous distribution of these substances is unstable, since any small local elevation of the activator concentration – resulting, perhaps, from random fluctuations – will be amplified by the activator autocatalysis. The inhibitor, which is produced in response to the increasing activator production, cannot halt the local increased activator production, since it diffuses quickly into the surrounding tissue and there suppresses activator production outside the activated center (Fig. 1). Thus, the locally increased activator concentration will increase further until some limiting factor comes into play, for instance the loss by diffusion is equal to the net production. A stable activator and inhibitor profile is ultimately obtained, although both the substances continue to be made, to diffuse, and to be broken down. Such a simple system of two interacting substances is, therefore, able to produce a stable, strongly patterned distribution from a nearly homogeneous initial distribution.

B. Regeneration and Induction

The local high concentration of the activator or inhibitor can serve as a signal for the formation of a particular structure by differentiating cells, initiating evagination, etc. Characteristic of many embryonic systems is

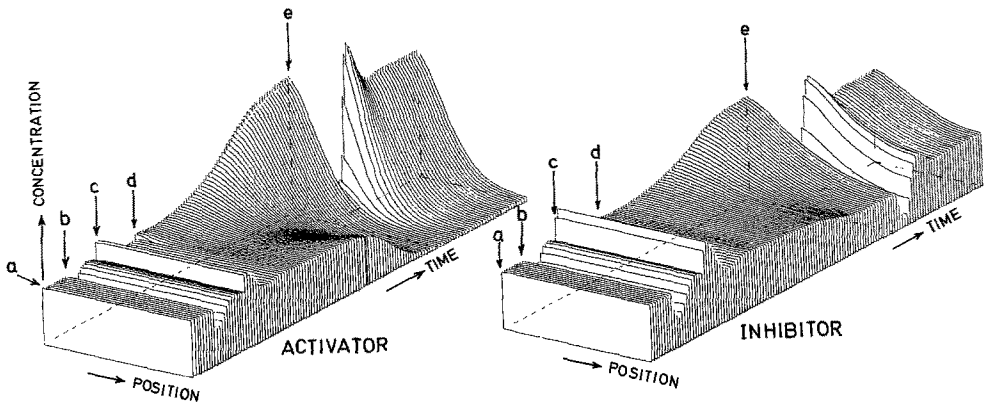


Fig. 1. Generation of a patterned distribution of an autocatalytic substance – the activator – and its more rapidly diffusing antagonist – the inhibitor. Assumed is a linear array of cells and the activator and inhibitor concentration is plotted as function of time. A completely homogeneous distribution of both substances is stable (*time a*), an artificial activator decrease (*time b*) or increase (*time c*) disappears following a compensating change in the inhibitor concentration. But a small local activator increase (*time d*) or even a random fluctuation cannot be compensated, since the additionally produced inhibitor diffuses quickly into the surroundings. In the activated region, the activator concentration will increase further until a steady state is reached in which the gain by production and the loss by diffusion and decay is balanced. The inhibitor is similarly distributed, only the slope is shallower due to the higher diffusion rate. The stability of such a distribution is very high, for instance after removal of the activated area (*time e*), the production site of the inhibitor is also removed, the remnant inhibitor decays, and the activator gradient "regenerates." To avoid oscillations, the inhibitor has to decay more rapidly than the activator (computed with Eq. 4; adapted from *Meinhardt*, 1974)

their ability to regenerate parts after removal. One possibility is a reprogramming of the remaining tissue (morphallaxis), for which the model provides an explanation. Removal of the active site also removes the site of inhibitor production, the existing inhibitor decays, and the remaining activator will induce a new maximum (Fig. 1, time e). An example will be given in connection with the regeneration of the hydra head in Figure 7.

Frequently, a second structure can be induced at a distance from an existing one. *Spemann* (1938) and his co-workers have made very detailed studies with induction of a second embryo in an amphibian blastula. It was surprising and disappointing to discover that very unspecific stimuli lead to an induction; even cell poisoning, injury, or implantation of killed or foreign tissues are sufficient. *Waddington* et al. (1936) proposed that this unspecificity results from the removal of an inhibitor. In the model, a local activator maximum is necessarily surrounded by a field of inhibition; the inhibition decreases with distance from the maximum. At higher distances, any artificial inhibitor decrease – perhaps by leakage due to an injury or due to destruction by UV irradiation – can induce

a new activation. The UV induction of double abdomen in the early insect development (Fig. 12) will later be given as an example of this. On the other hand, a specific induction is possible by a local increase in the activator concentration; the induction of a second embryo by the transplantation of a piece of dorsal lip of an amphibian blastopore may have this origin.

In the model, a low activator concentration can be maintained indefinitely by a basic (activator-independent) inhibitor production ρ_1 (Table 1). This offers the possibility that the gradient-forming mechanism is "asleep" until a contact is made with a particular other tissue, during which some activator may be supplied or inhibitor removed. The resulting high activator concentration will then appear exactly at the location of the contact. This renders possible a gradient formation in a precise spatial relationship to other presently existing structures.

C. Dependence of the Pattern Formed on the Tissue Size and the Generation of Polarity

An area that is small compared to the diffusion range of the activator (which depends on the diffusion rate and the lifetime) will attain a stable, uniform, and defined activator and inhibitor concentration, because the small size prevents an expression of the different diffusion rates. But with increasing size, the homogeneous distribution becomes unstable (Fig. 2a). In a field with a size of the order of the activator range, a fluctuation at the boundary can initiate the pattern formation process, whereas a fluctation at the center (Fig. 2b) cannot, since in the latter case, the inhibitor does not have enough space into which it can spread out, assuming the boundaries to be tight. The accumulating inhibitor will choke the incipient activator center. Therefore, the high activation will appear at one end. This is not a result of special properties of the tissue at the boundaries, but rather a consequence of the pattern-forming process itself; the highest and lowest concentrations arise at the maximal distance from each other. If the size increases further by growth, the activated area will remain at the same boundary.

The generation of a high concentration at one boundary and a low concentration at the other corresponds to a property found in many biologic systems. A polarity exists or develops; both ends are or become different from one another. The polarity of a developing structure within an embryo is, of course, not random but controlled by the surrounding tissue which assures a coordinated development. Such an influence of the surrounding tissue has been demonstrated with experiments in which amphibian limb buds (*Harrison,* 1921), or eye cups (*Stone,* 1960) are

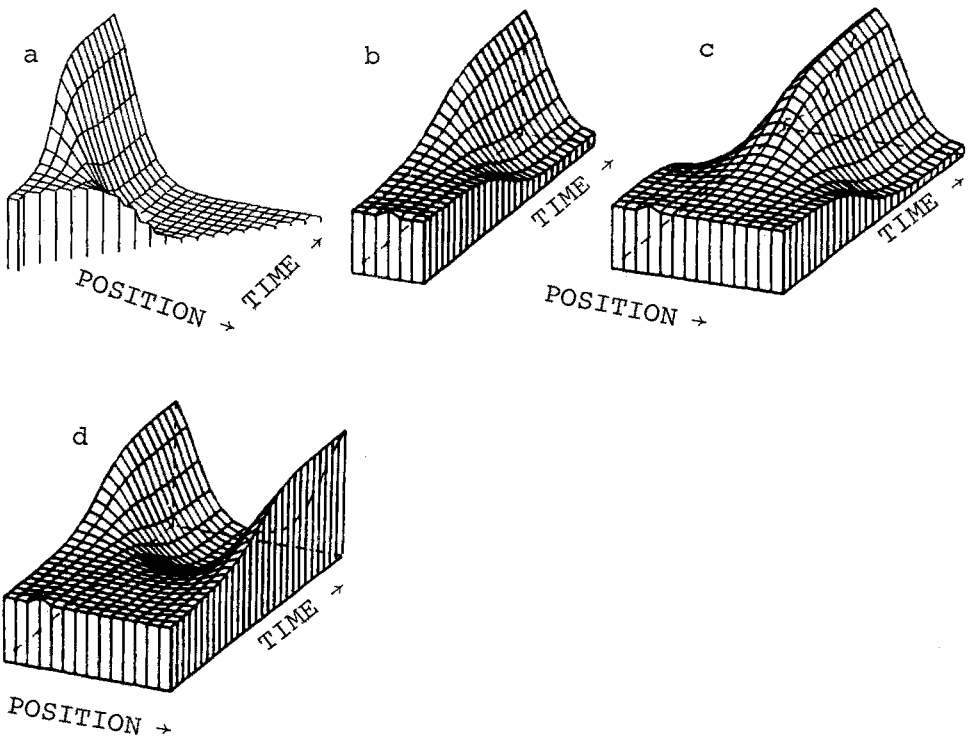


Fig. 2a - d. Formation of monotonic and symmetric patterns. (a and b) In a small field, the high activator concentration appears at one of the boundaries, since, due to the lateral inhibition mechanism, the highest and lowest concentrations develop at maximal distance from each other. No assumptions of special tissue properties at the boundaries are required. The result is a monotonic activator or inhibitor gradient as required for many developmental systems. (a) The homogeneous activator distribution becomes quite abruptly unstable if a certain size is exceeded. Random fluctuation can trigger the maximum first at one boundary where it remains during further growth. (b) In an area of the size of the activator range, even an artificial central activation will disappear and random fluctuation will trigger a marginal maximum. A marginal activation is, therefore, a property of the pattern-forming interaction. (c and d) In a larger field, a symmetric development is preferred, a maximum appears either in the center or at the two margins (calculated with Eq. 4 with 1% random fluctuation in the constant c; $\rho,\rho' \equiv 1$)

cut out and – after 180° rotation – reimplanted. If such a rotation is made at an early stage, the influence of the surrounding tissue is strong enough to reorient the polarity in the rotated tissue. But if the rotation is made at a later stage, such reorientation is no longer possible and, for instance, the developing limb may grow out in inverted orientation with respect to the body.

The model proposed provides an explanation of such behavior. After the structure has reached a certain size, the activator-inhibitor system is in an unstable equilibrium, and minor differences imposed by the sur-

rounding tissue determine at which end the high activation will occur. By autocatalysis and lateral inhibition, minor differences become amplified. If this amplification has proceeded to a certain point, the gradient is no longer reversible, even if – after the rotation – the external influences have the opposite orientation. Interestingly, the transition from the reversible to the irreversible stage of a *Xenopus* retina also appears in tissue culture in the absence of the influence of the surrounding tissue (*Hunt* and *Jacobson*, 1973a). This supports the view that the internal amplification of the previously imposed difference is involved. (For the simulation of retina-rotation experiments, see *Meinhardt* and *Gierer*, 1974).

The eggs of most species are formed in an asymmetric environment and are, therefore, also asymmetric, which assures a particular orientation of the embryo within the egg. A rare exception is the almost homogeneous egg of the brown algea *Fucus* (*Child*, 1946). The outgrowth of the rhizoid can be directed by light, electric current, and differences in pH or temperature between the different sides of the egg, or by mutual attraction from other nearby eggs (*Jaffe*, 1968). The variety and the unspecificity of the possible stimuli support the view that there is an unstable equilibrium and that any local disturbance can orient the location of the signal which initiates the outgrowth of the rhizoid. In addition, in the absence of any orienting effect, the outgrowth appears at random but much delayed. This is also in agreement with the proposed mechanism, since the time required for the activator peak formation is shorter for a larger deviation from the semistable equilibrium.

If the pattern-forming process is initiated when the tissue has already attained a size somewhat larger than the activator range, a symmetric distribution is favored with either the high activation at the center or at both sides (Fig. 2c and d). Which one is realized depends on small variations of the initial conditions. These two possible symmetric forms have been observed after the experimental disorganization of an initiated development, after the system had obtained a certain size. *Yajima* (1960) found that after centrifugation of insect eggs, a development of symmetric embryos with either an abdomen or a head at both ends occurred. The formation of compound retinas by grafting together retina tissue (*Hunt* and *Jacobson*, 1974) resulted in symmetrical, double nasal, or double temporal retina specification.

D. Size Regulation

The size of a particular substructure may be regulated in relation to the total size of the organism. An example is the extent of the region of the presumptive stalk cells in the slug of the slime mold *Dictyostelium dis-*

coideum (*Raper*, 1940; *Bonner* and *Slifkin*, 1949). In the model, the size of the activated region will be proportioned to the total size of the field if the maximum activator concentration is limited to a level lower than would be obtained if the limitation arises only from the activator diffusion. Such limitation, as introduced in Equation 1b, can arise from the saturation of an enzyme involved in the autocatalytic reaction and has the consequence that the activator concentration peak cannot grow further in height. Increased activator synthesis will result in an extension of the activated area. The activator production depends upon the total available space into which the inhibitor can diffuse – and decay. As long as the total size is not larger than the range of the inhibitor, the activated region is, therefore, essentially proportional to the total size (Fig. 3a and b). This mechanism also works when the pattern is already established, e.g., the removal of the nonactivated part leads to a shrinkage of the activated area (Fig. 3c).

Different modes of size regulation will be discussed below in connection with periodic structures and the interpretation of gradients.

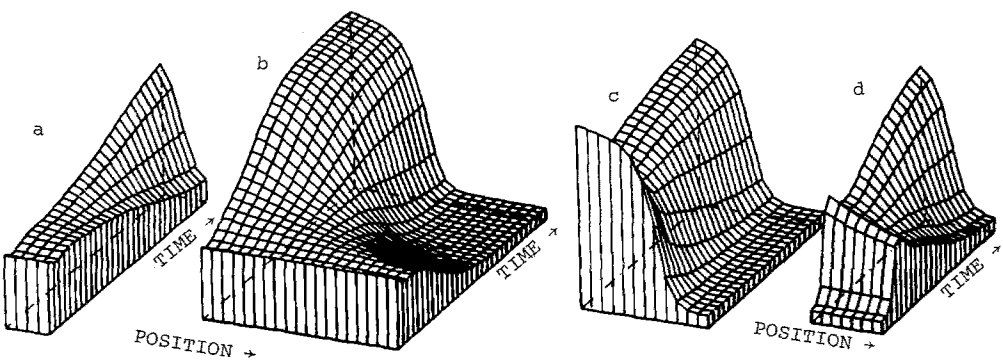


Fig. 3 a - d. Size regulation of the activated region occurs if the maximum activator production is limited (eq. 1b and c, Table 1). Due to the limitation, an increase of the total activator production can occur only by enlargement of the activator-producing area. The total activator production depends on the space into which the inhibitor can diffuse and decay. The acitvated region will be, therefore, roughly proportional to the total area as long as the inhibitor spreads out into the whole area by rapid diffusion or convection. (a and b) Rows of cells of different length develop an activated portion roughly proportional to their size. (c) After removal of the nonactivated part from a distributions as shown in (b), the activated area shrinks until the corresponding size is obtained due to a build-up of inhibitor within the more confined space. (d) Removal of the activated part leads to a regeneration of the activated region corresponding to the smaller size

E. Periodic Structures

A possibility for the generation of periodic structures by coupled biochemical reactions has been shown in the pioneering paper of *Turing* (1952). To make analytic solutions possible, *Turing* had linearized his equations, which led to some stability problems. Numerical solutions of *Turning's* nonlinear equations show, however, periodic distributions that are stable in time (*Martinez,* 1972). *Wilcox* et al. (1973) have explained the periodic appearance of heterocyst cells in the alga *Anabaena* by means of an induction-lateral inhibition mechanism.

In the proposed theory, periodic structures are formed if the total area becomes larger than the range of the inhibitor; a small basic activator production (ρ_0 in Table 1) can trigger a new activator maximum at a distance from an existing one. The resulting pattern will be fairly regular if the pattern formation mechanism has been working throughout the growth period, as shown in Figures 4a, 4b and 5. A new activator peak is formed whenever the distance to the nearest active center exceeds a critical distance. However, if the pattern formation has begun only after

Fig. 4 a - c. Formation of periodic structures. Periodic structures are formed if the range of the inhibitor is smaller than the size of the field. (a and b) Regular spacing of activator peaks occurs if pattern formation works during growth. New maxima appear in an area remote to the existing maxima, since the inhibition there is too low to suppress the onset of the autocatalysis of the basic activator production ρ_0. (a) Intercalary growth leads to insertion of new peaks after the distance between the peaks has been approximately doubled. The number of peaks are therefore, in a certain range size-independent (*Gierer,* 1977). (b) In the case of marginal growth new peaks are successively formed in the growing area. (c) A somewhat irregular pattern arises if pattern formation begins to work only after a certain size is obtained. Maxima appear first too close together and some are finally suppressed. A certain minimal and maximal distance is observed. A delay of the pattern formation is possible by an increased basic (activator-independent) inhibitor production ρ_1 (Table 1) ▶

Fig. 5 a - i. Regular spacing of activator peaks as model for phyllotaxis. A growing shoot is simulated by doubling the cells at the upper end of a cylinder; random fluctuation may determine the location of the first maximum (a) which can be used as a signal to initiate a leaf. After further growth (b), the next maximum (c) appears due to the inhibition emanating from the first maximum on the other side; the final result (d) is an alternate (distichous) arrangement. Opposite (decussate) pattern (e) is formed if the diameter of the cylinder is higher or the diffusion range of the inhibitor is smaller, especially if an inhibitory influence from the apex prevents new centers from arising near the apex. Parallelly arranged activator maxima (f) are formed if the growth is fast enough so that cells have some memory that their ancestors were originally activated or if the diffusion of the activator is facilitated in an axial direction. If the cylinder simulation is supplemented by also taking into account the cone-shaped apical meristem, helical arrangements of activator peaks (signal for leaf initiation) can arise. Examples of alternate and opposite leaf arrangements are given in (g) and (h). The parallelly arranged leaflets shown in (i) may arise from an activator pattern as shown in (f). (Fig. 5 a - e according to *Meinhardt,* 1974) ▶

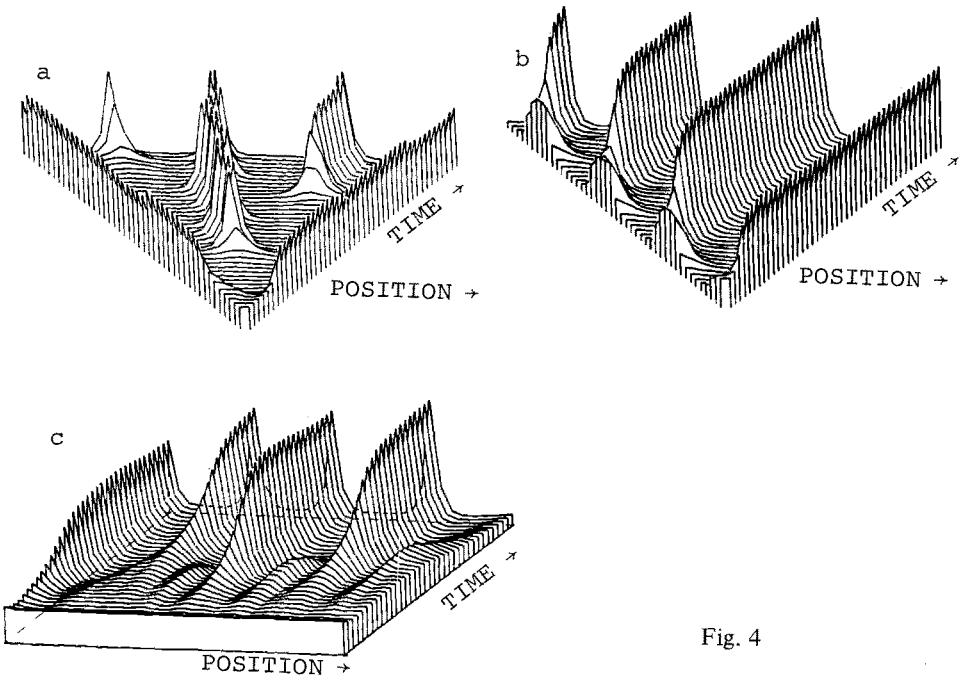


Fig. 4

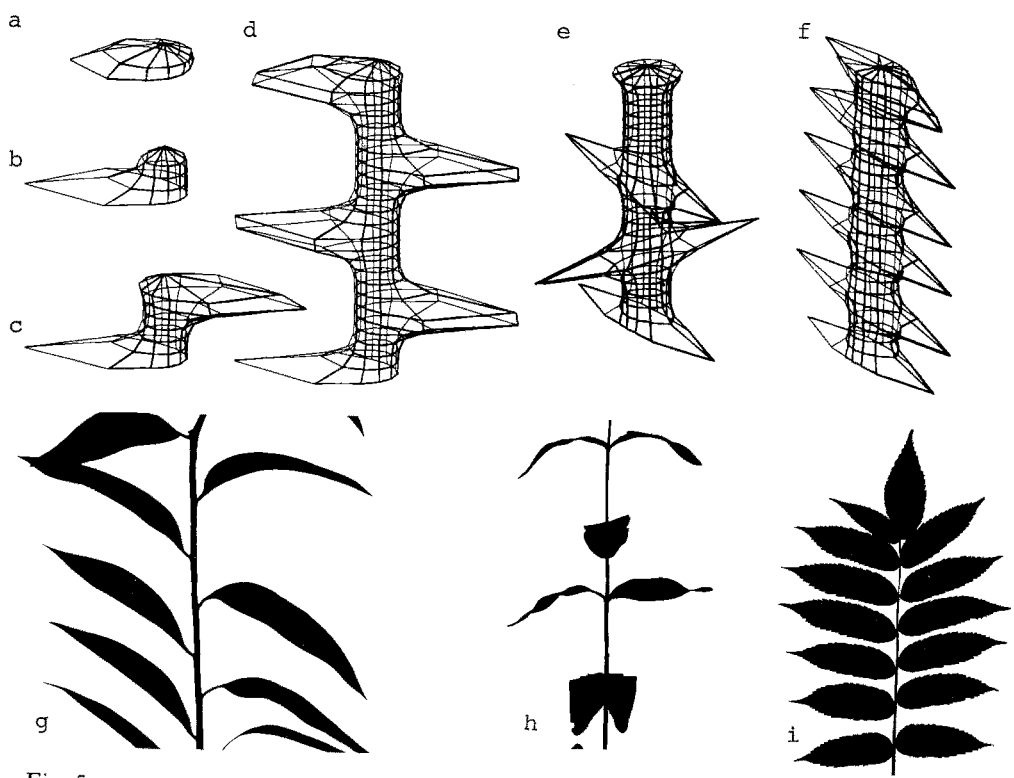


Fig. 5

certain extension has been obtained, the spacing will be somewhat irregular (Fig. 4c); local maxima initially appear too close to one another, since the inhibition originating from each developing center is initially small. With increasing activator concentration, the mutual inhibition also increases and, therefore, some of the initially present activator peaks are, in the course of time, suppressed. An irregular spacing arises, but a maximum and minimum spacing is nonetheless observed. Such a delay in the pattern formation can result from a high constitutive inhibitor production (ρ_1, Table 1).

Examples of both types of periodic pattern can be observed in botany. The leaf primordia are formed during cell proliferation in the shoot apical meristem. It has long been argued that the spacing results from inhibitory fields around each existing primordia and that a new primordia will appear at the location of the lowest inhibition (*Schoute,* 1913). That is exactly what the model can afford. The simulation of a growing shoot by approximation to a growing cylinder (Fig. 5) shows alternate, opposite, or parallel arrangements of activator peaks as signals for leaf formation. An example of a more irregular pattern is the spacing of the stomatas, the apertures in the epidermis of leaves which are used for gas exchange. *Bünning* and *Sagromsky* (1948) pointed out that the stomata formation begins only after the leaves have obtained a certain size and after the cell division in the epidermis has almost ceased, which implies that the concentration of a growth hormone has dropped below a critical level. But cell division once again occurs adjacent to the stomatas. The growth hormone seems to be distributed similarly to the inhibitor in the proposed theory, suggesting that they may be identical: initially the formation of the activator peak is suppressed by a constitutive inhibitor (growth hormone) production (ρ_1 in Table 1) over the entire leaf. Only after the switching off of this production activator peaks (signal for stomata formation) are formed with an irregular spacing (Fig. 6a). Each peak is surrounded by a cloud of inhibition (growth hormone) – leading to further cell divisions here. After ceasing cell division, the leaf grows further by expansion of the cells. If the stomatas become too remote from one another, new stomatas are formed at optimal spacing between the existing ones. Figure 6b shows the generation of new centers according to the theory.

Wigglesworth (1940) and *Lawrence* (1966a, 1970) similarly explained the spacing of bristles in the integument of the bugs *Rhodnius* and *Oncopeltus.* To explain the actual distances, *Lawrence* concluded that the inhibitory circles have no fixed extension but that some normally distributed range of extension is required. According to our theory, the spacing will naturally show some variations. Additional activator centers arise if the inhibitor concentration is below a certain level and not at a

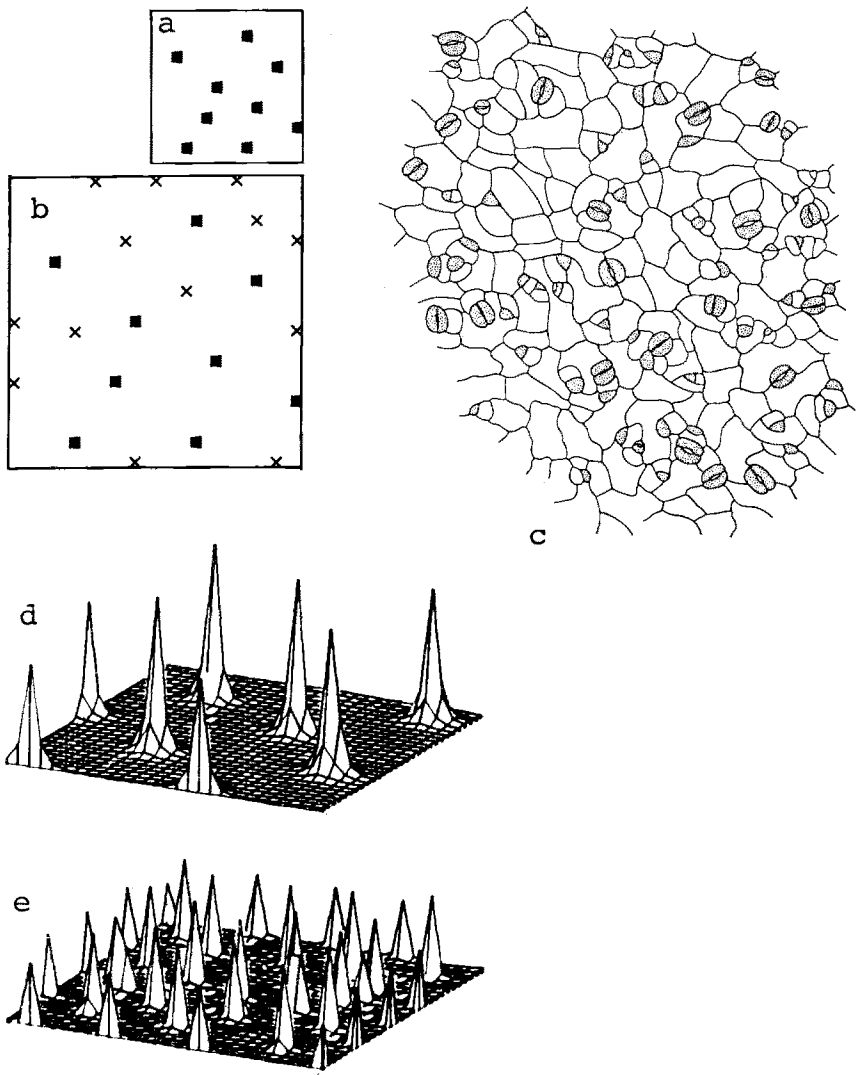


Fig. 6 a - e. Formation of bristle-like patterns. (a and b) Irregularly spaced activator peaks in two dimensions appear if the pattern formation starts after a certain size has been obtained (similar to the linear case, Fig. 4c). The spacing of stomatas on leaves or of the bristles on the sternite of the insects *Oncopeltus* or *Rhodnius* (see *Lawrence*, 1970) closely resembles such a pattern. If, in addition growth occurs, new centers (x) appear at optimal spacing (b). (c) For comparison, the epidermis of the leaf of *Alliaria* (*Bünning* and *Sagromsky*, 1948); the older stomatas (two dark cells) have obtained some distance from each other and new stomatas (one dark cell) are initiated.(d and e) Additional activator peaks can arise also if the range of the inhibitor shrinks. This may be caused by further subdivision into more and smaller cells or by closing intercellular junctions. The accumulation of the less diffusible inhibitor in the activated cells leads to a reduction of the activator peak height (e). Such a mechanism may be responsible for the formation of hairs and bristles in *Oncopeltus* (*Lawrence*, 1970). During the first larval stages, sensory bristles are formed which are remote from one another. During the fifth stage, hairs are formed in between the bristles. The reduced activator concentration may be the signal to form hairs instead of bristles

fixed distance from other centers. More remote centers will also contribute to the inhibitor concentration and have, therefore, an influence on the spacing. A fully developed activator peak is surrounded by a high inhibitor concentration, a new center can be formed only at a large distance from an existing one, whereas the initially lower inhibitor concentration of two simultaneously arising peaks allows their formation in closer proximity.

It is interesting that in leaves, as well as in the insect epidermis, the spacing of different structures is controlled presumably by only one inhibitor; in leaves these structures are stomatas and hairs, in insects (*Oncopeltus*) bristles and hairs. In *Oncopeltus* bristles are formed only during the first four larval stages; hairs are determined in the last, or fifth, larval stage. *Lawrence* (1970) concluded that the distribution of hairs among the bristles is obtained from a shrinkage of the inhibitory fields. According to our theory, after such a shrinkage the newly formed peaks would be of considerably lower peak height (Fig. 6d and 6e) which may signify the change from the signal "make bristles" to "make hairs."

F. Regulation of the Number of Structures in a Periodic Pattern

Waddington (1954) has argued that the hypothesis of the formation of periodic structures by coupled chemical reactions implies a fixed distance between the maxima, the "chemical wavelength." The number of maxima should, therefore, increase with size. But, as a contrary example, he pointed out that a smaller hydra may have the same number of tentacles as a larger one or that the number of somites in amphibia is, to a large extent, size-independent (*Cooke,* 1975a).

Gierer (1977) has shown that if the periodic structure is formed during intercalary growth, an increase in size of nearly a factor two is necessary to allow for the insertion of new activator peaks. This is detectable in Figure 4a where, for instance, the number of peaks remains three despite a doubling of the length.

Another possibility for regulation of spacing would be an adaptation of the inhibitor range to the total size. If the inhibitor lifetime is determined mainly by the loss of inhibitor through the outer surface of the organism into the environmental medium, the inhibitor lifetime would be shorter for a smaller object, since a smaller object usually has a higher surface to volume ratio. This regulates the spacing of the activator peaks within some limits.

G. The Sources of the Activator and Inhibitor and Their Polarity-Determining Influence on the Pattern

Up to now, we have assumed that the tissue is initially completely homogeneous, every cell is able to synthesize the activator with the same efficiency. In fact, it has been one of our aims to show the possibility of pattern formation out of an otherwise unstructured tissue.

However, for the synthesis of the activator and inhibitor there may be certain prerequisites necessary; for instance, a particular messenger or enzyme, ribosomes, energy-rich substances, such as ATP, or the presence of a certain cell type to which the synthesis is possibly restricted. We have called these necessary components "sources." The activator and inhibitor concentrations decide to what extent the sources are active. The source densities enter into the equations simply as factors in the autocatalytic terms (ρ (x), ρ' (x) in Table 1).

The main purpose for considering the source density here is that inhomogeneously distributed sources can provide the asymmetry which decides the orientation of a developing activator distribution. Axial differentials in respiration, in oxidations – reduction reactions – in the permeability, or in electric potentials have been detected in protozoa, eggs, embryos, hydroids, and some algae (see *Child,* 1929, 1941). If a local inhomogeneity has any influence on the activator-inhibitor production, this gradient will orient the activator gradient. Also, each gradient in a substructure will be so oriented, assuring that, for example, the retina, the fore, and the hind leg of an amphibia obtain the correct antero-posterior orientation within the developing embryo.

The distribution of the differentiated cells or cell constituents is a relatively stable tissue property, and a change requires much more time than the change of an activator distribution. Since the source distribution determines the slope of the activator, the source distribution is the (relatively stable) polarity-determining tissue property. This view is supported for hydra by cell dissociation and reaggregation experiments (*Gierer* et al., 1972) in which the formation of a new head is strongly correlated with the location of cells derived from a near head region.

The influence of a graded source distribution can be illustrated with a simulation of hydra transplantation experiments (*Wolpert* et al., 1971; *Wilby* and *Webster,* 1970a,b). A high activator concentration is assumed to induce head formation. The assumption of a graded source distribution is unavoidable for the simulation of these experiments. It is possible that activator and inhibitor production (or release) is confined to the nerve cells which are graded within the animal. In this case, the nerve cells would be the sources of the activator and inhibitor. An alternative possibility would be that the activator and inhibitor are produced in the

Fig. 7 a - i. Simulation of transplantation experiments with Hydra (*Gierer* and *Meinhardt*, 1972). The simulation is intended to demonstrate the polarizing effect of a shallow source distribution, the suppression of an activator peak if induced too close to another one, and the time requirement for the diffusion of the inhibitor. High activator concentration is assumed to be the signal for head formation (dotted in the schematic drawing). The nerve cells are assumed to be the sources of activator and inhibitor ($\rho=\rho'$ in Eq. 4), the assumed distribution is indicated ▲▲▲▲. a: Formation of the graded activator concentration which is used as the initial concentration before experimental interference in (b-g). The schematic drawing illustrates the terminology: H = head or hypostome, 1-4 = gastric column, B = bud, P = peduncle, D = basal disc. (b) Regeneration of a 1-2 piece. The source distribution assures regeneration in the original polarity, even if the activator distribution is completely homogeneous. (c) A small piece from near the head,1,grafted onto a body column regenerates only one head due to the lateral inhibition. (d) A larger piece, 1-2, grafted onto the body column develops two heads. (e) The second head can be suppressed if the original head is left on the first piece. (f) If the first piece is longer and, therefore, the distance between the head and the site of the graft (containing a source density discontinuity) higher, the inhibition of the head may be too low and a new head can be formed. (g) If the head is removed and grafted at the opposite end of the body column, the inhibitor neads too much time to diffuse through the animal; regeneration of a head will take place. (h) If, in a similar experiment, the original head is removed no earlier than 4 hours after implantation of the second head, there is enough time available for the inhibitor to diffuse through the animal and, after removal of the original head, a regeneration is suppressed. (i) If the head of such a hydra with apparently reversed polarity is removed, the regeneration appears according to the original polarity; the source distribution, not the remnant activator concentration, is decisive for the location of the new activation.

This simulation agrees with the experiments of *Wolpert* et al. (1971) (c-f) and *Wilby* and *Webster* (1970 a,b) (g-i)

epithelial cells and that the ability of these cells to synthesize both substances is a graded tissue property.

Figure 7a shows the rapid formation of an activator peak under the influence of the graded source (nerve cell) distribution. The resultant concentrations in the individual parts are used as initial conditions in the simulations of the experimental manipulations. After the complete removal of the activated area (Fig. 7b), the remaining activator distribution is nearly flat. But even the shallower source distribution in this area quickly orients the regenerating activator distribution. A comparison of Figure 7a and b shows that the maximum activator concentration (but not the inhibitor concentration) is independent of the absolute source density (valid only for Eq. 1). Discontinuities in the source distribution can induce a new activator peak (Fig. 7d and f) if an existing activator maximum is not too nearby (compare Fig. 7e and f). An artificial reversal of the activator distribution by grafting a head at the lower end of a body column need not lead to a polarity reversal; a removal of the grafted head, if removed not too much later (less than 24 h), leads to a regeneration according to the original polarity, since the source distribution and not the remaining activator distribution will in this case determine the slope of the regenerating activator distribution.

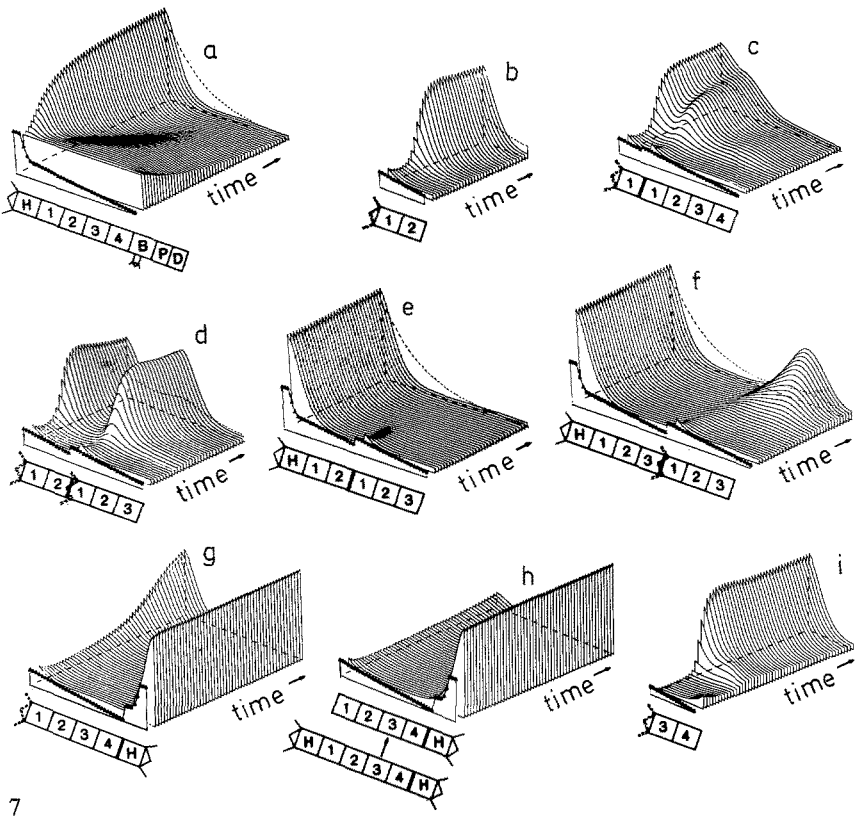


Fig. 7

In very short pieces of hydra tissue, a regeneration on either end can occur (*Barth,* 1940). According to the model, the differences in the source density are too minute to orient the gradient, and differences in the healing wounds may be the decisive factor.

It may appear that a circular argument has been creeping in. We assume that the graded source distribution orients the activator gradient, but what then is the origin of the source gradient? We have seen (Fig. 2a) that in a growing area a polar activator distribution will appear even if the sources are completely homogeneously distributed. If now, for instance, the high activator concentration increases the probability for the differentiation of interstitial cells (stem cells for nerve cells and nematocytes) into nerve cells, the initially homogeneous nerve cell distribution will become graded. The permanent intercalary growth and the long lifetime of the nerve cells lead to a graded nerve cell distribution throughout the animal. The incorporation of growth and the feedback of the activator on its own source into the model (*Meinhardt* and *Gierer,* 1974) allow the description of an indefinite repetition of head removal and regeneration without exhausting the polarity-determining source gradient.

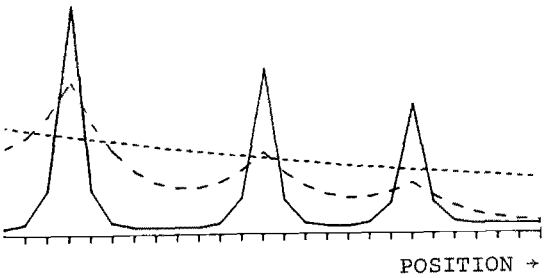


Fig. 8. Formation of a periodic activator distribution with decreasing maximal concentrations. For the explanation of the pattern of head bristles of *Drosophila, Maynard Smith* and *Sondy* (1961) have demanded a periodic prepattern with decreasing maximal amplitude. Such a pattern will be formed in a field of graded source density if the activator (——) concentration at the steady state depends on the source density (---) and the range of the inhibitor (— —) is smaller than the total area. Interactions which lead to source-dependent activator concentrations are the interconversion model (Eq. 2, Table 1) or the interaction according to Equation 1 if the activator source ρ is graded but the inhibitor source ρ' is evenly distributed. In contrast, if, in an interaction according to Equation 1, both sources have the same distribution, the activator concentration is independent of the absolute source density

Depending on the type of interaction, the final activator concentration may (Eq. 2, Table 1, Fig. 8) or may not (Eq. 1, Fig. 7) depend on the absolute source density. An example of a source-dependent activator distribution is given in Figure 8. A periodic structure formed in an area of a graded source density leads to a series of periodic maxima with decreasing peak height. A biologic example may be seen in the formation of head bristles in *Drosophila.* The number of bristles can be affected by mutations. *Stern* (1956) has shown with genetic mosaics that the prepattern in these mutants is unchanged, but that the response of the cells to the prepattern is affected. *Maynard Smith* and *Sondhi* (1961) explained the mutations with a periodic prepattern wherein each maximum is the signal for one bristle. Instead of two bristles, a mutation can show only one at a normal location or a superficial bristle. This indicates that each individual maximum has a decreasing peak height and that the threshold is different in the individual mutants, such that one, two (wild type), or three head bristles are formed.

H. Activator-Depleted Substrate Model

The long-range inhibitory effect need not come from a physically existing substance but can be derived from a depletion of a substance (*Barth,* 1940) necessary for the activator production. A possible interaction which leads, according to the theory (*Gierer* and *Meinhardt,* 1972), to

pattern formation is given in Equation 3, Table 1. Figure 9 shows the development of a localized activator concentration according to this interaction. This mechanism has some properties different from the activator-inhibitor system which may allow an experimental distinction. In an activator-inhibitor system, an induction of a secondary activator peak is possible by an unspecific decrease of the inhibitor, e.g., by UV treatment or cell poisoning. Such unspecific induction is not possible in an activator-depleted substrate interaction, since neither the removal of the activator nor the removal of substrate will induce a new activation. This unspecific induction indicates the existence of a real inhibitor.

With increasing size, the concentration of the substrate in the non-activated area increases. Therefore, the distribution of the substrate in the vicinity of the activator maximum increases and also becomes steeper; the activator production near the maximum may become higher compared to that at the maximum itself. The location of the activated area at a boundary then becomes unstable and shifts into the center of the area where a substrate supply from both sides of the activator peak is possible (Fig. 9). With further growth, the activator maximum would split into two, both maxima separating from each other. A case in which such a process may be used is the formation of the growth zone of the murein sacculus of the bacterium *E.coli*. If the size of the bacterium surpasses a certain length, the growth zone first increases in activity and then divides

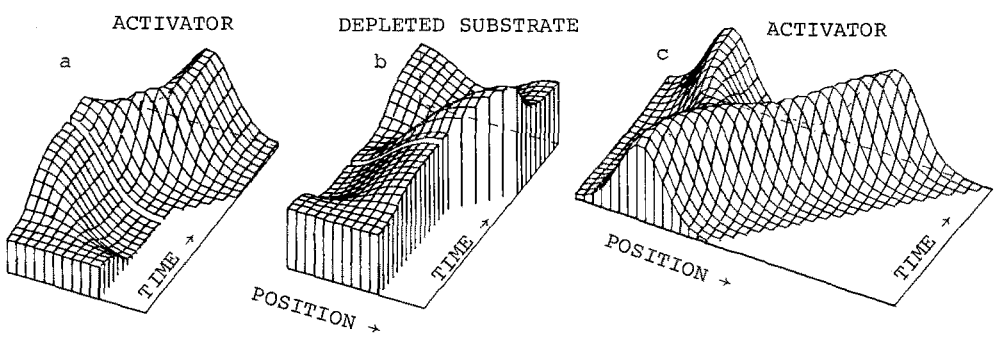


Fig. 9 a - c. Activator-depleted substrate model. An even simpler pattern-forming reaction does not require an inhibitor. A reaction in which the production of an autocatalytic substance (activator, left) proceeds at the expense of a more rapidly diffusing substrate (e.g., a precursor) can lead to pattern formation (Eq. 3, Table 1). When the field is small, only a marginal activation is possible. During growth, the substrate concentration in the nonactivated part can increase so that the location of the activator peak becomes unstable; a shift to the center follows. After further growth, the central peak will be split and the resulting two maxima separated (c). This can subsequently repeat itself. Such an activation-depletion mechanism may be involved in the selection of the zone of growth in *E.coli* (*Schwarz* et al., 1975). Equation 3 is used with the following constants:

$\rho \equiv 1$, $c=0.01$, $\mu=0.01$, $D_a=0.025$, $c_o=0.01$, $\nu=0.0001$, $D_s=0.4$

into two (*Schwarz* et al., 1975), thereafter the pole cups are formed and the division is initiated.

Thus, in the activator-depleted substrate system, the regular distribution of activator maxima is obtained by splittings and shifts of previously existing activator centers as the size of the organism increases. This is in contrast to the activator-inhibitor system where wholly new activator centers arise at a distance from previous centers.

I. Oscillating Patterns and Their Use in Chemotactic-Sensitive Cells

An activator peak, once formed, is very stable, and a shift in space by changed external influences is nearly impossible, since any activator peak is shielded by a "cloud" of inhibition. This stability is desirable for many situations; for others it is not. As already mentioned, small external influences can direct the location of a developing activator maximum. This mechanism offers a possibility for a chemotactic-sensitive cell to detect weak concentration differences. But the stability of an activator peak once formed would prevent a continuous adaptation to the changing external conditions. A possible solution would be an oscillating establishment and decay of an activator peak, whereby at each oscillation, the activator peak can be newly localized at the best position available. Periodic formation of the activator peak is obtained if the lifetime of the inhibitor is longer than that of the activator (*Meinhardt* and *Gierer,* 1974) or, in the activator-depleted substrate model, if the substrate concentration equilibrates too slowly. The periodic appearance of an activator maximum according to the latter mechanism is shown in Figure 10. Soon after the initiation of the activator production, the substrate is used up so rapidly that the activator production collapses. The localization at which the maximum appears depends on small external influences and may be changed from one oscillation maximum to the next.

An example where an oscillating maximum is used for the detection of an external gradient may be the aggregating ameba of the slime mold *Dictyostelium discoideum* (*Raper,* 1940; *Bonner,* 1947; *Gerisch,* 1968; *Gerisch* and *Hess,* 1974) which find each other by chemotaxis after a shortage of food.

In the model, the cells would have a sensitive phase for the orientation of the activator peak shortly before the next activation occurs. The short lifetime of the activator within the cell may reflect a secretion of the activator into the medium, thus providing a signal for other cells. The longer lifetime of the inhibitor or the time necessary to restore the substrate concentration leading to a lag phase before the next oscillation can be stimulated. Increasing activator concentration in the medium can

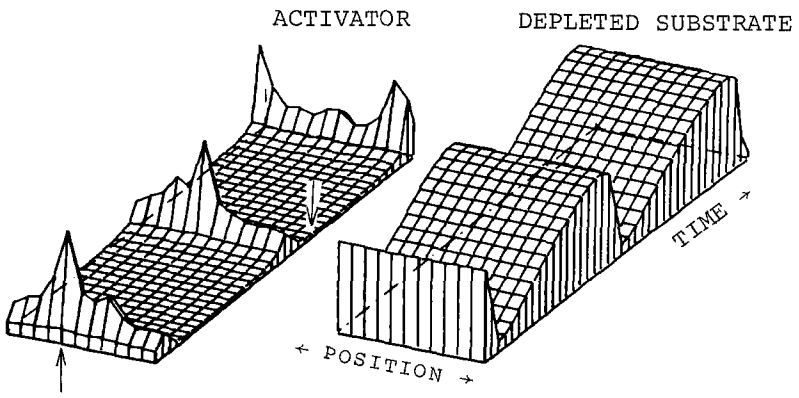


Fig. 10. A model for chemotactic sensitivity. The high amplification of small local differences which is inherent in the activator-lateral inhibition mechanism, can be used to detect the direction of an external gradient. A periodic adaptation to changing environmental conditions is possible if the activator concentration oscillates, as is demonstrated here with the activator-depleted substrate model. Oscillation occurs if the substrate concentration S adapts too slowly to a changing activator concentration. The calculation is made for a circular object; to allow a space-time plot, the circle is cut up, the left and right ends of the represented distributions are, therefore, in reality adjacent neighbors. Small environmental differences (assumed here to consist of 1% difference in ρ_0 across the circle with highest concentration in the direction of the arrow) will trigger a sharp local maximum which could then be used as a signal to draw out pseudopods at this side. All the substrate is then consumed and the next activation is possible only after a high S concentration has again been accumulated. If the direction of the external gradient has been changed (arrow), the location of the activator peak will change accordingly. Such a mechanism may be the basis for the chemotactic sensitivity of aggregating ameba of the slime mold *Dictyostelium discoideum*. Equation 3 is used with $c = 0.01$, $\mu = 0.03$, $D_a = 0.005$, $c_0 = 0.01$, $\nu = 0.0001$, $D_h = 0.4$, $\rho = 1 \pm 1.5\%$ random fluctuation. That signifies that a concentration difference of 1% across the cell can be detected even if, for instance, the receptor density on the cell surface varies considerably

shorten the time until the next oscillation occurs which allows the synchronization of the individual cells. Therefore, in addition to the ability to respond in a directional way, this simple mechanism has all the properties demanded by *Cohen* and *Robertson* (1971) for the relay mechanism in *Dictyostelium*.

A similar process may be going on in the growth cone of an extending nerve fiber. The growth cone has to find a particular target cell, presumably by following a signaling gradient. *Harrison* (1910) has shown that nerve extension is not a continuous process but proceeds stepwise. The reason may be the described permanent change between the measurement of the gradient and – after the internal amplification – growth in the thereby defined direction. More details about the formation of netlike structures will be given below.

III. Control of the Formation of Several Structures by a Morphogen Gradient

In many cases, several structures are determined in one developmental process. It has long been argued (*Bovery,* 1901; *Child,* 1929, 1941) that such spatial organization could be accomplished by the graded distribution of a substance – a morphogen. *Wolpert* (1969, 1971) has developed this idea further into the concept of positional information. He demonstrated that the size of an embryonic system is small when determination occurs, of the order of 1 mm or 100 cells across. *Crick* (1970) has shown that by diffusion and local production and destruction at each end, a gradient of this size can be formed within a few hours. This order of magnitude seems reasonable. Gradient formation in an area with a dimension of the order of 1 cm, on the other hand, would require a full day. It is thus tempting to speculate that the spatial development of an organism or of parts of it is controlled by a graded distribution of a substance during a stage of development where the extension of the region is of the order of 1 mm, and that the local concentration determines the further developmental pathway of each cell.

There are several ways to set up a graded distribution. The assumption of a morphogen source and/or sink alone would only shift the problem of morphogenesis to another level as long as no explanation is provided as to how they arise in an undifferentiated tissue. The mechanism described in the preceding paragraph can account for the formation of local high concentrations which may act as sources or sinks, and, in addition, explains why they usually appear at the boundaries of the system. A linear gradient can be obtained with a source at one side and a sink at the other (*Crick,* 1970), but such an arrangement has some disadvantages. First, the size regulation is poor; the concentration around the source depends on the mutual distance of the source and the sink (Fig. 11a) as long as no special mechanism is available by which the source strength is increased for smaller sizes. Secondly, the relative morphogen increase per unit length is high at low morphogen concentration and low at high morphogen concentration. The cell must be able to measure high concentrations much more precisely than low concentrations if it is to achieve the same spatial accuracy throughout the tissue. Both problems are avoided if the morphogen decays not only at the terminal sink but, to some extent, everywhere (Fig. 11b). The concentration around the source is then nearly independent of the total size and determined mainly by the local decay rate. The slope is steeper in the area of high morphogen concentration with an approximately constant change per unit length as would be desired for uniform reading accuracy. In addition, the time required to reach the steady-state concentration is much shorter, since only a diffusion into

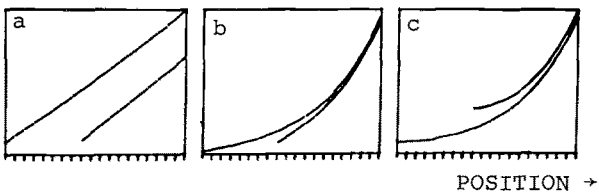


Fig. 11 a - c. Concentration profiles of morphogenetic substances, generated by different source-sink arrangements and their dependence on field size. (a) A linear gradient can be formed by a source (here at the right side) and a sink (left). The concentration around the source will depend very much on the distance between the source and the sink, as demonstrated by the long and the short curve. (b) A uniform breakdown in addition to that at the terminal sink leads to an approximately size-independent concentration at the source and sink, since the concentration around the source depends more on the local decay rate. The relative concentration increase per unit length is approximately constant which facilitates the discrimination between the different concentrations during the interpretation of the positional information by the cells. (c) A simpler system consists only of a terminal source and a uniform breakdown. The advantage of simplicity is counterbalanced by the problem that at the end opposite to the source the concentration is size-dependent and shallow. Nevertheless, the central and source-containing portion of the gradient can be used to supply positional information; an asymmetric location of the determination of the structures is then to be expected. This mechanism seems to be used in the determination of segments in insects or of the digits in chicken limbs

the immediate environment is necessary for the adjustment of the concentration around the source. Therefore, an approximately exponential gradient formed by a terminal source and sink with an additional overall decay of the morphogen is much more convenient for cell specification than a linear gradient produced by a morphogen source and sink alone.

The question arises if, in addition to the homogeneous destruction of the morphogen, a terminal sink is required at all. For the formation of a local sink a separate activator-inhibitor system would be necessary. A relatively simple pattern-forming system would, therefore, consist of a local source only and a uniformly distributed decay of the morphogen but without a local sink. The price paid for this simplicity is that the gradient at the end which does not contain the source is shallow (assuming the boundaries are impermeable), and the absolute concentration here is size-dependent (Fig. 11c). Therefore, appropriate positional information can be supplied only in the central region and that portion of the tissue containing the source of the gradient. In other words, only a certain concentration range of the gradient can be used. An advantage of using only a fraction of the gradient is that the mechanism then becomes insensitive to a size variation of the tissue over a certain range, since the fraction of the gradient used will be present both in a larger and a smaller field (Fig. 11c). Indeed, if only one organizing center is involved, the area opposite to the organizing center seems, in most cases, not to be used for the specification of structures. Two examples are the determina-

tion process in early insect development and the determination of the digits in chicken limb buds (*Tickle* et al., 1975) which will be discussed in detail below. The unused cells in the portion of tissue where the gradient is beyond the limit utilized for the relevant development may become necrotic and the constituent material recycled into the growing tissue. Or – vice versa – the utilization of the full region between the terminal boundaries of a diffusible gradient is a first indication that the gradient is set up by a terminal source and sink.

Hörstadius (1939) has proposed a double gradient system for the explanation of his combination experiments with sea urchin embryos. A source of vegetativizing and animalizing substances at the poles are assumed to be such that two gradients with opposite polarities would be formed. Further, he assumed that the local ratio of the two substances determines the developmental pathway of the cells. But a source-sink mechanism would also be compatible with the experiments, since a removal of a part of the source or a part of the sink would lead to a general decrease or increase in the gradient level – with the consequence of subsequential vegetalization or animalization as observed.

A. A Gradient Model for the Early Insect Development

A very convenient system for studying the determination of several structures within one process is early insect development (for review, see *Sander,* 1976; *Counce,* 1973). After fertilization, the dividing nuclei in the egg spread out into the cytoplasm (cleavage stage) and migrate finally to the egg periphery, coming to rest in a well-defined layer (syncytic blastoderm stage). Only then are cell walls formed between the nuclei, leading to the cellular blastoderm. The embryo proper – the germ band – is formed out of a fraction of this blastoderm. The segments of the larvae are linearly arranged and become individually distinguishable during germ band formation. The final pattern can be experimentally disturbed by centrifugation, ligation, thermocauterization, puncture, or UV irradiation. The reaction of the system to such interference should provide information on how normal development is controlled. Since the egg is well-supplied with nutritional substances, a development into recognizable structures is possible even after severe experimental disturbances. A large amount of experimental results have been accumulated for different species, providing challenge to any model.

It has been shown (*Meinhardt,* 1977) that a gradient, formed by an autocatalytic mechanism as discussed above, can account quantitatively for many irradiation, plasma-shift, and ligation experiments. This model should be described here in some detail.

In adapting the proposed mechanism to insect development, the first question that arises is at which end the morphogen is produced. *Seidel* (1929) found evidence in *Platycnemis* for the local production of a substance necessary for the organized development of the embryo at the posterior pole (see also *Sander,* 1976). An exclusion of the posterior tenth of the egg by a ligation suppresses embryonic development, but a narrow channel left by an incomplete ligation – too small for cells or nuclei to pass – allows normal development in the anterior part. This indicates that a diffusible substance, essential for the organization of the development of the insect spreads out from the posterior pole. The assumption of one organizing center at the posterior pole is supported by the fact that the embryo in many species is formed out of the more posterior portions of the blastoderm, while the cells in the more anterior portions develop extraembryonally. This supports the model that only a fraction of the gradient can be used; the fraction varying considerably from species to species (see *Krause,* 1939).

As shown above, a local high concentration at one end of an extended structure can be formed by an activator-inhibitor mechanism. Any substance whose production is controlled by the localized activator could act as a morphogen. Since the inhibitor production is activator-controlled and since the inhibitor, due to its higher diffusion rate, has a graded distribution throughout the total area, the inhibitor is a reasonable candidate for the morphogen. Our assumption will be, therefore, that a high activator concentration is formed at the posterior pole and that the cells or nuclei and their immediate plasma environment "learn" from the local inhibitor concentration which segment they must form. The explanatory power of this model can be illustrated by comparison of experimental results with the behavior of the model.

1. Formation of Posterior Structures at the Anterior Pole

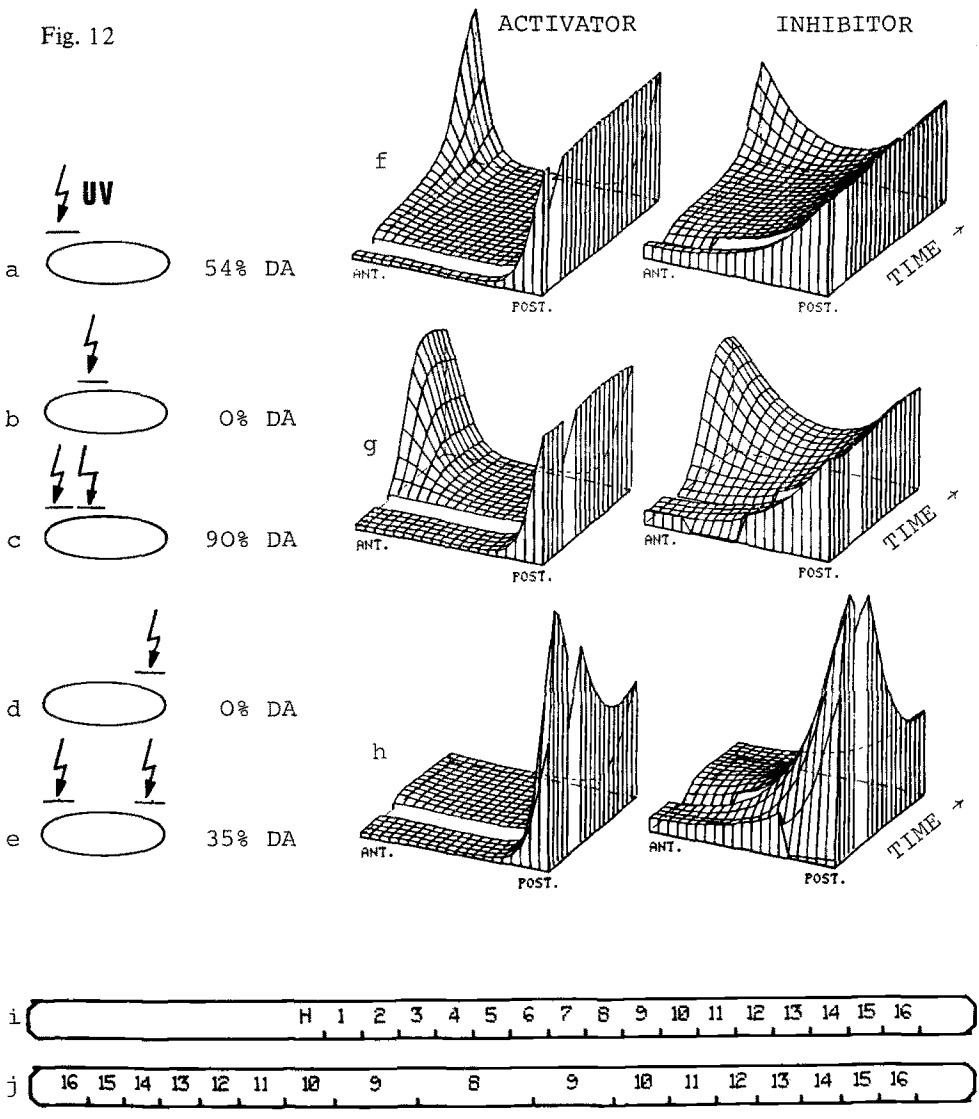
After experimental interference, many species form abdominal structures instead of head structures in their anterior portion. Frequently, a completely symmetric development is observed. The experimental treatments evoking such "double abdomen" malformation are quite diverse: UV irradiation (*Yajima,* 1964; *Kalthoff* and *Sander,* 1968) or puncturing (*Schmidt* et al., 1975) of the posterior pole, temporary ligation of the egg of *Callosobruchus maculatus* fabr. (*van der Meer,* personal communication), and centrifugation (*Yajima,* 1960). Double abdomen formation has also been found in a maternal effect mutant of *Drosophila* (*Bull,* 1966).

In the model, the abdominal structures are formed where the inhibitor concentration is high. The formation of additional posterior struc-

Fig. 12 a - j. Double abdomen (DA) formation in *Smittia* – comparison of experimental results (a-e) and the explanations given by the theory (f-j). Irradiation of the anterior quarter of a *Smittia* egg can lead to a completely symmetric embryo with one abdomen at each pole (*Kalthoff* and *Sander,* 1968). (a-e) The results of the experiments of *Kalthoff* (1971a). The dose of the irradiation of the anterior quarter was adjusted to yield about 50% DA, the dose of additional irradiations was somewhat smaller in order to minimize the number of eggs which fail to develop at all. Experiment: The irradiation of the anterior quarter leads to a symmetric embryo (a). Model: It is assumed that during the oogenesis, a local high activator concentration (see Fig. 2a) is formed at the posterior pole, and activator and inhibitor have already attained the steady state. The inhibitor is assumed to be UV-sensitive. A reduction of the inhibitor concentration at the anterior pole caused by an irradiation allows an increase of the activator concentration which can, via autocatalysis, develop into a full second maximum (f). The inhibitor (positional information) has a mirror image of the posterior pole distribution at the anterior pole.
Experiment: While an irradiation of the second anterior quarter is without effect (b), applied together with an irradiation of the first quarter, it considerably increases the probability of double abdomen induction (c). Model: The removed inhibitor in a central area is rapidly replenished by the nearby source (g) and, therefore, without effect. But such a removal delays the restoration of the inhibitor concentration after an irradiation of the anterior quarter. Therefore, the activator increase after an irradiation of the anterior half is much more rapid (g), and the probability of reaching the critical level for the DA formation is increased.
Experiment: An irradiation at the posterior pole is without serious effect (d), but such an irradiation reduces the probability of a DA induction by an anterior irradiation (e). Model: Inhibitor reduction at the activated site leads to an overshoot of the activator concentration and, consequently, also in the inhibitor concentration. This is without serious effect, since all concentrations necessary for the determination of any particular structure remain present. But the overall increased inhibitor concentration reduces the activator increase after "irradiation" of the anterior end; the activator increase may not be sufficient to reach the threshold for further autocatalysis and may, therefore, disappear. The approximate location of the determination of the segments (i) can be concluded from the late blastoderm ligations (see Fig. 14a-b). If the normal inhibitor concentration is responsible for such a determination, the symmetric inhibitor distribution after DA induction (f) would lead to a segment pattern as shown in (j) which is essentially in agreement with the experiments of *Kalthoff* and *Sander* (1968). Simulations according to *Meinhardt* (1977)

tures at unusual locations would indicate the activation of a second morphogen source. An especially favorable location for the formation of a second activation is the anterior pole, since here the inhibitor has its lowest concentration and any unspecific additional reduction of the inhibitor concentration may be sufficient to induce a new center of activation in agreement with the unspecific modes of double abdomen induction already mentioned. The midge, *Smittia,* where double abdomen formation can be induced by UV irradiation (*Kalthoff* and *Sander,* 1968) or by a puncture (*Schmidt* et al., 1975) at the anterior pole may serve as an example. According to the model, the UV irradiation may either destroy the inhibitor or the inhibitor-producing structures. The results of a very instructive set of experiments by *Kalthoff* (1971a) are shown in Figure 12, together with their explanation with the theory assuming that the

Fig. 12



inhibitor is UV-sensitive. Due to the inhibitor reduction, the activator concentration increases. If this activator increase is sufficiently high, a new activator maximum develops via autocatalysis, even if the inhibitor concentration is rapidly restored. If the activator concentration fails to reach the critical level, the activator increase will disappear (Fig. 12h). In agreement with the experiment, the formation of a second activation is an all or nothing event. Substantial support for the postulated activator-inhibitor interaction can be derived from the fact that a posterior irradiation *reduces* the probability of induction of a double abdomen by irradiation of the anterior pole. A reduction of the inhibitor at the activated (pos-

terior) site produces an overshoot of activator. As a result, more inhibitor is subsequently produced which spreads out quickly by diffusion and then acts to reduce the probability of triggering a second activation center at the anterior pole (Fig. 12h).

The central segment formed in a double abdomen embryo can be predicted if the diffusion rates, lifetimes, and approximate positions of the segments are estimated from ligation experiments (see below). The segment eight or nine is expected (Fig. 12j) which essentially agrees with the experimental observation.

Kalthoff (1971b) found further that the probability of double abdomen formation can be reduced by a second irradiation with visible light or with UV of longer wave length (photoreversal). The second inactivation can still be effective with a delay of several hours after the first irradiation (*Kalthoff* et al., 1975). Recently, *Kandler-Singer* and *Kalthoff* (1976) found that RNAase treatment of a puncture of the anterior pole induces double abdomen very efficiently, indicating unambiguously that RNA is involved in this pattern-forming process. This finding can be incorporated into the model by the assumption that this RNA is a part of the inhibitor-producing system, for instance, a mRNA coding for the inhibitor. With the constants used for the calculation of Figure 12, a reduction of the basic inhibitor production (ρ_1 in Eq. 4 of Table 1) of less than 50% is sufficient for the triggering of a second activation.

The dynamic behavior of the system may help to distinguish whether it is the inhibitor or the inhibitor-producing structures which are UV-sensitive. The inhibitor must have a relatively high turnover rate, otherwise a new steady state could not be attaind within a few hours – as is indicated by the fact that a double abdomen can be induced quite late in the preblastoderm (*Kalthoff* et al., 1975). Thus, the inhibitor would be rapidly restored after destruction by irradiation (see Fig. 12g). The experiments shown in Figure 12a and c demonstrate a higher yield of double abdomen if the anterior half is irradiated. If the inhibitor is the UV-sensitive molecule, a sequential irradiation of the two anterior quarters with a 1 h delay should reduce the probability of the double abdomen formation to the level expected after an irradiation of the anterior quarter alone, since due to the high turnover, the inhibitor concentration would be restored in between the two irradiations. But, if instead, the more stable inhibitor-producing structures are hit, a sequential irradiation of the two anterior quarters should lead to the same probability of double abdomen formation as an irradiation of the anterior half. Pilot experiments of *Kalthoff* (personal communication) seem to support the latter mechanism.

Further evidence that the source is dynamically activated by autocatalysis and lateral inhibition and that it is not only a result of prefor-

mation during oogenesis is available. A ball of symbionts is located at the posterior pole of the egg of the leaf hopper *Euscelis* (*Sander,* 1959, 1960, 1961a, b, 1975b). A dislocation of this posterior pole material in anterior direction has a dramatic effect on the further development of the animal. For instance, after a shift and a ligation, up to three abdominal structures can be formed within one egg, some with reversed polarity. These experiments are explainable by the theory assuming that some activated plasma is shifted with the symbionts (*Meinhardt,* 1977). Traces of this activated plasma can develop a fully activated source, preferentially at the physical boundaries of the (ligated) egg. In contrast, a stable morphogen source subdivided into two or three parts would only yield a much reduced morphogen concentration and no abdominal structure would be expected – in contradiction to the experiment. Similarely, *Seidel* (1929) found in *Platycnemis* that up to 10% of the egg at the posterior pole can be removed and a complete germ band can still be formed. For early ligations, this is an all or nothing effect. Again, with a static morphogen source, one would expect a gradual drop in the morphogen concentration if increasing portions of the source were eliminated. But an autocatalytically activated source will regenerate (see Fig. 1) as long as sufficient activator is included in the anterior portion.

2. The Interpretation of the Positional Information

So far, we have assumed that the positional information is contained in a graded distribution of a diffusible substance. The cells, of course, must convert this labile information into a more permanent form by switching genes on or off. Some hints as to the functioning of this process can be derived from ligation experiments.

Ligating an egg during the cleavage stage leads to an omission of segments in many species; for example in *Euscelis* (*Sander,* 1959), *Calliphora* (*Nitschmann,* 1959), *Bruchidius* (*Jung,* 1966), or *Protophormia* (*Herth* and *Sander,* 1973). As illustrated with the results of *Sander* (1975a) with *Smittia* in Figure 13, the terminal structures remain present after a ligation, but the more central segments, normally formed out of cells located in the blastoderm on either side of the ligation, are omitted. In terms of the model, a ligation during the cleavage stage leads to an accumulation of the morphogen in the posterior part and consequently to a shift of the location of the determination of any particular structure there in an anterior direction (Fig. 14). The segments which would be normally determined just posteriorely to the ligation are omitted. From the experimentally known omission of segments in the posterior portion and the minimum time in which a second activation can be formed, one can estimate the diffusion rate and the lifetime of the inhibitor at $5 \cdot 10^{-9}$ cm^2/s and

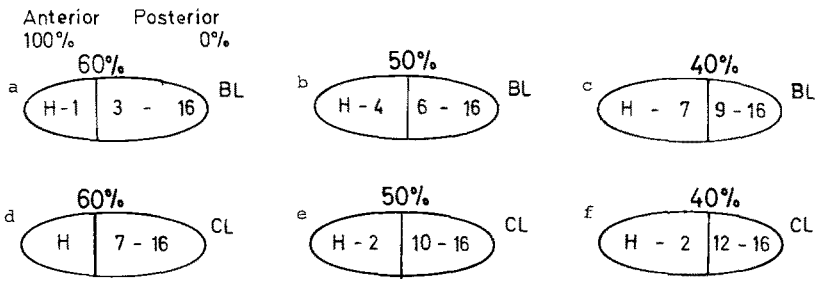


Fig. 13 a - f. Schematic drawing of experimentally observed germ band fragments after the ligation of eggs of the insect *Smittia* (*Sander,* 1975b). Details about the gradient and its interpretation can be derived from the aberrant pattern observed after a ligation. The segments are designated H (head lobe), 1....16. The first and last segment formed in each germ band fragment are indicated. (a-c) After ligation during the blastoderm stage (BL) very few – if any – segments are omitted: the egg behaves as a mosaic. (d-f) If, however, the ligation is made earlier, during the cleavage stage (CL), many segments are omitted, but the terminal segments always remain present. Note that the gap formation is asymmetric: if a ligation is performed more anteriorly (d), the segments omitted belong more to the posterior portion, while in the anterior part, a head lobe is formed nearly independent of the time of operation. But if the ligation is performed at a more posterior location, more segments are lost in the posterior part than gained in the anterior part. The explanation in terms of the model is given in Figure 14

1 h, respectively. However, a description of the omission of segments in the anterior portion with these parameters is not in agreement with the experiment. From the estimated short lifetime, one would expect a relative fast decay of the inhibitor such that no segments at all would be formed in the anterior part (Fig. 14). On the contrary, the head lobe is always formed. As Figures 13a and 13d show, a ligation at 60% EL (%EL = % egg length, 0% = posterior pole) of a *Smittia* egg leads to the same segments being formed in the anterior part independent of whether the ligation is made early, during the cleavage, or late, during the blastoderm stage. In such a ligation experiment, a particular cell or nucleus seems to be already determined at the time of the ligation if located anterior to the ligation, but the final pathway can be changed if located posterior to the ligation. Extensive reprogramming seems to be possible so that a more anterior structure can be reprogrammed to form a more posterior structure but not vice versa.

The explanation I have proposed for this stipulates the nature of the action of the morphogen on a cell: Originally, all cells are programmed to form the most anterior structure. Under the influence of the morphogen, the cells proceed stepwise to higher determination levels – "head," – "thorax," – etc. – until the level of determination corresponds to the local morphogen concentration. A step in the determination is – as in other development systems – irreversible. Determination – or commit-

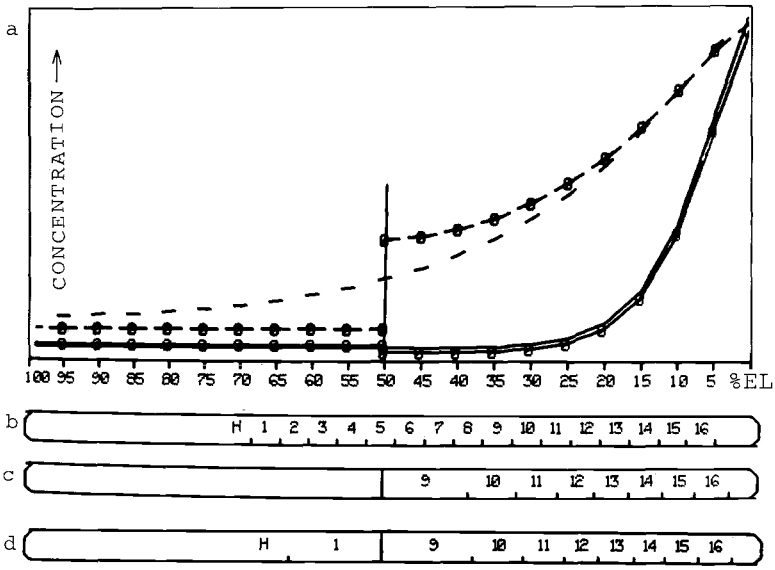


Fig. 14 a - d. The influence of a diffusion barrier (ligation) upon the gradient and the pattern it controls. Undisturbed activator (——) and inhibitor (---) distributions are shown together with their adjustments after a ligation (curves marked with a circle). (b) Segment pattern assumed to be formed if an undisturbed gradient is present. (a and c) After a ligation, the inhibitor accumulates on the source-containing posterior side of the ligation, which leads to a shift of the segments in an anterior direction. The segments normally determined posterior to the ligation would be no longer formed; in this example, the elements 5-8 would be omitted. In the anterior portion, on the other hand, the inhibitor (morphogen) concentration decreases to a level which normally never occurs, and no element would be expected to be formed. But this is at variance with the experimental observation (see Fig. 13e). This contradiction has forced the assumption that the nuclei are initially programmed to form the most anterior structure and that, under the influence of the morphogen, the determination proceeds stepwise to more posterior structures until the determination corresponds to the local morphogen concentration. The structures formed in the anterior part after a ligation reveal how far the determination has already advanced at the time of the ligation. Therefore, after an early ligation, only a head lobe is formed anteriorly and this result is fairly independent of the position of the ligation (Fig. 13d and f). On the other hand, the pattern in the posterior portion depends on the extent of morphogen accumulation and the time available to adapt the determination to this increased morphogen concentration. With this, an explanation of the asymmetry noticed in Figure 13 is given and the simulation (d) (*Meinhardt*, 1977) agrees with the experiment

ment – of a group of cells to form, say, a head lobe must consist of switching on a particular set of genes. If, instead, a neighboring structure should be determined by a higher morphogen concentration, the corresponding genes must be switched on; but the "head genes" must also be turned off. Instead of many specific inducers or thresholds, each activated gene may try to activate the genes necessary for the determination of the next posterior structure. But for this switch in gene activity,

an additional unspecific signal "proceed" would be required. The signal proceed would be produced in proportion to the local morphogen level and in some way progressively inactivated as the determination proceeds to more and more posterior structures. The sequential determination will come to rest at a stage where the determination corresponds to the local morphogen concentration. If, during this process, the morphogen disappears, the proceed signal will no longer be formed, and the determination would remain unchanged at the stage already reached. Such a situation exists in the anterior part after a ligation. But if the morphogen increases artificially, for instance by the accumulation of the morphogen in the posterior portion after a ligation or after the induction of a second activation by UV irradiation, the determination can proceed, and structure corresponding to more posterior positions would then be formed.

3. Further Spatial Specifications

The proposed mechanism allows the subdivision of the organism into several segments, perhaps as many as 16. An indication of a further subdivision shortly after the blastoderm stage is the specification of the imaginal disc cells in *Drosophila* (for review, see *Gehring* and *Nöthiger,* 1973). *Lawrence* (1973) found evidence for the formation of segment borders shortly after the completion of the blastoderm: genetically marked cells can move within a certain territory, but cannot cross an otherwise invisible border. *Locke* (1959) demonstrated repetitive gradients within the segments of the larvae of *Rhodnius,* and *Lawrence* (1966b) concluded from the orientation of hairs in the tergit of *Oncopeltus* that the segment borders are impermeable to the morphogen and that a discontinuity must exist across the segment borders. Transplantation experiments within a particular segment with pieces of epidermal tissue in *Rhodnius,* with a ripple pattern as indicator (*Lawrence* et al., 1972; *Locke,* 1959), have shown that an anterior piece of tissue grafted in a more posterior position adapts much more rapidly to the new environment than vice versa. It is thus tempting to speculate that after the formation of the segment borders, the remains of the primary gradient trigger subgradients within each segment, possibly also with the high concentration at the posterior end, and that the interpretation of the positional information thus formed obeys the same rules as segment formation (see also *Sander,* 1975a).

But the intersegmental pattern formation also shows pronounced differences from the primary pattern formation of the segment determination. In the above-mentioned investigation, *Lawrence* (1966b) found that a segment border of *Oncopeltus* can occasionally show a gap. In such a case, the orientation of hairs changes in such a way that one has to

assume that morphogen flowed through the gap in the segment border, leaving a decreased morphogen concentration on one side and creating a cone-shaped region of increased concentration on the other (the sand model). This suggests, that the intersegmental gradient is not formed dynamically by autocatalysis and lateral inhibition, since a diffusion of activator through such a gap into a nonactivated region would trigger a second activation there resulting in a symmetric pattern within a segment. This is clearly not what happens in the experiment. The intersegmental gradient is, therefore, presumably maintained by a previously fixed tissue property (homeostasis, see also *Lawrence* et al., 1972), possibly, especially of the segment border.

Another reason argues against the generation of the intersegmental gradient by an activator-inhibitor mechanism. The length of the individual segments in the antero-posterior dimension is small compared with their breadth. As we have seen earlier, an activator-inhibitor gradient will orient itself in the direction of the largest spatial extension available. After the subdivision into segments, this is no longer the antero-posterior axis but rather, say, the dorso-ventral axis. The subdivision itself, therefore, produces the condition which renders the generation of a gradient in a new orientation possible. In agreement with this consideration is the fact that, at least in *Euscelis* (*Sander,* 1971) and *Tachycinis* (*Krause,* 1935), the dorso-ventral organization takes place after the interpretation of the anterior-posterior axis is finished, i.e., after blastoderm formation. Longitudinal ligation before blastoderm formation may cause a reorganization of the dorso-ventral axis and parallel-oriented twins may develop, one in each portion.

Early insect development shows a great variability among the different insect species (see *Sander,* 1976), but here it has only been possible to demonstrate the model with a few examples. Some parameters can easily be changed to cover other experimental observations. The diffusion range of the morphogen and limits of concentration used for pattern specification would decide to which extent the blastoderm is used for the formation of the embryo proper. If the activator range is increased, a larger portion at the posterior pole of the egg can be removed and a complete germband would still be formed. The gap phenomenon is more pronounced for higher morphogen diffusion rates; the asymmetry of the gap would be higher if the interpretation were a faster process. The induction of double abdomen is rendered more difficult if the basic activator production is smaller.

So far, changes in the pattern observed have been accounted for solely by a change in the prepattern, i.e., a change in the primary signal the cells have received. But one must also consider the possibility that the "memory" of the cell – i.e., the way in which they register which signal

has previously been received – is altered. This is especially likely in the case where structures which normally are never formed in adjacent locations are formed in touch with each other. In such a case, the jump in the positional specification may be partially compensated for by a process similar to the intercalary regeneration.

B. Organization of the Antero-Posterior Axis in Vertebrate Limb Development

The proposed model for early insect development may be applicable to other developmental systems as well. *Saunders* and *Gaseling* (1968) have discovered a "zone of polarizing activity" (ZPA) in the chick wing bud. An implantation of this area into other regions of a wing bud can evoke the formation of additional posterior wing structures. Whereas during normal development, the digits 2,3, and 4 are formed (4 is the most posterior structure), after an implantation of a second ZPA, the sequence 234434 may be observed. *Tickle* et al. (1975) have shown that the results are interpretable to a large extent by a positional information model. According to these authors, a diffusible morphogenetic substance is produced in the ZPA. An implantation of the ZPA leads to an increase of the morphogen concentration in the surroundings. Tissues close to the graft will develop into the most posterior structures. Those cells further away will develop into more anterior structures. The model of *Tickle* et al. (1975) can explain most of the resultant pattern, including the symmetric development around a graft and the partially reverted polarity of the digits. However, a few characteristic difficulties remain which can be easily dealt with by the proposed theory. Problem one: if two ZPAs are grafted close together, one would expect a 2344 digit pattern. However, as a rule, there is no 4th digit formed at all. This is especially surprising, since two morphogen sources close to one another should easily produce a concentration above that necessary to determine the 4th digit. The explanation in terms of our model: two activator peaks grafted in close proximity generate a high inhibitor concentration which then acts to dampen the activation centers, regulating the final morphogen concentration to a level below that of a single activation center. The reduced concentration is only sufficient to determine the 3rd digit. Problem two: a ZPA grafted close to the anterior boundary should lead to a structure 432234, but only a 32234 or 2234 pattern is observed. The formation of the 3rd digit shows that the tissue around the graft is indeed sensitive to the morphogen. The remaining question is why a 4th digit cannot be induced at the anterior boundary. The explanation in terms of the model: the tissue at the anterior boundary is at a low determination level. After

the raising of the morphogen concentration by the implantation of the ZPA, the tissue is forced to proceed to higher determination levels, but this is a time-consuming process, possibly because a certain number of cell divisions are required. If the total available time is too short to proceed through all the determination steps, only a digit 2 or 3 is formed instead of the digit 4. However, in the center of the bud, the tissue is already at a higher determination level; fewer steps are required and the digit 4 can be attained. A further indication that a time deficiency is the reason why no 4th digit can be formed at the anterior boundary comes from an observation of *Summerbell* (cited in *Wolpert* et al., 1975). A graft has to be in contact with the host tissue for at least 10 h to exert an effect indicating that the reprogramming is a slow process.

According to *Tickle* et al. (1975), after the removal of the ZPA, the digit 4 is missing, but the digits 2 and 3 are normal. The situation is obviously completely analogous to that deduced from insect development: after removal of the source, the cells maintain the previously attained determination level.

It is also interesting that, in a central area (but, as mentioned, never at the margins), two 4th digits can be formed, at some distance, one with opposite polarity in respect to the host. This indicates that the morphogen concentration was not only sufficient to attain the 4th level but was substantially higher. This is understandable in terms of the model. In a non-marginal peak, the inhibitor can diffuse away in all directions, and the peak height is considerably higher than that of a marginal peak, as can be seen in Figure 4.

The possibility for the induction of additional structures also indicates that in this system only a part of the gradient is utilized and that the usual location of the structures is asymmetric and closer to the activated source, as would be expected if a field is organized by only one center.

In conclusion, the concept of dynamic formation and interpretation is a powerful supplement to the positional information concept and represents a further step toward a molecular understanding of development.

IV. The Recursive Type of Pattern Formation

One has to distinguish between two types of gradient formation. The first type has been discussed in detail in Chapter III. A gradient is formed by a diffusible morphogen and maintained by sources and sinks. The gradient is assumed to be unchanged until genes in the individual cells have been switched on and off according to the local morphogen level. But a systematic change of a certain cell property which is related to the

cells position within the tissue can also be achieved if at one boundary of a growing structure new cells are added by a blastema – a zone of rapidly dividing cells. The cells leaving the blastema must differ in some specific property from the cell population which they then contact and which have already left the blastema. The determination of the cells along the proximo-distal direction of the vertebrate limb (*Saunders,* 1948) seems to be of this type, as well as regeneration processes in which a blastema is formed (Epimorphosis), such as, for instance, the regeneration of imaginal discs (*Bryant,* 1971, 1975), insect legs (*Bohn,* 1970), and vertebrate limbs (*Summerbell* et al., 1973). The stem cells for more distal structures remain for a longer time span in the blastema. *Summerbell* et al. (1973) have proposed that this longer time could be ultilized to change the positional value of the cells such that the cells leaving the blastema one after another have a more and more distal positional value. Another possibility would be that the cells leaving the blastema adapt their own positional value so that an existing gradient in the existing cells is "extrapolated." *Gierer* (1977) has discussed the recursive type of gradient formation in more detail and proposed a mechanism to explain how the cells can sense a discontinuity in the positional values. Such a mechanism is required to account for regeneration after the removal of an intermediate region, for instance of an insect leg (*Bohn,* 1970).

While the molecular basis of positional values is still unknown, the existence of such a recursive pattern-forming system appears necessary. Since the lateral inhibition mechanism is controlled by diffusion, only a small area of the order of 1 mm or 100 cells across (*Wolpert,* 1971; *Crick,* 1970) can be organized. The possible geometry of the field is restricted because the dimension to be organized should not be smaller than a dimension perpendicular to it. Each change in the geometry would change the local morphogen concentrations. To avoid this, the geometry must be maintained until the interpretation of the gradient is completed. Therefore, for larger and growing fields, the recursive mechanism is a necessary supplement to the activation-lateral inhibition mechanism.

It seems that many connections exist between both mechanisms. *Stern* and *Tokunaga* (1967) found a mutant of *Drosophila* in which the segment border between the first and second tarsal segment of the foreleg is missing and in which many more sexcombs are formed. With genetic mosaics they have further shown that the prepattern in these mutants is changed and not the response of the cells. Such a prepattern change is expected since a missing segment border leads to an enlarged space into which the inhibitor can diffuse and decay, and the result is an enlarged morphogen peak. On the other hand, the experiments of *Bohn* (1970) with insect legs indicate clearly that the regeneration is of the recursive type.

As discussed above, the experiments concerning the axes of the retina (see p.55) have indicated that an autocatalytic amplification of small influences from the surrounding tissue is involved. At the time that the axes of the retina are fixed, only 2% of the cells of the mature retina are yet present, the others are added by cell division in the ring-shaped margin (*Straznicki* and *Gaze,* 1971). However, regeneration experiments with eye fragments (*Feldman* and *Gaze,* 1975 with Appendix by *MacDonald)* show the complementary behavior typical of the recursive mode. The fragment which contains the center of the retina regenerates to a normally specified retina, whereas the smaller fragment duplicates in such a way that a double nasal or double temporal specification results. A completely analogous situation is given in the regeneration of the imaginal discs (*Bryant,* 1975).

It is, thus, tempting to speculate that such structures are formed out of an initially small field in which a primary gradient is formed by an activation-lateral inhibition mechanism. This gradient formation is a fast process compared with the changes of the geometry of the field due to growth. This primary gradient is first translated into a sequence of more stable determination levels (whatever that means in molecular terms). This sequence is continued in the same direction during the subsequent marginal growth by the recursive mechanism which is less well understood.

V. Formation of Netlike Structures

Netlike structures are quite different from the kind of structures we have been discussing. They are common in almost every higher organism. The vascular system, the lymphatic system, the nervous system, the tracheae of insects, the veins of leaves and insect wings are examples. Such netlike structures can be used to supply a tissue with nutritional substances, oxygen, and water. They can provide information (as nerves) or mechanical stability or used to remove certain substances from an area. The filamentous elements of a net consist of either linearly arranged differentiated cells or long fibers formed from single cells. A positional information mechanism is certainly not appropriate for interpreting net formation as essentially a one step process in which the complete network is determined in all its ramifications. This would require an enormous number of threshold values in each cell. Moreover, in many net systems, regulative processes have been observed. For instance, new tracheae grow into a field of artificially evoked oxygen deficiency (*Wigglesworth,* 1954). *Kühn* (1948) has found a mutant of the insect *Ptychopoda seriata* with a

missing vein in the wing in which the other veins are rearranged in such a way that the missing vein is compensated for and there is no large gap in the pattern. Some substances are known to have an influence on the formation of a net: auxin on leaves (*Jost,* 1942), the nerve growth factor (*Levi-Montalcini,* 1964) on adrenergic nerves, and a tumor angiogenesis factor (*Folkman* et al., 1971; *Folkman,* 1976) on blood vessels. These findings indicate that elongation and branching are locally controlled processes. The question remains as to which biochemical interactions allow the formation of elongated structures. How, for instance, can an initially spheric neuroblast receive a signal on one side to extrude a filament? How can the elongation be directed toward a particular target area? How is the very small surface area selected in which a new branch is initiated? Or, as in the case of leaves, how can the differentiation of cells into members of the vascular system proceed along a line so that a certain distance to other vascular elements is maintained? How can these processes be encoded in the genes?

The mechanism I have proposed for the formation of netlike structures (*Meinhardt,* 1976) should be explained in some detail for the special case in which the filaments are formed by ordered differentiation within a field of undifferentiated cells. A generalization will be given later.

We have seen above that it is easy to generate a local high concentration of a substance (activator). Such a sharp maximum can act as a signal for the cell at this location to differentiate. To generate a filament, we must arrange for the shifting of the signal to a neighboring cell, this cell would then also differentiate. To obtain differentiated cells arranged in a line, the signal has to be shifted to the front of the tip of this incipient line, not to its side. This is made possible by postulating a repulsive force of the already differentiated cells on the signal which has induced its differentiation. Filaments are, therefore, formed as a trail behind a wandering filament-inducing signal. The wandering comes about through the repulsive interaction of the filament on its own inducing signal. This repulsion can also be used to regulate the distance between the individual filaments.

A. Formation of a Filament

To transform this idea into a molecularly interpretable, mathematically formulated model, we have to supplement the activator-inhibitor mechanism with a description of the differentiation process and a mechanism for the activator shift.

Differentiation means the irreversible transition from one stage to another under the influence of a (activator) signal. The state of differentiation can be characterized by the substance Y; the concentration of Y

would be low in the undifferentiated state and high in the differentiated state. The transition from the low to the high concentration under the influence of the activator can happen in the following way. The activator produces Y, but Y also has a positive feedback to itself which saturates at high Y concentrations (Eq. 8, Table 2). If, under the influence of the activator, a certain Y concentration is once attained, a further increase in Y is activator-independent (for details, see legend Fig. 2 in *Meinhardt*, 1976).

Table 2. Interactions of substances which can lead to the formation of lines and nets

The change of the local concentrations (or, more precisely, the time derivatives) of the activator A, the inhibitor H, and the substances S and Y is given as function of the local concentrations of these substances.
Interactions according to Equations 5 and 6 or 9 and 10, respectively, allow the formation of local high activator concentration which, according to Equation 8, induces the transition from a low to a high concentration of Y (differentiation) at that location. The differentiated cells remove the substance S (Eq. 7 or 10) and this depletion of S induces a directional drive of the activator peak away from the differentiated cells. Interaction according to Equations 5 - 8 allow lateral branching and reconnections (Fig. 15 and 18d) whereas that according to Equations 8 - 10 without a separate inhibitor – allows only bifucations (Fig. 17 and 18b) (dichotomous pattern). Equation 6a or 6b leads to different regulations of the final net density (see Chap. VI)

$$\dot{A} = cA^2S/H - \mu A - \mu' AY + D_a\Delta A + \rho_0 Y \quad (5)$$

$$\dot{H} = cA^2S - \nu H + D_h\Delta H + \rho_1 Y \quad (6a)$$

$$\dot{H} = cA^2 - \nu H + D_h\Delta H \quad (6b)$$

$$\dot{S} = c_0 - \gamma S - \epsilon SY + D_s\Delta S \quad (7)$$

$$\dot{Y} = dA - eY + Y^2/(1+fY^2) \quad (8)$$

$$\dot{A} = cA^2S - \mu A + D_a\Delta A \quad (9)$$

$$\dot{S} = c_0 - cA^2S - \gamma S - \epsilon SY + D_s\Delta S \quad 10)$$

Let us assume that the purpose of the net is the removal of a substance S which is produced everywhere in the tissue and that the activator production depends on this substance. Around each differentiated cell, a depression in the S concentration will appear. In a newly differentiated cell – which still has a high activator concentration – the S concentration and, therefore, the activator production will decrease. Some activator diffuses out from the peak into the neighboring cells and – since the S concentration is higher there – the activator production of a just-differentiated cell becomes lower than that of its neighbors. Due to the mutual competition of all the neighboring cells, only one of these cells will develop a new activator maximum. The result is a shift of the activator maximum into a neighboring cell. Moreover, the shift will occur into that neighboring cell which has the highest S concentration. This is

normally that cell which is most remote from all other differentiated cells which is the cell in front of the tip of the filament – as required for the elongation of the filament (Fig. 15).

B. Formation of Lateral Branches

An essential element in the formation of a net is the branching. A branch can be formed either by a splitting of the growing point or by a formation of a new growing point along an existing filament. As the length of a filament increases, the inhibition – arising mainly from the high activator concentration at the growing tip – may no longer be sufficient to suppress the basic (activator-independent) activator production in the filament component cells ($\rho_0 Y$ in Eq. 5, Figs. 15f and g). By autocatalysis, a new activator maximum will be formed on the filament, but, since the concentration of S is higher in the environment of the filament, the activator maximum is immediately shifted to a cell at the side of a filament and a branch is initiated.

C. Limitation of the Maximum Net Density

The net density will increase, since any branch can be the origin of other branches. The ultimate net density can be controlled in two ways. If the activator but not the inhibitor production depends on S (Eq. 6b), then with increasing net density and, therefore, with a decreasing average S concentration, the maximum activator concentration also decreases. Therefore, if a certain net density is obtained, the activator maximum will be too low to induce further cell differentiation. The final net density will be proportional to the local production of S; the net density will increase if more of the substance to be removed is present (see also Fig. 19). An example may be the growth of tracheae into a region of experimentally induced oxygen deficiency (*Wigglesworth*, 1954). If both the activator and the inhibitor production depends on S (Eq. 6a), elongation and branching are independent of the absolute S concentration. The final net density can be controlled by a constitutive inhibitor production by the differentiated cells (ρ_1 Y in Eq. 6a). This creates a background inhibitor concentration proportional to the local net density. Filament elongation or the formation of new branches will cease if the activator production is suppressed as the level of background inhibitor concentration surpasses a certain level. This type of regulation will lead to an S-independent spacing.

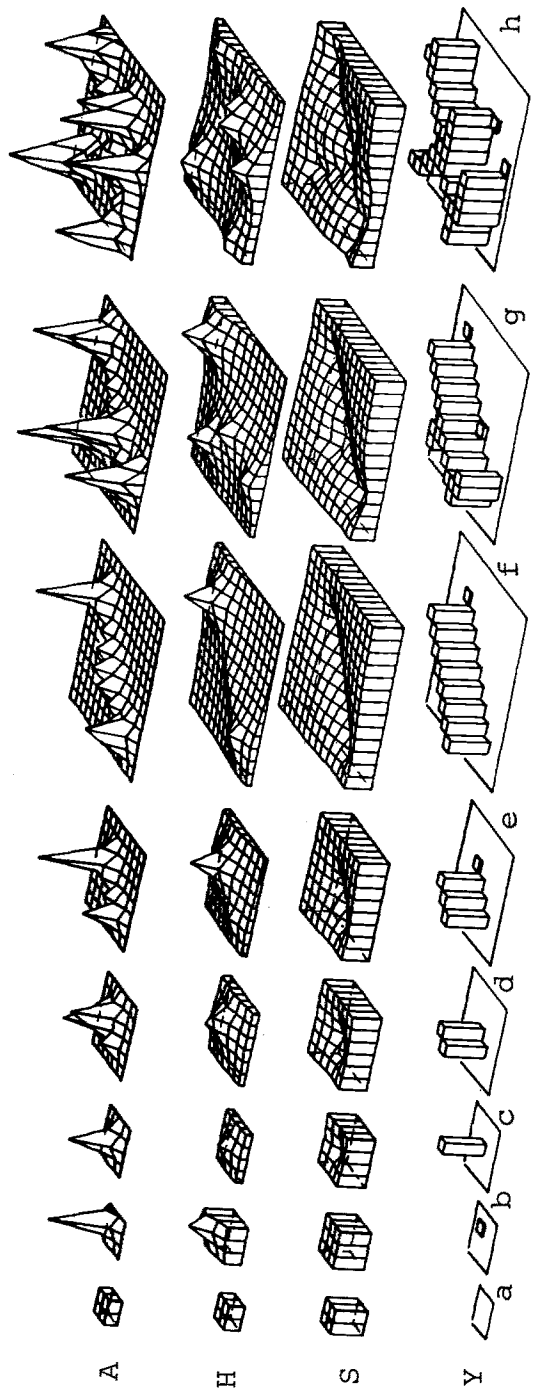


Fig. 15 a - h. Formation of a filament of differentiated cells and the initiation of a branch. By the interaction of an activator (A, upper row) and an inhibitor (H) a local high activator concentration is formed (a-b) which is used as a signal to differentiate the corresponding cell (c) (switching Y from low to high concentration). The differentiated cell removes the substrate S which is produced everywhere. The activator production, assumed to depend on S, escapes from the S depression, the activator peak is shifted to a neighboring cell which is thereupon also differentiated (d). Indefinetely long filaments of differentiated cells can be formed by repetition of these steps (e-f). If the growing trip of the filament becomes sufficiently remote and enough space is available, the basic activator production of the differentiated cells can trigger a new activator maximum in the filament which escapes, as quickly as possible, the S depleted valley which is centered around each filament, i.e. under a right angle (g-h). Equations 5-8 are used with c=0.004 with 5% random fluctation from cell to cell, μ=0.03, μ'=0.09, ρ_O= 0.04, D_a=0.02, ν= 0.04, D_h=0.19, ρ_1=0.0003, c_O=0.02, γ=0.02, ϵ=0.2, D_s=0.06, d=0.0013, e=0.1, f=10. Growth is simulated by successive divisions of the cells at two margins

D. The Finding of Particular Target Cells by a Growing Filament

In the proposed theory, the elongation proceeds in the direction of the highest concentration of S which is, in the case of a homogeneous S production, usually located in front of the filament tip. But, if to the contrary, the substance S is produced only by a particular cell, then this cell will be surrounded by a graded S concentration and the filament will follow this S gradient upward and home to the target cell.

E. Formation of Reconnections

In leaves, most of the finer veins end blindly, but some of the veins reconnect with other veins to form closed loops.

In the model, filament elongation is directed into the largest available free space; a growing filament will, therefore remain distant from existing ones. A record of this mutual avoidance during the growth can be seen in the final pattern of a leaf (Fig. 16a). It is, therefore, reasonable, while retaining the avoidance mechanism described above, to look for a mechanism which can occasionally overcome this repulsion. The repulsion results from two different inhibitory factors on the activator production. A very strong factor is the inhibitor which is centered around a growing tip and is used to keep the activator localized. A weaker factor results from the depletion of the substance along an existing filament which provides the drive for the shift of the activator peak away from the differentiated cells. A growing tip will be strongly repelled by another growing tip, but only much more weakly repelled by an existing filament. Reconnections are then possible by the strong deflection of two growing tips so that the weaker repulsion arising from an existing filament is overcome. Two examples of such reconnection are sketched in Figs. 16b and c.

According to *Avery* (1933), reconnections in the tobacco leaf are formed only after the transition from marginal to intercalary growth. That is understandable from the model since, during intercalary growth, two activator peaks can arise quite close together. As they develop more fully, a strong mutual repulsion will come into play as they come under the influence of one another's inhibitors. They can thus be forced to elongate in such a direction that they contact other, older filaments. The midvein and the main lateral branches are formed before intercalary growth begins, which explains why usually only branches of a higher order form reconnections.

This mode of reconnection is only possible in a two-dimensional system. If a third dimension is available, the deflected filament would circumvent the existing veins by passing underneath or overhead. Indeed,

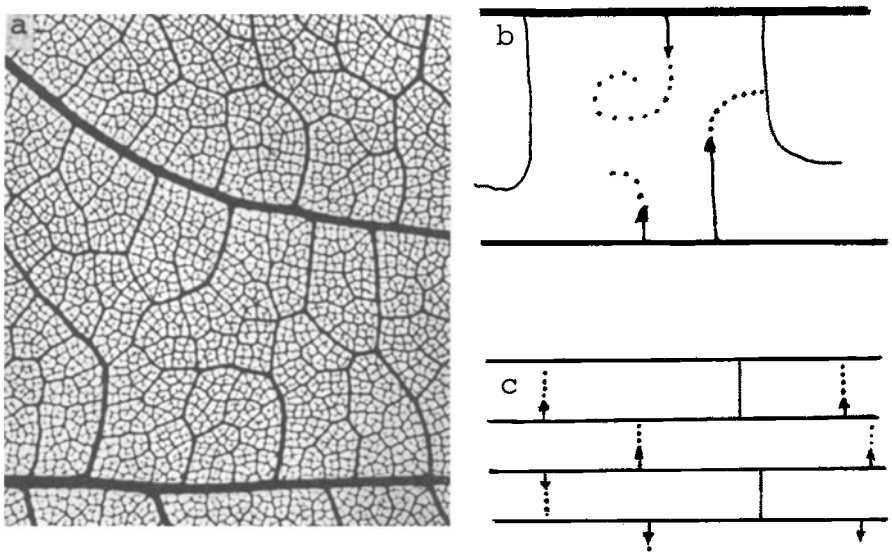


Fig. 16 a - c. Avoidance orientation and the formation of reconnections. The basic principle of the proposed line formation consists of a repulsive action of the differentiated cells on the differentiation-inducing signal. The elongation of a filament will be oriented away from other filaments into the largest available free space. (a) A record of this avoidance reaction can be seen in this maple leaf. However, reconnections between veins are also possible. (b and c) According to the model, two growing tips (arrow heads) show a strong mutual repulsion, whereas the repulsion which an existing filament exerts onto a growing tip is of a more moderate degree. Connections are possible as the strong withdrawing movement (dashed lines) of two growing tips overrides the weaker repulsion arising from an existing filament. A reaction as shown in (c) may be involved in the veination of grasslike leaves

three-dimensional networks consisting of only one cell type, such as trachea or lymph capillaries, usually end blind. Extensive reconnections in three dimensions are possible, however, if two cell types are involved, for instance, veins and arteries. Each cell type can form its own network by the repulsive interaction mentioned above and the growth of the capillaries toward each other can be obtained if one cell type produces a substance which accelerates the activator production in the other cell type.

F. Formation of a Dichotomous Branching Pattern

The question may arise of whether this mechanism of line formation is the simplest possible one. The answer is that it is not. As just discussed, two inhibitory actions are involved in line formation, one to localize the activation at the growing tip and one which determines the direction in which the center of activations will migrate. As discussed in Chapter

II, Section H, the inhibitor may be replaced by a substrate which is depleted during activator production. Therefore, both tasks, the formation and the shift of the activator peak, can be mediated by one and the same substance. Therefore, including the activator, only two substances are necessary for controlling differentiation here (Eqs. 9 and 10, Table 2). A pattern formed according to this interaction is given in Figure 17.

The main difference here from the pattern formation discussed above is that lateral branching is no longer possible. To provide sufficient drive, the differentiated cells have to be so inhibitory that secondary activator peak formation along an existing filament is no longer possible. But the growing tip can still split into two, allowing a binary fission of an extending filament. It seems that this is the evolutionary older form of leaf

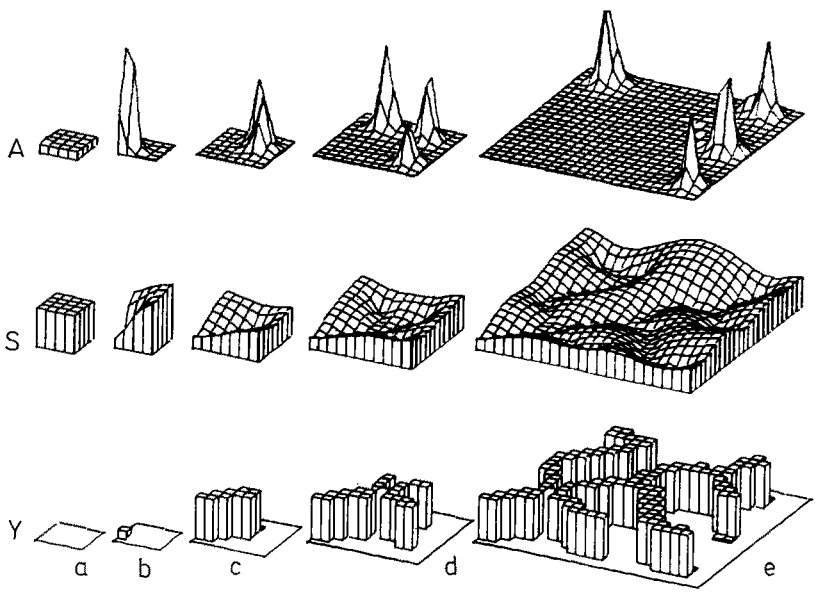


Fig. 17 a - e. Simulation of an evolutionary earlier leaf pattern. The simpler dichotomous leaf pattern shows only bifurcation of the growing veins but not lateral branching later on. Such a pattern can still be found in the ginkgo and in some ferns (Fig. 18a). Its simulation requires only two controlling substances (*Meinhardt*, 1976). Local high activator (A) concentration is formed by the autocatalysis and the inhibitory action of the depleted concentration of the substrate S (a, b). The local high activator concentration differentiates (b) irreversibly the corresponding cells (switching Y from low to high concentrations). Since the differentiated cells also remove the substrate, the activator maximum wanders away from the differentiated cells (c). If enough free space is available, the activator maximum can split (see also Fig. 9), leading to a bifurcation (d and e). To obtain a sufficient drive for the shift of the activator peak, the differentiated cells have to remove a substantial amount of the substrate S. They are, therefore, so inhibitory that no secondary activator maximum can be formed along an existing filament, and lateral branching and reconnections are impossible. A biologic example of such a pattern is given in Figure 18a

vascularization – dichotomous branching – which can still be found in leaves of some ferns or in the leaves of the ginkgo tree. If this view is correct, the evolutionary step from the dichotomous (Fig. 18a) to the normal leaf pattern (Fig. 18c) would be the separation of the two inhibitory effects by the invention of a separate inhibitory substance. The evolutionary gain that lateral branching has to offer is that it opens up the possibility of intercalary growth. New branches can grow into the increasing free spaces providing the necessary nourishement of the tissue. In addition, the disruption of one vein is of less danger to the tissue as a whole, since the network has closed loops and other pathways are available to be utilized.

G. Filaments Formed by Oriented Cell Divisions or by Extensions of Single Cells

Elongation of a line by accretion of newly differentiated cells is only one of several possible modes of line formation which can be described by the proposed theory. The high activator concentration at the filament tip can be used to initiate cell division and the activator increase in front of the tip can orient this cell division. In this case, the network is formed by an organized proliferation of the constituent cells.

In the case of the nervous system and trachea system, the elements of the filaments consist of long extensions of single cells. The formation of such a net can be explained by the model, assuming that a local high activator concentration is formed on the cell surface, particularly, perhaps, on the surface of the growth cone, and that this high concentration acts as a signal to produce pseudopodes. As in the orientation of a chemotactic-sensitive cell (see p. 68), the precise localization of the activator peak during the fiber elongation is facilitated by a periodic formation and decay of the signal. *Harrison* (1910) has shown that, indeed, the growth of a nerve fiber is not a continuous process but that phases of fast elongation alternate with phases of searching for a new direction. Several pseudopodes may be sent out at the same time, although most of them will be retracted later on.

H. Substances Influencing the Formation of Nets, in View of the Proposed Theory

As already mentioned, there are several substances known which influence the formation of particular nets. Some are identified, some only partially purified. All these substances are of the type of the proposed shift sub-

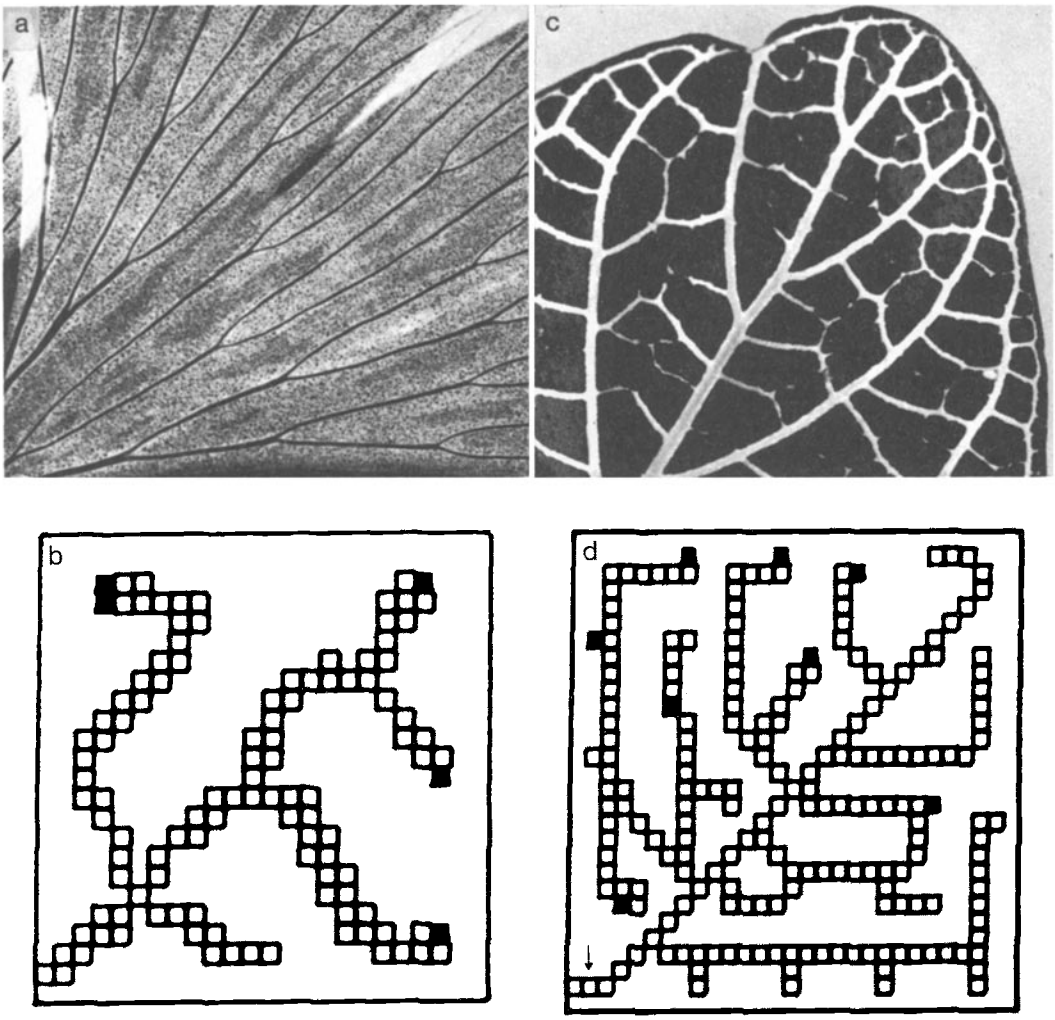


Fig. 18 a - d. Comparison of branching pattern of leaves with their simulations. (a) An evolutionary older form of branching is the dichotomous branching wherein a growing line can split into two, forming a fork, but lateral branching is not possible. (b) A simulation of dichotomous branching is possible under the assumption of only two controlling substances (see Fig. 17), the forking pattern is reproduced. (c) Lateral branching in a leaf of *Fittonia Verschaffelti* where, by a caprice of nature, the major veins appear white. (d) In the simulation one differentiated cell was initially assumed (arrow). The first vein is oriented towards the largest available space, in the diagonal. The first lateral branches try to grow out at 90° but are repelled by the margins and establish, therefore, an angle of 45° with the midvein (see Fig. 15). The following lateral branches are repelled by the first and appear, therefore, also at 45° but branches of higher order grow out at 90°. The margins are avoided since the inhibitor cannot diffuse past the margin and is, therefore, accumulated here, especially when a growing filament approaches. Reconnections are occasionally possible between higher order branches. Whereas the main lateral branches are quite straight, the higher order branches are more curved since they are frequently deflected by other growing tips. The simulation of a leaf with 30 · 30 cells can be only a crude approximation of the reality (even such a relatively simple simulation requires 28 h on a relatively fast PTP 11/40 computer), but does demonstrate that such a complex appearing pattern can be formed by the interaction of only a few substances

stance S, whereas none of the activator-inhibitor type is known. The reason for this is that substances of the type S must be constantly produced or present in all the cells into which the filaments should grow. In contrast, the proposed activator-inhibitor substances are produced only very locally and possibly only during short time intervals. Further difficulties in observing these substances result from their strong mutual interaction (see Chap. VI).

In leaves, additional vascular elements are formed after application of auxin at an injury (*Jost,* 1942). Auxin is known to be actively transported by the vessels from the leaves to the roots. Therefore, the veins remove auxin from their environment. Similarly, nerve growth factor (NGF) (*Levi-Montalcini,* 1964), necessary for the outgrowth of adreneric nerves, is actively transported from the filaments to the cell body (*Hendry* et al., 1974). The highest NGF concentration a particular nerve will detect is presumably at its endings, since more surface elements are available to remove NGF along a fiber. This would lead, as long as no other constraints are imposed, to a linear elongation of the fiber. The valley of NGF centered around each fiber would cause fibers of the same type to maintain a certain distance from each other. If higher amounts of NGF are present in a particular area, this area would attract growing fibers. According to the model, NGF (and similarly, auxin) would be a cofactor for the activation of fiber elongation, for instance in the formation of a growth cone. The activation itself has to have autocatalytic properties with an element of lateral inhibition.

The rapid growth of a tumor is only possible if it is extremely well-nourished by a plethora of newly formed vessels (*Algire* and *Chalkley,* 1945). Obviously, a malignant tumor is able to overcome the body's control of blood vessel formation and the understanding of how the vessel density is controlled is of great importance. *Folkman* et al. (1971) and *Folkman* (1976) have isolated a tumor angiogenesis factor (TAF) from different tumors which induces ingrowth of vessels into normally avascular areas, such as the cornea or the epidermis. According to the model, the density of the net can be controlled by the substance S concentration (see Chap. IV, Sect. C). Examples are given in Figure 19. Low, medium, and high S concentration leads to corresponding density of the filaments, which may correspond to the situation of an avascular tissue, a normal tissue, or a tumor. TAF may itself be or may induce the synthesis of a substance which is removed by the vessels and which is a cofactor in the local activation for an elongation or induction of a new branch. On the other hand, *Folkman* et al. (1971) have isolated a factor from the cartilage – an avascular tissue – which suppresses the ingrowth of vessels. This factor could be the proposed inhibitor since externally supplied inhibitor can suppress activator production and in this way elongation and branching even in the presence of high S concentration. (Fig. 19c).

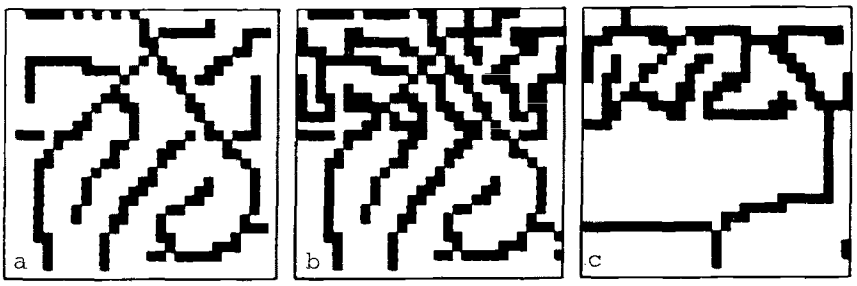


Fig. 19 a - c. Regulation of the density of a net. According to the model, the density of a net can be regulated by the concentration of a substance S which is produced everywhere in the tissue and removed by the net (Eq. 6b, Table 2). (a) "Normal" net density. (b) A two-fold higher S production in the upper half area leads to a much higher vessel density. This may be the situation in a tumor with its excessive vascularization. (c) As (b) but an inhibitor-producing cell layer is assumed in the center of the field; no vessels can grow through the inhibited area, but can circumvent it. This situation may be similar to that reported by *Folkman* (1976) where a piece of cartilage tissue has a strong inhibiting effect on the vessel formation and only occasionally can some vessels circumvent the hindrance to be then attracted by the tumor tissue

VI. Isolation of Activators and Inhibitors

For steps toward the isolation of both substances biologic tests are necessary, and attention should be called to some precautions. The general problem can be illustrated by an analogy. Let us assume that we would like to detect the mechanism whereby a few people become rich and others remain poor. Perhaps, for this investigation, we could arrange for a complete separation of all the rich people from the rest of the population. Instead of finding the mechanism, the so-separated rich people will become poor and new rich ones will emerge. The property we sought to investigate has disappeared, since its maintenance requires a permanent interaction with an environment. Correspondingly, isolated small activated areas would lose their activation quite soon, since the inhibitor can no longer spread out and, in this way, the activation itself would be suppressed.

For the maintenance of an inhomogeneous activator and inhibitor distribution, a continous production and decay of both substances are necessary. It is, therefore, to be expected that the cells produce the substances only in small quantities and that the cells are sufficiently sensitive. To give an example, the slime mold *Dictyostelium discoideum* is sensitive to a cAMP increase of as low as 10^{-8} M (*Gerisch* and *Hess*, 1974). If it is necessary to add the substances in larger amounts in order to obtain an effect, the probability is high that only a secondary effect will be observed.

The application of one of the substances can mimic the effect of the other. If, for instance, activator is added in a quantity which is large compared to that which occurs naturally, the activator peak disappears, since the activator level is elevated everywhere. In addition, due to the crosscatalysis, the inhibitor concentration will also increase tremendously. After the removal of the activator excess, the pattern is quickly restored, perhaps with an activator peak at a new location. The same result would have been observed if inhibitor were supplied in large quantities. The local application of the activator or inhibitor at the active center, as well as the removal of inhibitor there, could suppress the formation of a secondary activation. A local application of activator at a distance from the activated center can induce a second center, but one has to take care that the effect does not arise from an unspecific loss of the inhibitor resulting from the treatment. These difficulties may explain why, in spite of intensive research, the real pattern-forming reaction has not yet been found.

Perhaps the best method for testing a substance, if it is a candidate to be the activator or inhibitor, is the application in low dosage. If the existing activator center has first been removed, the system would be especially sensitive to the addition of small amounts of activator or inhibitor. If a periodic structure regenerates – as in the case of a hydra head with its tentacles – small amounts of activator can increase the number of the maxima. Further, it should be noticed that the necessary autocatalysis must not be connected with the feedback of the activator on its own synthesis but can consist of an autocatalytic release. Activator and inhibitor can be stored in a bound, inactive form and free activator can cause a release of both substances into the free form. A molecular interpretation of Equation 1 would imply that two activator molecules can release one activator molecule or one inhibitor molecule. One inhibitor molecule can block the release of the activator but is without influence on its own release.

Schaller (1973) has purified a substance which stimulates head and tentacle formation in hydra, and *Berking* (1977) found a substance which is able to suppress bud formation in hydra. Head-activating substance is active in very small concentrations (10^{-10} M) and is stored in larger amounts in vesicular structures, presumably within the nerve cells (*Schaller* and *Gierer*, 1973), but whether or not they are directly involved in the autocatalytic reaction is an open question.

An essential condition for pattern formation was the low diffusion rate of the activator. If the total area is small, for instance, if the pattern is formed within a single cell, it has to be expected that the activator is bound to a nondiffusing structure, such as the cell cortex.

VII. Concepts in Embryology in View of the Proposed Theory

The meaning of the terms polarity, boundary region, dominant region, fields, etc. which are used to describe aspects of embryonic development can be more precisely defined by the proposed theory.

Polarity denotes a certain asymmetry in the tissue which leads to a predictable asymmetric development. According to the model, asymmetry can have two different molecular bases. The first would be a graded distribution of an autocatalytic substance, such as is presumably formed during the early development of *Fucus* (see p. 56). The second would be a graded distribution of the activator sources which determines the location of an activator peak. This mechanism presumably causes the regeneration of a hydra head always at the distal end of the body column. The source density is a quite stable tissue property, while the location at which an activator peak will appear is more labile and can be influenced by experimental interference. As already mentioned, the more stable source distribution can arise through a positive feedback of the more labile activator peak on its own sources. Both causes for asymmetric development can be brought to conflict if an activator peak is experimentally induced in the region of lower source density, for instance, by grafting a hydra head or an activated piece of tissue at the proximal end of the gastric column. As a rule, an established activator peak will be dominant over the source distribution. Either the morphogen gradient itself or the "extrapolation" of this gradient during growth (see Chap. IV) may be translated in a more stable, space-dependent surface property, which makes cells otherwise homogeneous "nonequivalent" (*Lewis* and *Wolpert,* 1976).

Another property is described by the terms boundary region (*Wolpert,* 1971), dominant region (*Child,* 1941), and organizer (*Spemann,* 1938; *Child,* 1946). According to the theory, two situations must be distinguished here. In some cases there exists an autocatalytic center. Such a center will regenerate after it has been removed or it can be induced at a distance from the existing center by rather unspecific stimuli. Examples are the dorsal lip in amphibians, the tip of the slime mold slug, or the head of a hydra. Each autocatalytic center is necessarily surrounded by an inhibitory field. We have seen that, if the total area is small, such an organizing center is forced to appear at the physical boundary region. In other cases, organizing regions exist which do not have this autocatalytic property. Examples are the animal and vegetal pole of a sea urchin egg or the borders of insect segments. It may be that irreversible changes have been established under the influence of a previously formed autocatalytic center. Usually, after the removal of a noncatalytic organizing center, parts of the structures will be missing, since frequently no regeneration takes place.

A field can be defined as an area which is controlled by a set of diffusible morphogens. The insect egg, the insect segments, the imaginal discs, and the segments of an insect leg which develops out of a single disc demonstrate the ever finer subdivisions of the fields and in this sense, the terms "compartment" (*Garcia-Bellido,* 1975) and "field" may become congenial.

VIII. Conclusion

It was our intention to show that the emergence of pattern during development can be explained by relatively simple coupled biochemical interactions. All the ingredients used, mutual activation and inhibition of biochemical reactions, and of course diffusion, are known to exist in other biochemical systems. An explanation of a variety of phenomena can be given without the addition of unreasonable assumptions. Even if a biochemical proof of the existence of such pattern-forming mechanisms must wait for future investigation, the calculations have shown the consistency of the theory. For the insect and leaf organization, many models initially taken into consideration turned out to be unable to account quantitatively for the experimental observations in the computer simulations. But conversely, after a model consistent with the initially chosen experiments was found, such a model frequently could also account for phenomena for which it was not originally designed. This is, of course, not a proof of the model but suggests that it has something to do with what is realized in development.

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Extraretinal Influences on the Lateral Geniculate Nucleus

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Contents

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List of Abbreviations

AP	action potential
ARG	autoradiographic
CA	catecholamine
CL	centralis lateralis nucleus } of the thalamus
CM	centrum medianum nucleus } of the thalamus
EMP	eye-movement potential
EPSP	excitatory postsynaptic potential
FW	fast wave (sleep)
GABA	γ-aminobutyric acid
HRP	horseradish peroxidase
5-HT	5-hydroxytryptamine (serotonin)
IPSP	inhibitory postsynaptic potential
LC	locus coeruleus
LGN	lateral geniculate nucleus
LGNd	dorsal nucleus of the lateral geniculate nucleus
MRF	midbrain reticular formation
NA	noradrenaline
NSC	nucleus subcoeruleus
OR	optic radiation
OT	optic tract
PGO	ponto-geniculo-occipital
PRF	pontine reticular formation
RF	reticular formation
RNT	reticular nucleus of the thalamus
SC	superior colliculus
SW	slow wave (sleep)
TR	transfer ratio
VA	ventralis anterior nucleus of the thalamus
VC	visual cortex

I. Introduction

It is no longer customary to think of the dorsal lateral geniculate nucleus (LGNd) as a simple relay station between retina and visual cortex. It is now accepted that there are extraretinal influences on the LGNd and these are concerned mainly with two functions: 1) tonic effects, related to changes in level of consciousness, 2) phasic changes, related to eye movements. In addition, it is probable that a third function is concerned with binocular vision. As far as the first of these functions is concerned, similar adjustments seem to occur in other specific thalamic nuclei as well as in the cerebral cortex (*Steriade*, 1970).

An appreciation of the effect of arousal on the LGNd dates back to 1956 when *Hernández-Péon* et al. in unanesthetized cats observed a decrease in the response in the LGNd to a photic stimulus when this was regularly presented every 5 or 8 s, although the response in the optic tract (OT) remained the same. Recovery of response was obtained after a rest or as a result of an intense acoustic or photic stimulus. Subsequently

Bremer and *Stoupel* (1958, 1959) and *Dumont* and *Dell* (1958, 1960) showed that the response of the optic radiation (OR) of the cat to electric stimulation of optic nerve or tract was enhanced during arousal, either spontaneous or as a result of sensory stimulation or by stimulation of the reticular formation (RF). *Arden* and *Söderberg* (1959, 1961) found that the discharge of a single cell in the LGNd of the *encéphale isolé* rabbit was affected by "arousing" stimuli, including noises, even when the retina was inactivated; this experiment clearly suggested an input to the LGNd additional to that from the retina. *Long* (1959) also showed that simulation of RF facilitated the LGNd responses to OT stimulation.

The phasic electric changes in the LGNd associated with the rapid eye movements of fast-wave (FW) sleep were noted in 1961 by *Mikiten* et al., but the changes occurring during waking eye movements were not described until 1968 (*Brooks; Cohen* and *Feldman*). Positive evidence for an extraretinal influence concerned with binocular interaction has only recently been obtained (*Schmielau* and *Singer,* 1977).

Subsequent research will be discussed in this review under several headings. In order to relate these findings to the structure of the LGNd and its connections with retina and visual cortex, our current ideas on these topics are first reviewed in Section II. Tonic effects are described in Section III, phasic effects in Sections IV and V. In Section VI the question of whether presynaptic inhibition plays any part in these effects is discussed. Section VII describes the effects of nonvisual sensory stimulation. An anatomic basis for the effects is examined in Sections VIII, IX and X. Section VIII deals with the influence of the reticular formation, the raphe nuclei and the reticular nucleus of the thalamus, Section IX with the cerebral cortex and Section X with other regions of the brain. Section XI attempts to correlate chemical, anatomic and physiologic results in a model of the LGNd. Section XII discusses the function of the LGNd and summarizes the role of extraretinal influences.

This review is concerned primarily with the LGNd. There are as yet insufficient data on the closely associated nuclei, the medial interlaminar nucleus and the ventral nucleus of the LGN, to make a discussion of extraretinal influences on these nuclei worthwhile; however, there is an input to both these nuclei from visual cortex (VC) (e.g., *Updyke,* 1975) and to the ventral nucleus from the RF (*Edwards* and *de Olmos,* 1976) and from the superior colliculus (*Altman* and *Carpenter,* 1961). The perigeniculate nucleus will be discussed in relation to theories of inhibition within the LGN (see Section II). The review will deal particularly with the cat since this has been most extensively studied but, where relevant, references will be made to other species; if in any reference no mention is made of the species it may be assumed that this is cat. The topic of

extraretinal influences on the LGNd has featured in several reviews in recent years (*Creutzfeldt* and *Sakmann,* 1969; *Steriade,* 1969, 1970; *Brindley,* 1970; *McIlwain,* 1972; *Freund,* 1973).[1]

II. Retinal and Interneuronal Connections of the LGNd

Before considering the effects of extraretinal influences on the LGNd it is desirable to review briefly our current ideas concerning connections between retina and LGNd and within LGNd. There are three groups of optic tract fibers in the cat – a fast group (t_1, Y, "type I" "brisk-transient,") a medium-speed group (t_2, X, "type II," "brisk-sustained") and a slow group (t_3, W) (*Bishop* and *McLeod,* 1954; *Enroth-Cugell* and *Robson,* 1966; *Bishop* et al., 1969; *Saito* et al., 1970, 1971; *Fukada,* 1971; *Cleland* et al., 1971b; *Stone* and *Hoffmann,* 1972; *Cleland* et al., 1973; *Hoffmann,* 1973; *Cleland* and *Levick,* 1974a; *Stone* and *Fukuda* 1974; *Fukuda* and *Stone,* 1974).

A note on terminology. The original Y/X classification defined Y retinal ganglion cells as those showing nonlinear spatial summation in their receptive fields and X cells as those showing linear summation (*Enroth-Cugell* and *Robson,* 1966). This categorization appears to be not only a satisfactory one but also the one that most clearly separates the retinal ganglion cells (*Hochstein* and *Shapley,* 1976). Furthermore, it seems also to apply satisfactorily to LGNd cells (*Shapley* and *Hochstein,* 1975). Unfortunately most workers do not apply tests for spatial linearity, and it is also evident that the equivalences listed above are only approximate (*Hochstein* and *Shapley,* 1976). Nevertheless, we shall adopt the Y/X/W terminology in this review and apply it as though the equivalences were exact. We think that the advantages of using a single terminology outweigh any inexactitude involved and that errors introduced in this way will not be serious. We believe the Y/X classification to be the best because it is independent of receptive-field-type or retinal eccentricity of the receptive field (*Hochstein* and *Shapley,* 1976). Undoubtedly progress in this area will be easier if the linearity test is applied wherever possible. Although this test could obviously be applied also to at least some of the slowest group of fibers, we shall restrict the Y/X classification to the fast and medium groups and call the slowest group the W fibers.

The Y fibers are the largest; their characteristic response to a sudden change of illumination or movement is a prominent transient burst of firing and they show a poorly sustained discharge during a maintained stimulus. They respond well to fast movement (*Cleland* et al., 1971b) and may function as "movement detectors." The X fibers have smaller receptive fields, are better represented in the central areas of the retina, do not respond well to fast movement, but have a well-sustained response to a maintained stimulus, hence they may function as "pattern detectors." The W fibers (*Stone* and *Hoffmann,* 1972), which are the smallest fibers,

[1] Since this manuscript was submitted the following review on a topic similar to this has appeared: *W. Singer*, Control of thalamic transmission by corticofugal and ascending reticular pathways in the visual system. Physiol. Rev. 57, 386-420 (1977).

do not constitute a homogeneous functional class (*Stone* and *Fukuda,* 1974; *Fukuda* and *Stone,* 1974). They include both concentric and non-concentric classes, the former subdivided into a "transient" and a "sustained" group and the latter into five subgroups of unusual receptive field properties (*Cleland* and *Levick,* 1974a, b; *Levick,* 1975).

The Y and X fibers have classic center-surround receptive fields and they connect with Y and X LGNd relay cells respectively, which likewise have center-surround receptive fields. These cells are in the upper magnocellular laminae (A, A_1 and C) of the LGNd of the cat, whereas the W fibers supply mainly or only the lower parvocellular laminae (C_1 and C_2) as well as C (*Cleland* et al., 1975, 1976; *Wilson* and *Stone,* 1975; *Wilson* et al., 1976). In the monkey, the magnocellular layers contain "Y-like" cells, whereas the parvocellular layers contain "X-like" cells (*Dreher* et al., 1976).

Very few X fibers go to the SC (*Cleland* and *Levick,* 1974a), but Y and W fibers supply separate regions of the SC (*Hoffmann,* 1973) and the W region sends fibers to the W region of the LGNd (see Section X). These facts provide some justification for treating W fibers as a group in spite of their nonhomogeneous properties.

The Y LGNd cells may correspond to the type 1 cells (*Guillery,* 1966) seen in Golgi preparations and the class I cells (*Ferster* and *LeVay,* 1976), which lack cytoplasmic laminar bodies but are labelled after injection of horseradish peroxidase (HRP) into the VC; X LGNd cells may correspond to the type 2 cells of *Guillery* (1966) and the class II cells of *Ferster* and *Levay* (1976), which possess cytoplasmic laminar bodies and are also labelled with HRP (see also *Fukuda* and *Stone,* 1976).

The Y and X cells both in retina and LGNd can have either *on*-center or *off*-center receptive fields. Y and X LGNd cells each receive an input from one or a few OT fibers but one OT fiber is usually dominant (*Cleland* et al., 1971a, b; *Singer* and *Bedworth,* 1973) and is capable of firing the cell unaided (*Bishop* et al., 1958; *Cohen* and *Vendrik,* 1972). There is very little excitatory interaction between X and Y inputs (*Cleland* et al., 1971b; *Hofmann* et al., 1972) or between contralateral and ipsilateral inputs (*Bishop* et al., 1959; *Grüsser* and *Sauer,* 1960; but see *Schmielau* and *Singer,* 1977). However, there is much inhibitory interaction. Inhibition will be discussed in some detail because brainstem influences on the LGNd appear to act chiefly by disinhibition, and cortical influences at least partly by inhibition.

In Golgi preparations, small neurons are found with axons ramifying close to the perikaryon (class 3 cells of *Guillery,* 1966) and constitute about 20% of all LGNd neurons in the cat (type III cell of *Ferster* and *LeVay,* 1976); the proportion of interneurons in monkey LGNd is estimated to be much less than in the cat (2 - 5.9% of all cells; *Norden,* 1975).

Tömböl (1969) has described two types of short-axon cell in the LGNd, in one of which (a) the axon ramifies within the dendritic arborization of its own cell; in the other (b) the axon extends much further and connects different laminae of the LGNd. *Guillery* (1969a) has described two types of nerve ending with flat vesicles (F_1, F_2), both presumably inhibitory. The F_2 terminal may correspond to the ID terminal (*Famiglietti* and *Peters,* 1972) or the P-bouton in the rat (*Lieberman* and *Webster,* 1974); the F_1 terminal may correspond to the IA terminal (*Famiglietti* and *Peters,* 1972) or the F-bouton in the rat (*Lieberman* and *Webster,* 1974). It is clear from the work of these authors that the ID terminals (P-boutons) are formed by presynaptic dendrites of interneurons, many of which in the rat apparently have no axons (but see also *Brauer* et al., 1974). The origin of the IA terminals (F-boutons) is not certain; they could originate from one of the interneurones described by *Tömböl* (1969) or from an extrageniculate source.

There is inhibitory interaction between *on*-center cells and between *off*-center cells ("synergistic inhibition") and between *on*-center and *off*-center cells ("antagonistic inhibition") (*Singer* et al., 1972). Inhibitory interaction between contralateral and ipsilateral inputs is quite extensive, up to 72% of relay cells excited from one eye being inhibited from the other eye (*Sanderson* et al., 1969, 1971; *Singer,* 1970; *Suzuki* and *Takahashi,* 1970, 1973; *Takahashi,* 1975). Inhibitory interaction between X and Y cells is also extensive. It is generally agreed that the Y system inhibits both X and Y cells (*Hoffmann* et al., 1972; *Rodieck,* 1973a; *Singer* and *Bedworth,* 1973) and this may be relevant to the phenomenon of "saccadic suppression." Whether or not the X cells inhibit the X or Y cells is not certain (*Hoffmann* et al., 1972; *Rodieck,* 1973a). It is accepted, however, that by several tests Y cells are less strongly inhibited than X cells (*Fukuda* and *Stone,* 1976). The relationships between Y cells and W cells or between X cells and W cells are at present not clear.

Thus the inhibitory mechanisms of the LGNd are much less specifically organized than are the excitatory connections. Nevertheless, they could function to produce lateral inhibition and so enhance contrast in the visual field. However, the functional significance of these inhibitory interactions is uncertain.

Two types of inhibitory circuit within the LGNd have been proposed (Fig. 1). A feedback circuit was proposed by *Fuster* et al. (1965) in the rabbit, by *Burke* and *Sefton* (1966a, b, c) in the rat, and by *Singer* and *Creuzfeldt* (1970) in the cat (Fig. 1a). *Sumitomo* et al. (1969) in the rat, *Singer* et al. (1972), *Coenen* et al. (1972), and *Stevens* and *Gerstein* (1976) in the cat presented evidence for a feed-forward circuit (Fig. 1b).

It is possible that both circuits exist. Stimulation of OR in rats and cats decorticated 1 week earlier (to produce degeneration of corticofugal

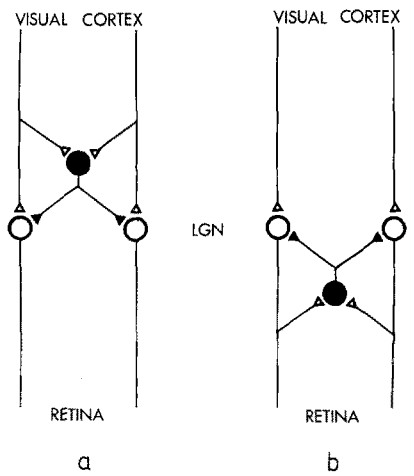

Fig. 1 a and b. Possible models of inhibitory connections within the LGNd. (a) Feedback inhibition. Relay cells (○) excite inhibitory interneurons (●) via recurrent axon branches. (b) Feed-forward inhibition. Inhibitory interneurons are excited by OT fibers

fibers) causes an inhibition comparable in time course to that produced by OT stimulation (*O'Sullivan* et al., 1976). This implies that the inhibition is mediated via processes of relay cells (Fig. 1a) although the interneurons need not be in the LGNd. On the other hand, *Cleland* and *Dubin* (1976) have recorded from cells monosynaptically activated from retina that are probably interneurons (not antidromically but transsynaptically activated from VC). *Eysel* (1976) found that there was a bimodal distribution of IPSP (inhibitory postsynaptic potential) latencies to OT stimulation – the longer-latency group consistent with a feedback circuit, the shorter-latency group consistent with a feed-forward circuit for the slower (X) OT input or with a feedback circuit for the faster (Y) OT fibers. There is anatomic evidence for direct innervation of interneurons by OT fibers. The OT terminals synapse with relay cell dendrites and presynaptic dendrites of interneurons in a special arrangement called a triad (see Fig. 9); in the triad the interneurons are postsynaptic to OT fibers and presynaptic to relay cell dendrites. Since the OT endings contain round vesicles it may be assumed that they depolarize both relay cells (P cells) and inhibitory interneurons (I cells). However, it is not clear whether the action on the I cells is inhibitory (presynaptic inhibition) or excitatory.

It has been suggested that the reticular nucleus of the thalamus (RNT) inhibits relay cells in many thalamic nuclei (*Scheibel* and *Scheibel,* 1966, 1967; *Scheibel* et al., 1972). The RNT receives collaterals from both thalamocortical and corticothalamic fibers and sends its output back into the thalamus (*Scheibel* and *Scheibel,* 1966). Using the HRP and autoradiographic (ARG) techniques *Jones* (1975) has shown that there is considerable overlap between the projections of the adjacent thalamic nuclei and between those of the adjacent cortical regions, and hence any inhibition relayed back into the thalamus could not be very specific. Several authors have shown that there is often a reciprocal firing pattern

between cells in part of the RNT and the corresponding thalamic nucleus (*Massion,* 1968; *Schlag* and *Waszak,* 1970; *Filion* et al., 1971; *Lamarre* et al., 1971).

Negishi et al. (1962) recorded from visually evoked cells in the RNT of the cat and described the very long-lasting discharges of these cells, both spontaneous and evoked. *Godfraind* and *Meulders* (1969) and *Meulders* and *Godfraind* (1969) recorded from cells in the perigeniculate nucleus, which may be the section of the RNT specifically concerned with the LGNd (*Szentágothai,* 1972), and found that they were excited by photic stimulation, had large receptive fields, often *on-off*, without periphery and easily fatigable. *Sanderson* (1971) also found that most of these cells were binocular and many had *on-off* centers, sometimes with inhibitory surrounds. *Cleland* and *Dubin* (1975) confirmed the *on-off* response of these neurons and reported that they were transsynaptically excited from the VC with latencies of 0.9 - 1.9 ms, consistent with activation via recurrent collaterals of Y cells in the LGNd. *Sumitomo* et al. (1975, 1976a, b) recorded from cells in the RNT of the rat; some cells appeared to be excited monosynaptically from the OT, others at a latency consistent with activation via recurrent collaterals of LGNd relay cells. Stimulation of these same regions produced inhibition in the LGNd and lesions placed in these regions substantially reduced the extent of inhibition produced by OT stimulation. These observations strongly support the original proposal of *Scheibel* and *Scheibel* (1966).

III. Tonic Effects in Normally Behaving Animals

It has been shown by many workers (*Palestini* et al., 1964; *Dagnino* et al., 1965, 1969, 1971; *Walsh* and *Cordeau,* 1965; *Maffei* et al., 1965; *Iwama* and *Sakakura,* 1965; *Sakakura* and *Iwama,* 1965; *Iwama* et al., 1966; *Kasamatsu* and *Iwama,* 1966; *Bizzi,* 1966a; *Sakakura,* 1968; *Malcolm* et al., 1970a; *Favale* et al., 1972) that the evoked field response in LGNd or OR to OT stimulation is modified according to the state of alertness. The amplitude of the presynaptic response (t) in the LGNd (see Fig. 2) increases with the change from alertness to nonalertness and increases further in sleep but during the PGO wave (see Section IV) of FW sleep it returns to alert values; these changes are paralleled by changes in the opposite direction in the antidromic response of the OT fibers (t(anti): Fig. 2), suggesting a relative hyperpolarization of the OT terminals during nonalertness and sleep. These changes are very small, not more than about 7% (*Malcolm* et al., 1970a) and may be without physiologic significance. Postsynaptic (r) changes are much greater. The ampli-

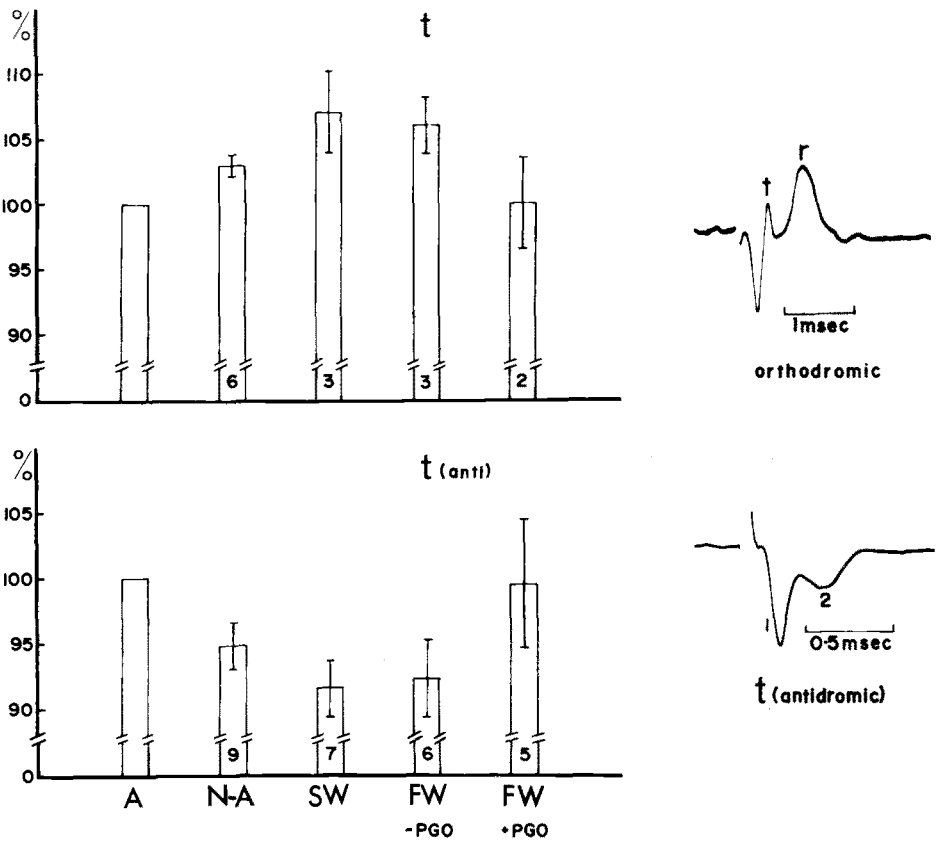

Fig. 2. Changes in amplitudes of the presynaptic (t) component of orthodromic field response (upper right) and of antidromic response of optic tract fibers (t(anti)) (lower right) in different states of wakefulness and sleep. Orthodromic response shows only response of the Y system whereas antidromic response shows response of Y fibers (wave 1) and X fibers (wave 2). Histograms show the relative amplitude of Y elements only, but changes are probably similar in the X elements. Vertical lines about the mean show ± SEM, the number at the base of each column the number of experiments. A = alert, N-A = nonalert, SW = slow-wave sleep, FW - PGO = fast-wave sleep between PGO waves, FW + PGO = FW sleep during a PGO wave. Changes in amplitude of the postsynaptic (r) component of orthodromic response are shown in Fig. 3 (from *Malcolm* et al., 1970a)

tude of the r response (see Fig. 2) decreases from 100% in the alert state to about 80% in the nonalert state and to about 50% in slow-wave (SW) sleep; in FW sleep it returns to about 90% with a further phasic increase to about 120% during the PGO wave (Fig. 3) (*Malcolm* et al., 1970a). *Sakakura* (1968) found that the firing probability of individual P cells to OT and VC stimulation was greatest in FW sleep and least in SW sleep (Fig. 3) and a similar conclusion was reached by *Dagnino* et al. (1969, 1971). *Maffei* et al. (1965) found that the discharge of single cells in the

LGNd of unanesthetized cats (midpontine pretrigeminal preparation) synchronized well with the sinusoidal changes in intensity of the stimulating illumination when the cat was awake (EEG desynchronized), whereas there was no correlation in SW sleep. Similar changes in response were obtained in records from single cells in the freely moving cat (*Mukhametov* and *Rizzolatti,* 1968). These changes could not be accounted for by variations in the OT response to light flashes which did not change (*Mukhametov* and *Rizzolatti,* 1970). Hence an extraretinal input to the LGNd was postulated as a means whereby the sensitivity could be adjusted.

Spontaneous discharges of LGNd P cells were originally studied in the intact unanesthetized cat by *Hubel* (1960), who found that the cells tended to fire in high-frequency clusters during SW sleep; *Hubel* also showed that clustered firing was not due to the presence or absence of light. These results were confirmed and extended by *Bizzi* (1966b), *Benoit* (1967), *Sakakura* (1968) and *Mukhametov* et al. (1970). *Sakakura* found that the mean rate of discharge was reduced in SW sleep compared with the awake condition and was highest in FW sleep (Fig. 3). Thus, the evoked response, both field and unit, changes in parallel with the spontaneous activity and also with the electric excitability determined by stimulation of OR (Fig. 3). *Sakakura* also found that I cells tended to reduce their discharge rate during arousal. *Sato* et al. (1973) observed decreases in firing rate of some LGNd cells, both Y & X, during EEG synchronization (indicative of SW sleep).

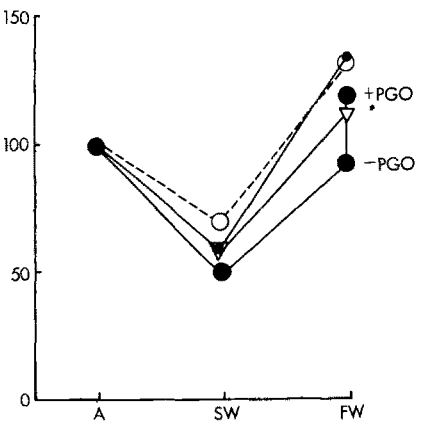

Fig. 3. Changes in responsiveness of LGNd neurons during different states of consciousness. In each case the response in alert state (A) has been taken as the reference state (100%) other responses expressed as a percentage thereof. (●) amplitude of postsynaptic (r) field response to a single stimulus to OT (from *Malcolm* et al., 1970a; see also Fig. 2). (○) rate of spontaneous discharge of LGNd relay cells; (▽) firing probability of single LGNd relay cells to stimulation of OT; (•) firing probability of single LGNd relay cells to stimulation of visual cortex (○ ▽ •) from *Sakakura* (1968). Abbreviations as in Fig. 2

However, the contribution of the optic fibers to these changes was in some doubt. *Benoit* (1967) claimed that the discharge of optic nerve fibers systematically decreased by 25 - 40% with the transition from the waking state to SW sleep with the animal in complete darkness. This relationship was not observed by *Kasamatsu* and *Iwama* (1967) nor by *Cavaggioni* (1968). *Cavaggioni,* however, stated that arousal always increased the rate of discharge. Whatever the contribution of the optic nerve, an extraretinal input to the LGNd must be postulated to explain the results of *Sakakura* and *Iwama* (1967) and *Mukhametov* et al. (1970). These authors established that differences in the pattern of discharge between different arousal states still existed when the cells were deprived of visual input by enucleation, while *Mukhametov* et al. (1970) confirmed that in OT fibers neither rate of discharge nor firing patterns were affected by the phases of the sleep-waking cycle.

Tonic changes in the LGNd resulting from learning have also been described. *Gould* et al. (1974) report that the evoked potential in the LGNd to a flashed pattern changed in waveform as the monkey reached criterion for pattern discrimination. These changes did not seem to be due to variations in arousal level nor to behavioral adaptation but it was not possible to analyze the mechanisms further.

IV. Phasic Changes in Sleep

Slow potential changes occur spontaneously in the LGNd, especially during FW sleep. These are now generally known as ponto-geniculo-occipital (PGO) waves (*Jeannerod* et al., 1965) because there is a synchronous electric change in the pontine reticular formation, LGNd and VC (*Jouvet* et al., 1959; *Mikiten* et al., 1961; *Mouret* et al., 1963). In fact, several other parts of the brain participate in this "event," notably the SC and oculomotor nuclei; PGO waves occur in synchrony with the rapid eye movements of FW sleep although they can occur in their absence, especially during SW sleep in the minute or so before FW sleep starts (*Thomas* and *Benoit,* 1967).

The PGO wave in the LGNd (Fig. 4a) is a predominantly monophasic negative wave of amplitude up to about 0.5 mV and duration up to about 300 ms (*Brooks* and *Bizzi,* 1963; *Sakakura* and *Iwama,* 1965; *Bizzi,* 1966a; *Brooks,* 1967a, 1968; *Cottee* et al., 1974). It occurs synchronously in both LGNds (*Sakakura* and *Iwama,* 1965; *Malcolm* et al., 1970b) and appears to be "all-or-nothing" (*Malcolm* et al., 1970b). It reverses to a positive wave below the LGNd (*Brooks,* 1967a; *Cottee* et al., 1974). This dipole (negative in the LGNd, positive in the OT) is con-

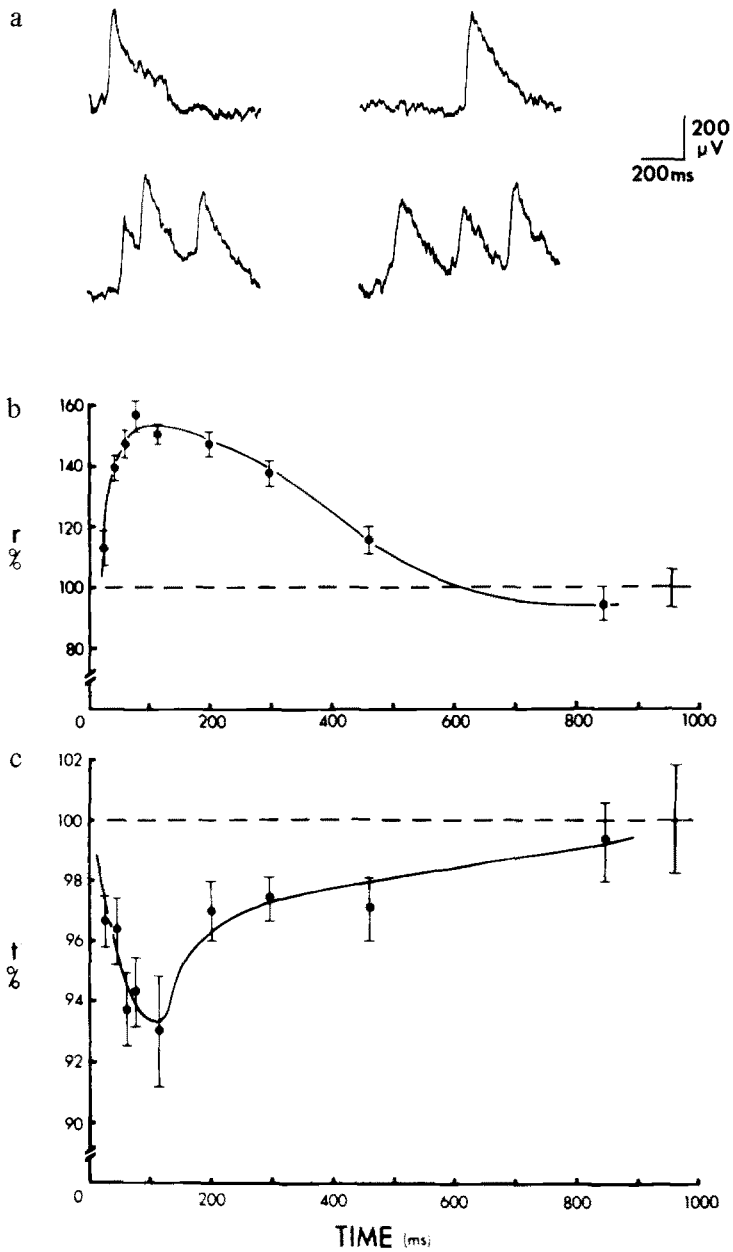

Fig. 4 a - c. PGO waves and changes in excitability in the LGNd during and following a PGO wave. (a) Spontaneous PGO waves in LGNd during FW sleep. Above, single waves; below, multiple waves. (b) Amplitude of r (postsynaptic) component of field response to an OT stimulus delivered at various times after commencement of a PGO wave, expressed as a percentage of a control response (between PGO waves). (c) Similar graph of amplitude of the t (presynaptic) component of field response. (a) from *Cottee, Van der Steen, Burke* (unpublished), (b) and (c) from *Cottee* et al. (1974)

sistent with a depolarization of OT terminals in the LGNd or, conceivably, a depolarization of cell bodies in the LGNd whose axons enter the OT. Since PGO waves are greatly diminished or disappear from the LGNd a few days after bilateral enucleation (*Jeannerod* et al., 1965; *Munson,* 1974a; but see *Brooks,* 1967b) the former possibility is the more likely.

Bursts of action potentials in P cells accompany the PGO waves even in darkness or in the acutely enucleated animal (*Bizzi,* 1966b; *Benoit,* 1967; *Sakakura,* 1968; *Mukhametov* et al., 1970; *Munson,* 1972; *Gardner-Medwin,* 1974). At the same time there are changes in the response of optic nerve terminals and LGNd cells to orthodromic excitation and in the excitability of these structures to direct excitation. In agreement with the depolarization postulated above, during a PGO wave optic nerve terminals show a reduced orthodromic response (Figs. 2, 4c) and an increased excitability (evidenced as an increased antidromic field response) (Fig. 2); LGNd cells show an increased response both to orthodromic (Fig. 4b) and antidromic activation (*Sakakura* and *Iwama,* 1965; *Bizzi,* 1966a; *Iwama* et al., 1966; *Kasamatsu* and *Iwama,* 1966; *Iwama* and *Kasamatsu,* 1966; *Malcolm* et al., 1970a; *Cottee* et al., 1974). *Laurent* et al. (1974b) have shown the close link between the PGO wave and the increased excitability of the optic nerve terminals in LGNd by suppressing both simultaneously through localized cooling of a pathway between the pons and the LGNd.

During FW sleep PGO waves occur singly or in a burst of up to six waves (Fig. 4a). *Pompeiano* and *Morrison* (1965) found that bilateral lesions of the medial and descending vestibular nuclei abolished the bursts although not the single waves, while *Marchiafava* and *Pompeiano* (1966) showed that stimulation of these nuclei or of the eighth cranial nerve enhanced the excitability of the OT endings in the LGNd. However, *Perenin* et al. (1972) reported that after extensive destruction of vestibular nuclei, rapid eye movements and PGO waves return; after 1 week their rate may be 70% of control values. Therefore they do not regard the vestibular nuclei as necessary for the generation of the phasic events of FW sleep but rather as a modulating influence. *Munson* and *Hurd* (1973) found that bilateral labyrinthectomy and cerebellar lesions had no effect on PGO waves in the LGNd.

There is now very conclusive evidence for a "PGO-wave generator" in the dorsolateral part of the pontine tegmentum (a region that is part of the pontine reticular formation [PRF]). This conclusion is reached from experiments which have shown that: 1) there is synchrony between waves in this region and in the LGNd (*Bizzi* and *Brooks,* 1963), 2) PGO waves occur in PRF up to 15 ms before the corresponding PGO wave in LGNd (*Jeannerod* and *Kiyono,* 1969a), 3) PGO waves in PRF could not be produced by stimulating LGNd whereas PGO waves in LGNd could be

produced by stimulating PRF (*Bizzi* and *Brooks,* 1963), and 4) transections below the pontine region did not prevent PGO waves occurring in the LGNd whereas transections above this region did (*Laurent* et al., 1972, 1974a).

Laurent et al. (1974a) have mapped out the PGO pathway between pons and LGNd in broad detail. They worked mainly not with sleep PGO waves but with PGO waves triggered by reserpine (PGO_R) waves, but there is good reason to think that these waves are essentially the same as the sleep waves (*Jeannerod* and *Kiyono,* 1969a, b; *Munson* et al., 1970; *Munson* and *Graham,* 1971; *Satoh* et al., 1974). Furthermore, the main results were confirmed on the *encéphale isolé* preparation. In the *encéphale isolé* reserpinized cat the PGO_R waves are correlated with activity in the various eye muscles and oculomotor nuclei (*Cespuglio* et al., 1975). Single PGO_R waves commonly have two peaks separated by about 80 ms, *Laurent* et al. (1974a) showed that these arise because there are two pontine generators, one on each side of the brain stem, each of which can work independently and initiate PGO waves in each LGNd; under reserpine each generator can also trigger the other with a latency of about 80 ms and this causes the appearance of the two peaks. The latency is probably less in sleep, and either the two peaks merge or there is refractoriness (*Malcolm* et al., 1970b). *Laurent* et al. (1974a) find that the ascending pontogeniculate system responsible for PGO_R waves coincides closely with the topography of the intermediate catecholamine pathway which ascends from the nucleus subcoeruleus (NSC) and crosses in the supraoptic commissure. However experiments with localized cooling at the level of the pontomesencephalic isthmus suggest that the identity of the two pathways is not complete (*Laurent* and *Guerrero,* 1975).

It has been mentioned that PGO waves can be elicited by stimulation of the PRF. The threshold for eliciting a wave is low only when PGO waves are occurring spontaneously. Slow negative waves can also be elicited by stimulation of the midbrain reticular formation (MRF) (*Angel* et al., 1965; *Sumitomo* and *Hayashi,* 1967; *Mass* and *Smirnov,* 1970; *Singer* and *Dräger, 1972;* Singer, 1973a; *Singer* and *Bedworth,* 1974; *Munson* et al., 1975; *Cohen* et al. 1969, in the monkey) (Fig. 6d); in one report intracellular recording from OT nerve endings has provided direct evidence that these terminals are depolarized with a time course similar to that of the slow wave (*Kahn* et al., 1967). This depolarization is presumably responsible for the occurrence of an increased excitability of the terminals to direct stimulation, the antidromic response being increased during both MRF- and PRF-evoked waves (*Angel* et al., 1965) (Fig. 6c and e).

Lesions of midline raphe nuclei caused a transient increase in PGO waves; parasagittal cuts separating the raphe nuclei from the PGO gener-

ator areas had a similar effect – after these cuts stimulation of the generator areas was more effective in eliciting PGO waves (*Simon* et al., 1973). These observations suggested that the raphe nuclei exert an inhibitory effect on the PGO generators. This conclusion is supported by experiments in which administration of reserpine (depleting the raphe of 5-hydroxytryptamine (5-HT)) was found to produce a continual discharge of PGO waves (*Delorme* et al., 1965; *Brooks* and *Gershon,* 1971, 1972). On the other hand, *Jacobs* et al. (1973) found that stimulation of n. raphe dorsalis suppressed PGO waves during FW sleep whereas stimulation of n. raphe pontis and magnus triggered PGO waves. They found that n. dorsalis stimulation in the conscious animal produced a mild arousal similar to that produced by MRF stimulation, and therefore considered that the suppression of PGO waves might have been due to this effect rather than to a more specific action. Thus, the role of the raphe nuclei in the generation of PGO waves is at present unclear.

Stimulation of nonspecific thalamic nuclei also gives rise to a slow negative wave in the LGNd (*Mass* and *Smirnov,* 1970). A slow negative wave in LGNd also results from stimulation of the optic nerve, but only if the RF is functioning normally (not blocked by drugs or surgical intervention); *Bonnet* and *Briot* (1970) suggest that this wave is identical with that produced by RF stimulation. Finally, in a sleeping cat a sudden "alerting" stimulus can elicit a slow wave that closely resembles the spontaneous PGO wave (*Bowker* and *Morrison,* 1976).

The functional significance of these phasic changes in sleep is obscure. However, the pathways and mechanisms involved in these events are becoming clearer (see Section VIII).

V. Phasic Changes in the Conscious Animal

A discharge of LGNd neurons frequently occurs at the time of an eye movement, even when the animal is in the dark (*Bizzi,* 1966b; *Sakakura,* 1968; *Lombroso* and *Corazza,* 1971; *Corazza* and *Lombroso,* 1970, 1971; *Singer* and *Bedworth,* 1974). This burst follows the eye movement by 15-100 ms (Fig. 5), and, if the animal is in the light, may precede any discharge coming from the retina as a result of the photic stimulation (*Corazza* et al., 1972). *Papaioannou* (1969, 1972a), *Jeannerod* and *Putkonen* (1970, 1971) and *Jeannerod* (1972) have also recorded increases (and occasional decreases) in the discharge of LGNd neurons occurring in the later part of an eye movement produced by vestibular stimulation in darkness. Such stimulation also modified the light-evoked discharge of LGNd neurons (*Papaioannou,* 1972b). Similarly, caloric

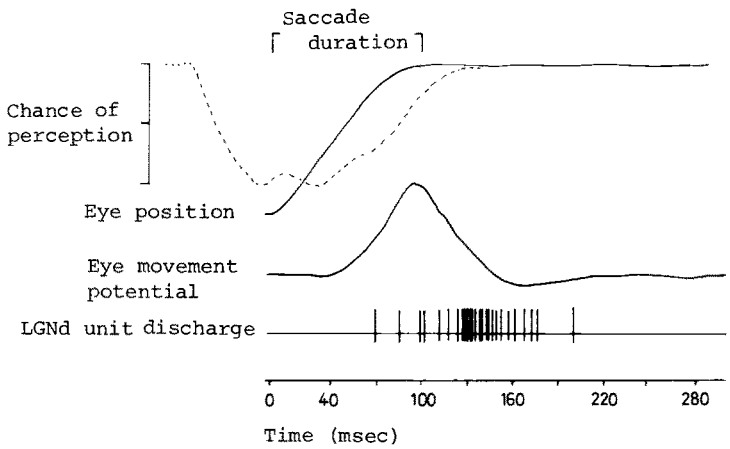

Fig. 5. Temporal relationship of saccade-related events. Dotted line indicates time course of saccadic suppression as tested psychophysically (taken from *Latour*, 1962). Entire diagram taken from *Jeannerod* (1972), slightly modified

labyrinthine stimulation excited or inhibited most LGNd neurons (*Papaioannou*, 1973a) or modified their light-evoked responses (*Papaioannou*, 1973b).

In the *encéphale isolé* rat the P-cell activity was reduced during and following a saccade in the light, whereas the activity of on-off cells (believed to be I cells) was increased (*Montero* and *Robles*, 1971). In this experiment a spontaneous nystagmus was induced by unilateral labyrinthectomy and the dominant eye immobilized by section of the extraocular muscles. Thus the LGNd cells excited from the dominant eye received no retinal discharge from that eye at the time of the saccade. Although it is conceivable that they might have received an indirect influence form the nondominant eye in the light, the same effects were obtained in the dark. These findings suggest that some vestibular-visual coordination occurs at LGNd level.

On the other hand, changes in firing rate related to spontaneous saccades were not observed in the LGNd neurons of the monkey (*Büttner* and *Fuchs*, 1973; *Duffy* and *Burchfiel*, 1975), nor in the intact alert cat in the dark (*Noda*, 1975b). These three experiments were performed in alert animals whereas in the experiments cited above the animal might not have been alert. Thus the saccade-related discharge may occur only when the LGNd excitability is not high.

There are also changes in responsiveness of LGNd neurons during and following an eye movement. In response to an electric stimulus to the OT the presynaptic response in the LGNd was unchanged but the postsynaptic response was reduced for about 200 ms following an eye movement, provided the animal was in the light (*Kawamura* and *Marchiafava*,

1966, 1968; *Malcolm* et al., 1970a; *Adey* and *Noda,* 1973; *Noda* and *Adey,* 1974; *Noda,* 1975a; *Ogawa,* 1972, in the monkey). A similar depression was produced by a quick displacement of a patterned object before a stationary eye (*Adey* and *Noda,* 1973; *Noda,* 1975a).

Rapid movement of the retinal image is a good stimulus for the Y fibers, excitation of which will in turn lead to inhibition of both X and Y neurons in the LGNd (see Section II) and thereby to a reduced orthodromic response. The degree of inhibition will depend on the background. The result obtained by *Michael* and *Ichinose* (1970) showing no decrease in the response to a flash during an eye movement could be explained if the visual background in their experiments was homogeneous or its level of illumination low. When photic effects are eliminated by placing the animal in the dark, the postsynaptic LGNd response shows either no change (*Malcolm* et al., 1970a; *Adey* and *Noda,* 1973; *Noda* and *Adey,* 1974; *Noda,* 1975a; *Cohen* et al., 1969, in the monkey; *Büttner* and *Fuchs,* 1973 in the monkey) or a small increase (*Singer* and *Bedworth,* 1974). In only one study was a decrease in the dark reported and to be detected this required data averaging (*Bartlett* et al., 1976, in the monkey). Thus, changes in postsynaptic responsiveness in the LGNd during eye movements in the light depend strongly on the retinal discharge and probably not in any important way on an extraretinal input to the LGNd.

Associated with an eye movement is an elevated visual threshold (*Latour,* 1962; *Volkmann,* 1962). This "saccadic suppression" commences about 50 ms before the eye movement and has a total duration of about 180 ms (Fig. 5). *Rodieck* (1973c), *Adey* and *Noda* (1974) and *Singer* and *Bedworth* (1974) have suggested that saccadic suppression may be at least partly attributed to the inhibitory effects in the LGNd of the retinal discharge produced by the photic stimulation during the saccade. In support of this idea is the observation of *Kulikowski* and *Leighton* (1977) that there is no elevation of threshold during a saccade if the viewing eye is curarized; this experiment also shows that the command signal for the saccade does not itself cause the suppression.

Tsumoto and *Suzuki* (1976) have found that stimulation of the frontal eye field in the cat in a region producing conjugate lateral saccadic eye movements also facilitates the response of LGNd X cells (but not Y cells) with a latency of 50 - 100 ms and a duration of about 500 ms, and they suggest that this may serve to cancel saccadic suppression and restore central vision in the new gaze position. This effect occurs in the dark and does not depend on the RF.

An eye movement in the conscious animal, whether spontaneous or due to nystagmus, or to stimulation of the vestibular system or PRF, is accompanied by a slow potential change in the LGNd and VC. This

potential change is similar to the PGO wave of sleep and hence has been termed PGO_W wave (W for wakefulness) (*Brooks* and *Gershon*, 1971) but it is more usually termed "eye movement potential" (EMP) (*Cohen* and *Feldman*, 1968; *Jeannerod* and *Sakai*, 1970) (Fig. 5). *Sakai* (1973) has distinguished three components of the EMP in the LGNd of the cat – an initial (positive) component commencing about 16 ms after the eye movement, a second (negative) component of latency 76 ms, and a third (negative-positive) component of latency 146 ms. The first component was present in the light when the animal viewed a patterned visual field and a similar potential resulted when the eyes were moved passively, or if a patterned visual field was moved in front of the eyes, but it was absent if the visual field was homogeneous or if the animal was in the dark (*Sakai*, 1973; *Feldman* and *Cohen*, 1968, in the monkey); thus it is probably due to the neural discharge originating in the retina. The second and third components were present in the homogeneous visual field and in darkness and equivalent potentials were observed in the paralyzed cat at times when eye movements would otherwise have ocurred, e.g., during caloric stimulation of the labyrinth (*Jeannerod* and *Sakai*, 1970; *Sakai*, 1973). The second component occurred during the eye movement and the third component commenced at about the end of the eye movement. Passive movement of the eye did not produce an EMP in a homogeneous visual field or in the dark (*Sakai*, 1973).

Similar potential changes during eye movements (spontaneous or evoked) have been recorded by *Brooks* (1968), *Jeannerod* and *Sakai* (1970), *Costin* and *Hafemann* (1970), *Brooks* and *Gershon* (1971), *Orban* et al. (1972), *Munson* and *Schwartz* (1972), *Okuma et al. (1973)*, *Munson* (1974a, b), *Singer* and *Bedworth* (1974), *Munson* et al. (1975) in the cat and by *Feldman* and *Cohen* (1968) and by *Cohen* and *Feldman* (1968, 1974) in the monkey and it is generally agreed that the EMP follows the onset of the eye movement in the dark by 40 ms or more. *Feldman* and *Cohen* (1968) noted that essentially similar potentials can also be elicited by nonvisual stimuli such as sounds and touch.

Finally, an increase in the antidromic response of OT fibers to stimulation in LGNd (or in SC) was obtained during tracking eye movements in the midpontine pretrigeminal cat by *Kawamura* and *Marchiafava* (1966, 1968).The effect decreased as the amplitude of the eye movements decreased ("habituation"), but was still present after curarization when the target was moved before the eyes, implying that it did not depend on eye movements. An increased antidromic OT response was also obtained by *Cohen* et al. (1969) in the monkey in darkness during an eye movement elicited by RF stimulation that was also accompanied by an EMP. These results suggest that the EMP (second and third components) is at least partly due to a slow wave of depolarization of the OT terminals. *Cohen* et al.

(1969) associated the effect with alerting because antidromic OT potentials were continuously enhanced after administration of amphetamine. As mentioned above, the effect in the midpontine pretrigeminal cat decreases with habituation of the eye movements and hence may likewise be associated with alerting; it seems probable that the RF is again involved. The linkage between EMPs and enhanced antidromic OT responses may not be inflexible, however. *Cohen* et al. (1969) pointed out that spontaneous rapid eye movements in the monkey did not affect the antidromic OT response although presumably they were associated with EMPs (*Feldman* and *Cohen*, 1968, *Cohen* and *Feldman*, 1968, 1974). They also showed that amphetamine did not affect the EMP induced by RF stimulation while reducing the increase in the antidromic OT response induced by the same stimulus.

To summarize, during and following an eye movement certain changes occur in the LGNd. There is a decreased responsivensess of LGNd relay cells when the eye movement occurs in a patterned environment in the light and this may partly account for "saccadic suppression." There are eye movement potentials and there may be changes in the neuronal discharge at the time of a saccade, even in the dark. Attempts have been made to interpret these events as the neuronal basis for perceptual adjustments. It was pointed out by *von Helmholtz* (1867) that the external world seems to remain at rest during a voluntary eye movement but appears to move if the eyeball is moved passively. *Sperry* (1950), *von Holst* and *Mittelstaedt* (1950) and *Ludvigh* (1952) proposed that at the time of a voluntary eye movement a signal would go to the perception centers at the same time as to the eye muscles. The signal, a "corollary discharge" (Sperry, 1950), would in some way ensure that an appropriate correction was made to the retinal signal to prevent it conveying the erroneous message of a moving environment. It would not be necessary for this corollary discharge to precede the eye movement but presumably it should reach the perception centers before or at the time of the discharge from the retina.

Although the saccade-related events in the LGNd are appropriately timed to represent corollary discharges, the evidence summarized above is not strongly in support of this idea. The main objection is that effects on synaptic transmission in the LGNd cannot be demonstrated if the animal is fully alert. It seems that there is facilitation of transmission (and associated firing of LGNd neurons, occurrence of an EMP and reduced threshold for antidromic excitation of OT fibers) only if there is some degree of nonalertness. There is an obvious advantage in facilitating transmission at the end of an eye movement in order to overcome saccadic suppression (*Tsumoto* and *Suzuki*, 1976) but this might not be necessary in the fully alert animal. There is better evidence for a corollary discharge to the VC (*Adey* and *Noda*, 1973; *Büttner* and *Fuchs*, 1973).

Eye movement potentials are at least partly due to depolarization of OT terminals and they are probably produced via an input from RF. Thus they resemble PGO waves. One important difference, however, is that a PGO wave is coincident with a rapid eye movement of sleep (*Jeannerod* and *Sakai,* 1970; *Cespuglio* et al., 1975), whereas an EMP (second and third components) follows a spontaneous eye movement by at least 40 ms (but an EMP evoked by MRF stimulation is coincident with the evoked eye movement (*Singer* and *Bedworth,* 1974)). Account should be taken of the facts that the spontaneous eye movement and EMP are initiated either by movement in the visual field or voluntarily (perhaps from the cerebral cortex) whereas the eye movement in FW sleep and the PGO wave are probably initiated from the PRF. The difference in time relations could readily be explained if the slow potential change was always mediated via the PRF; but the spontaneous eye movement occurred via a relatively fast pathway through cortex or tectum. EMPs also occur in FW sleep and are distinguishable from PGO waves by their longer latencies relative to PRF waves or to waves in the VI oculomotor nucleus (*Sakai* and *Cespuglio*, 1976).

VI. Depolarization of Optic Nerve Terminals and the Question of Presynaptic Inhibition

As already described, there is strong evidence that the PGO wave and EMP in the LGNd are at least partly due to a depolarization of OT endings in the LGNd. Similar arguments apply to the slow waves evoked by stimulation of MRF, PRF, VC (*Iwama* et al., 1965; *Suzuki* and *Kato,* 1965; *Angel* et al., 1967), the contralateral optic nerve (*Godfraind* and *Meulders*, 1966; *Marchiafava*, 1966; *Hayashi*, 1972); and other areas. It has been widely assumed that presynaptic depolarization could be taken as an index of presynaptic inhibition (*Schmidt*, 1971). However, there are at least two difficulties in applying this interpretation to the LGNd: 1) the presynaptic depolarization is occurring at a time when there is facilitation of the postsynaptic response (*Ogawa,* 1963; *Singer* and *Dräger*, 1972; *Tatton* and *Crapper*, 1972; *Heckenberg* and *Burke*, 1972; *Singer*, 1973a, b; *Cottee* et al., 1974; *Cohen* et al., 1969, in the monkey), 2) the postulated axo-axonal synapses that would mediate depolarization of the OT endings are absent (*Peters* and *Palay,* 1966; *Szentágothai* et al., 1966; *Guillery,* 1969a; *Famiglietti* and *Peters,* 1972; *Colonnier* and *Guillery,* 1964 (monkey); *Pecci-Saavedra* and *Vaccarezza,* 1968 (monkey); *Lieberman* and *Webster,* 1974 (rat)).

With regard to the first difficulty, there are reports of a reduction in the amplitude of the postsynaptic response preceding the postsynaptic facilitation (*Singer*, 1973b; *Pecci-Saavedra* et al., 1966 (monkey)) or fol-

lowing it (*Singer*, 1973b). *Singer* (1973b) has shown: 1) that the first inhibitory period in the cat precedes the slow negative wave and the period of enhanced excitability of the OT terminals, and he gives evidence that this is due to stimulation of bifurcating Y fibers running to SC (see *Hayashi* et al., 1967), 2) that both inhibitory periods coincide with a hyperpolarization of the P cell and that excitatory postsynaptic potentials (EPSPs) during the rising phase of this hyperpolarization can be reduced in amplitude but during the falling phase may be increased. These features strongly imply postsynaptic inhibition although some contribution due to presynaptic inhibition could not be ruled out. The second inhibitory phase *Singer* attributes to recurrent inhibition following the facilitation and resulting from it, because its magnitude was dependent on the degree of facilitation. In the monkey, *Pecci-Saavedra* et al. (1966) found that the early inhibition was blocked by picrotoxin which is known to antagonize γ-aminobutyric acid (GABA). *Curtis* and *Tebēcis* (1972) have evidence that the inhibitory transmitter from I cells to P cells is GABA. Therefore *Singer's* explanation for inhibition in the cat might also apply to the monkey, since the prediction would be that it would be blocked by picrotoxin.

Why does the presynaptic depolarization not seem to impair synaptic transmission? *Singer* and *Lux* (1973) suggest two reasons: 1) the depolarization may be too weak to affect transmitter release, 2) only the preterminal parts of the OT fibres may be depolarized, the terminals and boutons being protected from depolarization by the glial sheaths of the glomeruli in which they are enclosed. Since the glial sheaths presumably do not prevent an action potential reaching the terminals, it seems unlikely they could prevent them being passively depolarized. On the assumption that perhaps not all the fine terminal branches of OT fibres are normally invaded, *Singer* and *Lux* (1973) suggest that presynaptic depolarization might even facilitate transmission by allowing invasion of more of these fine terminals. This ought to lead to an increased orthodromic presynaptic response, whereas the opposite is the case (see Fig. 4). There is a more satisfactory explanation of the facilitation (see Section VIII). It is still possible that there is a reduction in transmitter output during this period of presynaptic depolarization but it is masked by the much greater postsynaptic effects of facilitation and inhibition.

Even if presynaptic inhibition may be ruled out, it is still necessary to explain the depolarization of the OT terminals. *Singer* and *Lux* (1973) have measured changes in extracellular potassium concentration in the LGNd after electric stimulation of MRF, VC, optic chiasm and photic stimulation of the retina. In all cases there was a transient increase in K^+ in the LGNd which had a similar latency, time course and cumulative property as the excitability change of the OT terminals. The increase in K^+

was attributed to increased neuronal activity. This suggests that the presynaptic depolarization may be a rather nonspecific effect, but whether it has physiologic significance is not yet clear.

VII. Effects of Nonvisual Sensory Stimulation on the LGNd

Nonvisual sensory stimulation can affect ongoing activity and evoked responses in LGNd. It is generally believed that these effects are mediated via the RF and are part of an arousal response. *Buser* and *Segundo* (1959) obtained both facilitation and inhibition of LGNd cells by electric stimulation of the paw of a cat anesthetized with chloralose. *Hotta* and *Kameda* (1963) stimulated the superficial radial nerve in cats anesthetized with chloralose and observed facilitation of the response of cells on the medial and dorsal borders of the LGNd. *Melzack* et al. (1968) and *Dubrovsky* and *Melzack* (1970) observed prolonged increases and decreases of firing of LGNd cells in anesthetized cats during somatic stimulation (rubbing of a paw, vibration of the skin or electric stimulation of the sciatic nerve). *Davidowa* et al. (1975) were able to affect the pattern of response of LGNd cells in rats to a flash by associating the flash with electric stimulation of the tail skin in a classic conditioning schedule.

Meulders, Godfraind and colleagues (*Meulders* et al., 1964, 1966; *Boisacq-Schepens* and *Meulders*, 1965) confirmed the mainly facilitatory effect of somatic sensory stimulation and also showed that this stimulation alone could excite LGNd cells. They have further shown (*Godfraind* and *Meulders*, 1967, 1969; *Meulders* and *Godfraind,* 1969) that both in the alert cat and in the cat under chloralose there is an enlargement of the visual receptive field of LGNd cells. This effect is probably mediated through MRF because extensive destruction of this region greatly reduced or abolished the effect, and the same effect was obtained with MRF stimulation (*Meulders* and *Godfraind*, 1969). *Godfraind* and *Meulders* (1969) also reported that cells that previously fired at the *on* of a visual stimulus could be made to fire at the *off* (and vice versa) by suitable timing of the somatic stimulation. It is difficult to explain this finding. *Levick* et al. (1972) found that, without exception, LGNd *on*-center cells were excited only by retinal *on*-center cells and LGNd *off*-center cells only by retinal *off*-center cells. A possible explanation of the effect of somatic stimulation is that it is mediated at the retinal level and that the photic stimulation was applied to the antagonistic surround. The reversal of response type would then be similar to that obtained with the transition from light-adaption to dark-adaption or vice versa (*Barlow* et al., 1957). *Godfraind* and *Meulders* (1969) were not able to detect a center-surround arrangement under the conditions of their experiments, so that it is not possible to say wheth-

er the stimulus would have been in the surround had the cat been light-adapted. More importantly, however, the evidence for retinal efferent fibers in the cat is not very good (cf. *Rodieck*, 1973b). *Chow* and *Lindsley* (1968) found that paw stimulation could affect the response of single units in the LGNd of the cat if eye movements were not carefully controlled. But *Meulders* and *Godfraind* (1969) rule out this possibility in their experiments.

Taira and *Okuda* (1962) used acoustic and olfactory "alerting" stimuli with similar effects to stimulation of the MRF. *Skrebitsky* (1969) reported that 49 out of 92 LGNd neurons in the anesthetized rabbit responded to an acoustic stimulus, 75 % showing an increased discharge. *Nicolai* et al. (1973) found that in the curarized alert rat 12 out of 59 LGNd cells responded to an acoustic stimulus, 19 out of 52 to an interoceptive stimulus (distension of the stomach) and four of these to both stimuli. *Chalupa* et al. (1975) found that they could reduce the response of some tonic (probably X) units in the LGNd of a cat to a flash by pairing the flash with a click given simultaneously or 100 ms earlier and in about half of these units the effect persisted for 2 - 3 min.; phasic (Y) units were unaffected. In the experiments of *Zippel* et al. (1976) rats were trained to learn a conditioned emotional reaction to a tone; pairing of tone and flash then resulted in the tone changing the reaction of LGNd units to a flash.

Evidence has been presented that somatic and auditory stimulation can affect both efferent and afferent units in the optic nerve (*Spinelli* et al., 1965; *Spinelli* and *Weingarten*, 1966; *Weingarten* and *Spinelli*, 1966), and so could influence the LGNd indirectly.

The LGN neurons have also been affected by caloric irrigation of the ear (*Kornhuber* and *Da Fonseca*, 1964; *Papaioannou*, 1973a, b). by rotation (*Magnin* et al., 1974; *Kennedy* et al., 1976) and by electric polarization of the labyrinth (*Jung* et al., 1963; *Kornhuber* and *Da Fonseca*, 1964). *Magnin* et al. (1974) reported that 61% of neurons in the lateral geniculate area (that is, including the ventral nucleus of the LGN and also the RNT) were influenced by rotation. Of these neurons, 63% changed their discharge rate (usually this increased) independently of the velocity or direction of rotation, in 30% the discharge was related to the direction of rotation, while 7 % were influenced only by changes in velocity, not direction, of rotation. However, all the cells in the latter two groups responded poorly to light, and a large proportion were in the ventral nucleus of the LGN. *Kennedy* et al. (1976) found that in some LGNd cells rotation diminished the antagonistic effect of the receptive field surround, probably a similar effect to the enlargement of the receptive field reported by *Godfraind* and *Meulders* (see above).

VIII. The LGNd and the Reticular Formation

Of all extraretinal influences on the LGNd most attention has been paid to that from the MRF. In the cat the effects of stimulating the MRF are greater on the VC than on the LGNd (*Bremer* and *Stoupel*, 1958, 1959; *Dumont* and *Dell*, 1958, 1960) whereas in the monkey the main effect is on the LGNd (*Doty* et al., 1973). In general, the effect of MRF stimulation in cats is enhancement of both spontaneous and evoked activity in LGNd cells. However, most workers have also reported inhibitory effects. There are also small changes in the response of the OT fibers. The amplitude of the t response to optic nerve or OT stimulation is reduced and that of the t(anti) response is increased, while there is a concurrent slow negative potential change in the OT (*Angel* et al., 1965). These three events run a similar time course (Fig. 6) and, as in the case of the PGO

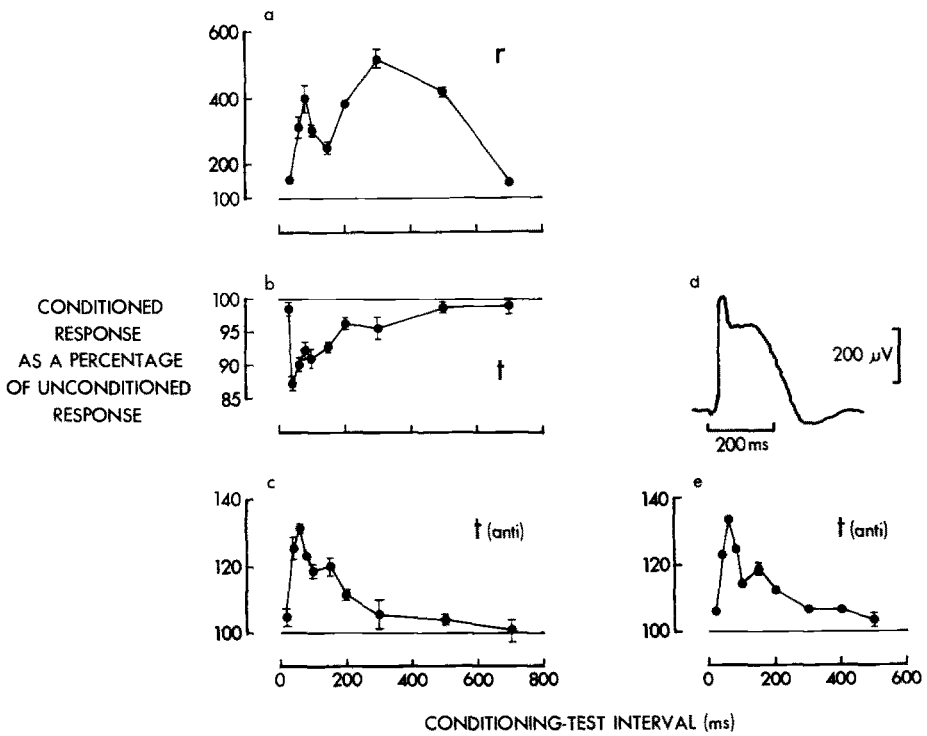

Fig. 6 a - e. Slow wave and changes in responsiveness in LGNd after MRF stimulation. Amplitues of r, t, and t(anti) responses (cf. Fig. 2) obtained at various intervals after MRF stimulation (6 shocks, 0.5 ms pulse width, 3.2 ms interval) plotted as percentages of the unconditioned response ±SEM. (a), (b), (c) from cat under N_2O anesthesia. (d) and (e) from another cat, also under N_2O anesthesia. (d) slow wave recorded between electrodes in LGNd and OT to same MRF stimulation as in (e), tracing of averaged response, negativity in LGNd upwards (from *K. Heckenberg* and *W. Burke*, unpublished)

wave, are interpreted as indicating a depolarization of the OT endings. The r response is facilitated at the same time, but the time course is usually somewhat different and frequently more prolonged, the relative effects on t and r resembling those during the PGO wave (Fig. 4). There is facilitation of both Y and X cells (*Fukuda* and *Stone*, 1976). For brief MRF stimulation the effect has a latency usually more than 20 ms and lasts several hundred ms (*Dumont* and *Dell,* 1960; *Suzuki* and *Taira,* 1961; *Okuda*, 1962; *Nakai* and *Domino*, 1968; *Satinsky*, 1968; *Tatton* and *Crapper*, 1972; *Singer* and *Dräger*, 1972; *Singer,* 1973a; *Foote* et al. 1974; *Fukuda* and *Iwama,* 1970, 1971, in the rat). Most of this Section will be concerned with the variations in effect or anomalies that have been reported.

A. Different Effects on the Flash-Evoked Response and on the Response to OT Stimulation

Bremer and *Stoupel* (1958, 1959) found that the LGNd postsynaptic response to OT stimulation in the *encéphale isolé* cat was facilitated by MRF stimulation whereas the response to a flash of light was diminished. *Hernández-Peón* et al. (1956) also described a reduction in the flash-evoked response. Other workers have reported no effect (*Fuster* and *Docter* 1962, in the unanesthetized rabbit) or an enhancement (*Buser* and *Segundo,* 1959; *Steriade* and *Demetrescu*, 1960; *Hotta* and *Kameda*, 1964; *Suzuki* and *Jacobson*, 1971, but only of the later slow negative wave and then only in the light). *Bremer* and *Stoupel* suggested that fibers from MRF and retina converge on to the same LGNd cells, that MRF fibers can themselves elicit firing of LGNd cells and that this could lead to some refractoriness; refractoriness might affect the light-evoked response more because the afferent impulses would be less synchronous. There is another explanation. The field response to a flash is dominated by a slow wave which may represent after-hyperpolarizations in relay cells (summed IPSPs). RF stimulation reduces or abolishes IPSPs (*Singer* and *Dräger,* 1972; see later) and hence could reduce the amplitude of the field response. The response to a single OT shock is more synchronous and so the fast (spike) components dominate the response and these are facilitated by RF stimulation.

B. Rate and Intensity of MRF Stimulation

In unanesthetized cats *Long* (1959) found that stimulation at low rates (about 0.5 Hz) tended to facilitate, stimuli at high rates (250 Hz) tended

to inhibit, the LGNd response to OT stimulation. In unanesthetized, mid-pontine-transected cats, *Tatton* and *Crapper* (1972) found that whereas weak stimuli had effects of duration comparable to those resulting from photic stimulation, strong stimuli (more than 650 μA for 0.1 ms pulses) caused a prolonged increase in the "spontaneous" discharge of LGNd neurons. *Theil* et al. (1972) reported that the primary component of the flash-evoked response in the LGNd of a freely moving rat was increased at low intensities of stimulation of MRF (area cuneiformis) but reduced at high intensities. One possible explanation for these differences is that there is more than one group of fibers from the MRF affecting the LGNd.

C. Stimulating Site in RF

Suzuki and *Taira* (1961) showed that the structures in the midbrain in which stimulation produced facilitation were fairly restricted, being roughly the region of the MRF. They found that stimulation close to the SC produced inhibitory effects probably by activation of fibers originating in OT and supplying both LGNd and SC. However, the SC may exert a direct effect on the LGNd (see later). *Hotta* and *Kameda* (1964) found that stimulation of the nearby mesencephalic lemniscus produced inhibitory effects. As yet however, there has been no description of separate facilitatory and inhibitory regions within the RF, and in the squirrel monkey facilitatory and inhibitory effects are maximal at the same locus (*Wilson* et al., 1973).

D. Locus of Effect

Stimulation of MRF produces pupillary dilatation and cortical activation (desynchronization) among other effects. Could the effects in the LGNd be due to these factors? Several workers have applied atropine to the eyes (*Singer*, 1973a) or kept the animal in the dark (*Doty* et al., 1973) without abolishing the effects. With the eye intact (albeit in the dark or atropinized) it is still possible that effects might arise in the retina due to circulatory changes or via retinopetal fibers. However, facilitatory effects are still obtained after enucleation (*Satinsky*, 1968). *Nakai* and *Domino* (1968) found that the RF facilitatory effect was reduced by enucleation. They therefore suggested that RF stimulation acts partly by disinhibition of an inhibitory influence dependent on the retina. However, this disinhibition could occur in the LGNd and indeed this seems probable because several groups have shown that MRF stimulation produces no comparable facilitatory effects in OT (*Suzuki* and *Taira*, 1961; *Okuda*, 1962; *Taira*

and *Okuda*, 1962; *Ogawa*, 1963). In any case there is a residual facilitatory effect after enucleation (*Nakai* and *Domino*, 1968). *Satinsky* (1968) reported that removal of eyes and visual cortices did not change the nature of the RF effect on the spontaneous firing of LGNd cells. It was also shown by *Tatton* and *Crapper* (1972) that cortical activation could be obtained by stimulation of certain regions of MRF and of the caudate-capsular region without effects on the firing of LGNd cells. Therefore cortical activation per se is not responsible for the facilitatory effects in the LGNd. Although it remains possible for the RF to exert an effect on the LGNd via retina or cortex the major effect does not depend on these regions.

E. Dependence of Effect on State of Arousal

Dumont and *Dell* (1960) found that the facilitatory effect of MRF stimulation was much greater in the sleeping cat than in the awake cat. This effect was confirmed in the rhesus monkey by *Cohen* et al. (1969). The parameter "transfer ratio" (TR) (*Coenen* and *Vendrik*, 1971, 1972; *Coenen* et al. 1972., *Singer* and *Dräger*, 1972; *Singer*, 1973a; *Fukuda* and *Stone*, 1976) has been used to explain the varying magnitude of the effects of RF stimulation. The TR is the ratio of the number of action potentials (APs) in an LGNd cell to the number of APs plus subthreshold EPSPs. The TR has been regarded as a measure of the cell's excitability. This is strictly valid only if each AP is elicited by a threshold EPSP and if the cell is driven by a single optic tract fiber (producing a unitary EPSP). The latter condition may apply in 75 % of LGNd cells (*Singer*, 1970). The former condition also does not always hold; in certain circumstances a single OT impulse may give rise to clustered firing of an LGNd cell (*Hubel* and *Wiesel*, 1961). Nevertheless, the TR appears to be a useful index in studying the extraretinal influences on the LGNd. By definition, the TR has a maximum value of 1.0. If the TR is high, as in the alert animal, MRF stimulation can produce little effect. If the TR is low, as in SW sleep, MRF stimulation can have a large effect. *Gijsbers* (1973) used a behavioral task which kept cats alert and found that MRF stimualtion had no effect on LGNd spontaneous activity (integrated multiunit record). In line with these ideas, RF stimulation has a strong facilitatory effect if it is applied at a time of synaptic depression in LGNd produced by a previous shock to the optic nerve (*Bonnet* and *Briot*, 1970; *Singer* and *Bedworth*, 1974), or a previous flash (*Singer* and *Phillips*, 1974).

A slightly different approach was used by *Taira* and *Okuda* (1962). They obtained the result that although the response of an LGNd unit to photic stimualtion might be increased, the baseline discharge could also be increased, so that the "signal/noise ratio" might even decrease. How-

ever, they showed that the variation of the response in the alert state was less than that in the nonalert state. Thus although the response might not increase significantly, its regularity was better, and hence reticular stimulation could be said to increase the reliability of information transfer.

F. Effect on Receptive Fields

Meulders and *Godfraind* (1969), using a *cerveau isolé* dark-adapted cat, found that MRF stimulation not only increased the response of an LGNd cell to a photic stimulus but also appeared to enlarge the receptive field of the neuron; these changes were accompanied by activation of the electroencephalogram. Receptive fields of most LGNd cells consist of a center and an antagonistic surround (*Hubel*, 1960; *Hubel* and *Wiesel*, 1961), and an outer region, always inhibitory, the "suppressive field" (*Sanderson* et al., 1971; *Levick* et al., 1972). *Singer* and *Creutzfeldt* (1970) have shown that center and surround exert inhibition by hyperpolarization of the LGNd cell, and this is probably true for the suppressive field also at least for the nondominant eye (*Suzuki* and *Kato*, 1966; *Sanderson* et al., 1971). When stimulation of the receptive field of a cell exceeds the center area and encroaches on the surround, the response of the cell decreases and the TR is reduced due to hyperpolarization; in these circumstances MRF stimulation reduces or abolishes the hyperpolarization and increases the TR (*Singer* and *Drager*, 1972). *Meulders* and *Godfraind* (1969) did not find an antagonistic surround in their experiments and they attributed this partly to the fact that the experiments were conducted under scotopic conditions. It is possible that there was a balance of excitation and inhibition in the periphery of the receptive fields of the LGNd cells from which they recorded. If so, RF stimulation might have removed the inhibition and appeared to enlarge the receptive field. Such an effect has been demonstrated by *Fukuda* and *Stone* (1976). By RF stimulation they could reduce the inhibition due to photic excitation of the surround or the suppressive field in X cells but because the inhibition from the periphery is much weaker in Y cells, so the RF effect was also very small. Similarly inhibition produced by stimulation of the nondominant eye is reduced by RF stimulation (*Fukuda* and *Stone*, 1976; *Singer* and *Schmielau*, 1976).

G. Pontine Reticular Formation

Stimulation of the PRF produces changes in responsiveness of presynaptic and postsynaptic elements in the LGNd similar to those produced by MRF

stimulation (*Sakakura* and *Iwama*, 1965; *Nakai* and *Domino*, 1968; *Malcolm* et al., 1970a; *Cottee* et al., 1974). Stimulation of PRF in the unanesthetized squirrel monkey produced slight enhancement of the response in the OR to OT stimulation (*Rapisardi* et al., 1974).

H. Pathways

There are several reports describing direct connections from RF to LGNd: *Scheibel* and *Scheibel* (1958) used the Golgi technique in newborn mice to demonstrate a projection from the magnocellular PRF to the LGNd; *Bowsher* (1970, 1975) stained for anterograde preterminal degeneration in adult cats to show a projection from midbrain tegmentum. *Pin* et al. (1968) and *Maeda* et al. (1973) have described the projection system of the catecholaminergic (CA) neurons of the tegmentum of the cat: two ascending CA pathways originate in the locus coeruleus (LC) and NSC and possibly supply the LGNd. *Leger* et al. (1975) used the HRP technique to confirm the projection from LC and NSC although in LC these HRP-labeled neurons seem not to be CA-ergic. The HRP technique has also been used by *Gilbert* and *Kelly* (1975) to demonstrate a projection to LGNd from MRF. *McBride* and *Sutin* (1976) have used the ARG technique and also Fink-Heimer staining for degenerating fibers to reveal a sparse projection from the LC to the LGN. In the LGNd of the rat there is a medium-to-high density of noradrenaline (NA) terminals and a low-to-medium density of 5-HT terminals (*Fuxe,* 1965). Some of the fluorescence is, of course, associated with arteriolar innervation (*Sercombe* et al., 1975). However, these fibers do not seem to originate in the LC (*Pickel* et al., 1974) nor in the VC (*Stenevi* et al., 1972).

The ARG technique was used by *Edwards* and *de Olmos* (1976) to show that in the cat the nucleus cuneiformis, which is part of the MRF, projects to the ventral nucleus of the LGN but not to the LGNd. In the rat the n. cuneiformis appears to send a cholinergic pathway to the LGNd (*Shute* and *Lewis*, 1967). Both in rat (*Shute* and *Lewis*, 1963, 1967) and in cat (*Phillis* et al., 1967b) acetylcholinesterase is in the nerve terminals and not in the cells of the LGNd which is therefore cholinoceptive but not cholinergic.

I. Mechanism of the Facilitatory Effect

Singer and *Dräger* (1972) and *Singer* (1973a) found that MRF stimulation eliminated hyperpolarizing potentials in LGNd cells whether these were "spontaneous" or IPSPs evoked by stimulation of OT or OR or pho-

tically (Fig. 7a). It seems probable that this effect is brought about by inhibition of I cells (see Section II). *Fukuda* and *Iwama* (1970, 1971) discovered that the activity of I cells in the rat LGNd, both spontaneous and evoked, was reduced or abolished by MRF stimulation, while MRF effects on P cells were largely facilitatory (Fig. 7b); they therefore suggested that the facilitatory effects are brought about by disinhibition.

Rather different results were obtained by *Nakai* and *Takaori* (1974) in the cat. They found that stimulation of the LC had opposite effects on two kinds of interneurons, while facilitating relay cells. Type A inter-

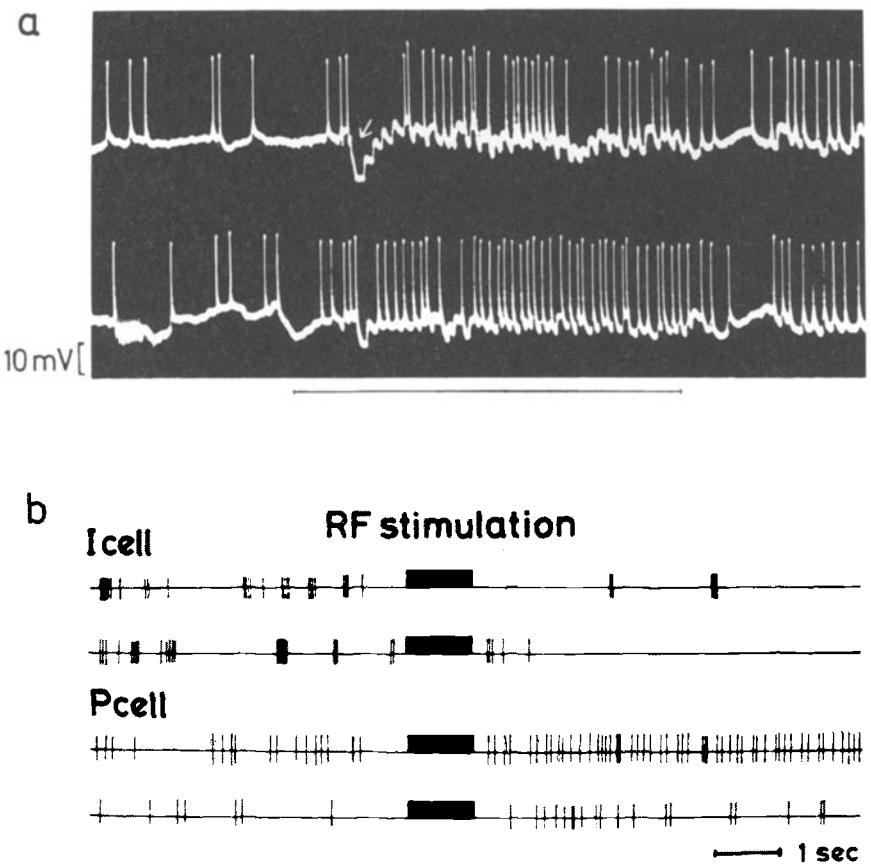

Fig. 7 a and b. Effect of RF stimulation on LGNd. (a) Response of an on-center LGNd cell in the cat to photic stimulation of the receptive field center and part of the surround for a duration indicated by the bar beneath the records (650 ms). Note hyperpolarization (upper trace) soon after commencement of stimulation (arrow) and TR falls to 0.66 during stimulus. In lower trace prior stimulation of MRF (stimulus artifacts near beginning of record) reduces phase of hyperpolarization and TR is now 0.9 during stimulus (from *Singer* and *Dräger*, 1972). (b) Effect of RF stimulation on two I cells (presumed to be inhibitory interneurons) and two P cells (presumed to be relay cells) in the LGNd of the rat. Note inhibition of I cell activity and facilitation of P cell activity (from *Fukuda* and *Iwama*, 1971)

neurons responded to stimulation both of OT and OR with three to five spikes and were therefore regarded as homologous with I cells in the rat; however, in contrast to the rat, these cells were facilitated by LC stimulation. Type B interneurons did not respond to stimulation of OR and gave a prolonged discharge of three to ten spikes; they were therefore regarded as interneurons possibly mediating forward inhibition as in the ventrobasal nucleus (*Andersen* et al., 1964). Type B interneurons were inhibited by LC stimulation. Although there may be a species difference between cat and rat, there are other possibilities. It may be that type A neurons are P cells. Certain P cells in the cat can discharge up to four spikes in a burst and when excited antidromically can also fire repetitively; these effects are best seen with threshold stimuli (*P.O. Bishop, W. Burke* and *R. Davis*, unpublished observations; see *Bishop*, 1960). The high proportion of "I cells" found in cat LGNd by *Ono* and *Noell* (1973) may also be explained on this basis. Type B interneurons may well be activated only from OT. However, there are neurons in cat LGNd that are activated orthodromically from OR, that seem to be the equivalent of I cells in the rat and that are inhibited by MRF stimulation (*Singer,* 1973a).

Can MRF stimulation directly depolarize P cells? *Singer* (1973a) found that most LGNd neurons with a TR of 1.0 did not increase their discharge rate with MRF stimulation. In a few neurons a slight depolarization was seen that could trigger one or more action potentials, but the rise and decay phases were much slower than those of specific EPSPs. He suggested that direct depolarization of P cells is much less important than disinhibition and that the responsible fibers are located peripherally on the cell. *Waring* (1974) has provided evidence that MRF-evoked facilitation in the rat may not be mediated wholly by disinhibition, in that facilitation is still obtainable in conditions when I cells apparently are silent. Figure 8a summarizes the disinhibition model.

J. Latency of Effects

Could the pathway from RF to LGNd be monosynaptic? A consideration of latencies is relevant. *Suzuki* and *Taira* (1961) reported facilitation and inhibition of individual LGNd cells occurring as early as 10 ms from the MRF stimulus. *Satinsky* (1968) measured the latency of effect in 86 LGNd cells; the shortest latency was 25 ms for both excitatory and inhibitory effects with a mode for excitation at 30 - 40 ms and for inhibition at 60 - 70 ms. *Fukuda* and *Iwama* (1971) in the rat found inhibition of I cells as early as 20 ms, inhibition of a few P cells also at 20 ms and facilitation at 40 ms.

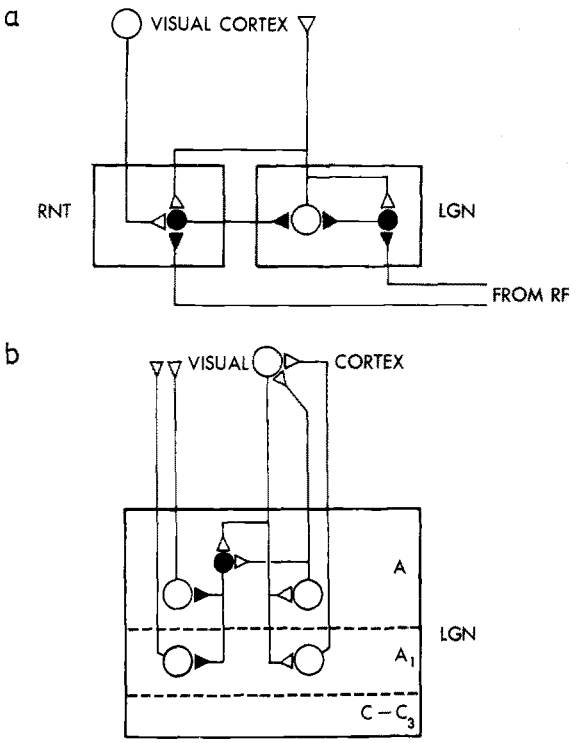

Fig. 8 a and b. Connections between LGNd and RF and between LGNd and VC. (a) Relay cells in the LGN (open circles) are inhibited by interneurons (filled circles) either in the LGN or extrinsic (RNT). RF acts by inhibition of these interneurons. (b) Corresponding relay cells in laminae A and A_1 of LGNd converge to a cell in the visual cortex; adequate simultaneous excitation of LGNd cells leads to positive feedback via VC cell and lateral inhibition via VC cell and interneuron. (Diagrams based largely on the work of *Iwama* and colleagues and *Singer* and colleagues; see text)

Tatton and *Crapper* (1972) reported a mode for excitation at 20 - 60 ms with the shortest latency at 7.5 ms; they did not give details for inhibitory latencies but these were said to be similar. Although *Singer* reported an early inhibition preceding the facilitatory effect with latency as brief as 2 - 3 ms, he attributed this to antidromic stimulation of bifurcating Y fibers entering both LGNd and SC (*Singer*, 1973b). *Singer* and *Bedworth* (1974) found the earliest facilitation at 30 - 50 ms in the *encéphale isolé* cat, whereas it was 60 - 150 ms in the anesthetized animal. *Foote* et al. (1974) obtained a minimum latency for inhibition at 7 ms and a mode less than 10 ms, while the minimum latency for excitation was 15 ms (mode 21 - 25 ms). They ruled out the possibility of stimulating fibers entering the SC. In squirrel monkeys and macaques, *Doty* et al. (1973) obtained inhibition commencing at 5 - 10 ms followed by facilitation.

If the facilitatory effect of RF stimulation is via disinhibition the shortest latencies will be for inhibitory effects on I cells and latencies for facilitation of P cells could be significantly longer. Unfortunately, with the exception of *Fukuda* and *Iwama* (1971), the studies cited above do not reliably distinguish between P and I cells. Nevertheless, in the cat there is clear evidence for certain cells being inhibited as early as or earlier than other cells are excited and at latencies of the order of 10 ms. If we suppose a conduction distance of 10 mm, conduction velocity for a direct pathway would be 1 m/s. This is a conservative estimate and some fibers could well have a higher conduction velocity. Taken in conjunction with the histologic evidence (see above), a direct connection remains a distinct possibility.

K. Raphe Nuclei

Closely related to the function of the RF is that of the midbrain raphe nuclei. There appears to be an interaction between raphe nuclei and PRF in the regulation of sleep and especially PGO waves (see Section IV). *Foote* et al. (1974) found that stimulation of n. raphe dorsalis produced excitation of certain LGNd cells and inhibition of others, the effect in any cell being identical to that produced by stimulation of the MRF.

The cells of the raphe nuclei are almost entirely serotonergic (5-HT) (*Dahlström* and *Fuxe*, 1964, in the rat; *Pin* et al., 1968). *Bobillier* et al. (1975, 1976) have shown, using ARG, that the raphe dorsalis sends a projection to the LGN, mainly to the ventral nucleus. The HRP technique was used by *Leger* et al. (1975) and *Gilbert* and *Kelly* (1975) to demonstrate a projection to the LGNd.

L. Reticular Nucleus of the Thalamus

As already described in Section II there is evidence that the cells of the RNT are responsible for inhibitory effects in the thalamus. The RNT receives an input from LC (*McBride* and *Sutin*, 1976) and from n. cuneiformis (*Edward* and *de Olmos*, 1976). *Szentágothai* (1972) has shown that the perigeniculate nucleus, which he regards as the part of the RNT related to the LGN, receives inputs from cerebral cortex and from RF and send fibers into the LGNd. He suggests that RF effects on the LGNd might be mediated through this nucleus.

IX. The LGNd and the Cerebral Cortex

The second major region of the brain with an influence on the LGNd is the cerebral cortex, especially the visual cortex. The anatomic relations between LGNd and VC are better understood than those between LGNd and RF but the reverse is true for the physiologic relationships.

A difficult problem in interpretation exists when considering the results of stimulation of the VC. Stimulation of this area usually produces inhibition of synaptic transmission in the LGNd (*Widén* and *Ajmone Marsan*, 1960; *Kwak*, 1965; *Suzuki* and *Kato*, 1965). However, in many cases this is due to antidromic activation of P cells leading to recurrent inhibition (see Section II). One method of activating corticofugal fibers without stimulating the P cells antidromically is to stimulate the cortex contralateral to the LGNd being studied. In this way both excitatory and inhibitory effects have been obtained in the cat (*Ajmone Marsan* and *Morillo*, 1961; *Kwak*, 1965) and the rat (*Sefton* and *Bruce*, 1971). These effects are probably mediated via the ipsilateral cortex (*Doty*, 1973) and are restricted to regions concerned with central vision.

Corticofugal fibers may also be activated by application of strychnine and this causes facilitation of the response in the LGNd to a flash of light (*Meschersky*, 1968). The influence of the VC on the ipsilateral LGNd may also be inferred from experiments in which the cortex is inactivated. From such an experiment, *Silakov* (1975) concluded that corticofugal fibers had two effects, an inhibitory action on relay cells and an inhibitory action on the mechanism of lateral inhibition.

Reversible inactivation of the cortex allows a single cell to be studied with and without cortical influence. This has been achieved by electric polarization of the cortex (*Vastola*, 1967), by KCl solution (*Meulders* and *Colle*, 1966) and by cooling (*Meulders* and *Colle*, 1966; *Kalil* and *Chase*, 1970; *Singer*, 1970; *Richard* et al., 1975; *Schmielau* and *Singer*, 1977; *Hull*, 1968 in monkey). *Vastola* (1967) and *Hull* (1968) found that their procedures in most cases reduced the LGNd response to OT stimulation implying that the cortex had a facilitatory effect on LGNd transmission. *Kalil* and *Chase* (1970) obtained mainly a decreased response to photic stimulation supporting this conclusion. On the other hand, *Meulders* and *Colle* (1966) found no change in the early response, only a reduction of the late slow wave to OT stimulation, and *Richard* et al. (1975) reported that cryogenic blockade of the primary visual cortex had no influence on LGNd responses.

The suggestion has been made that the corticogeniculate fibers are important in stereoscopic vision (*Pettigrew*, 1972). This idea is supported by recent work of *Schmielau* and *Singer* (1977) who report that the

corticothalamic cells are binocular. Using reversible cooling of areas 17 and 18, they also showed that the corticofugal fibers facilitated the responses of an LGNd cell when the centers of the receptive fields in both eyes were simultaneously excited, and inhibited the cell when the receptive field surround of the nondominant eye was excited; this inhibitory effect of the cortex is additional to the direct inhibitory effect of the nondominant eye (*Sanderson* et al., 1969, 1971). These findings suggest a way in which the contrast may be enhanced between objects on the fixation plane and those in the background or foreground. They also provide a coherent explanation for previous observations describing both facilitory and inhibitory corticofugal effects. Figure 8b depicts a model of LGNd-cortical interaction based on these findings.

Other cortical regions are also found to affect LGNd function. *Spinelli* and *Pribram* (1967) found that stimulation of the frontal lobe shortened the *off* discharge of *on-off* cells to a flash of light, while inferotemporal stimulation lengthened the *off* discharge. *Tsumoto* and *Suzuki* (1976) stimulated the frontal eye field in *encéphale isolé* cats and found a facilitatory effect on X units but no effect on Y units; units in the RNT were inhibited with the same time course as the LGNd X cell facilitation; units in the ventral nucleus of the LGN were also facilitated.

The special effect of OT terminal depolarization produced by cortical stimulation has been considered in Section VI.

A. Pathways

A direct pathway runs from visual cortex to LGNd in cat (*Beresford,* 1961, 1962; *Szentágothai* et al., 1966; *Guillery,* 1967; *Barron* and *Doolin,* 1968; *Garey* et al., 1968; *Jones* and *Powell,* 1969; *Niimi* et al., 1970a, 1971; *Holländer,* 1970, 1972a, b, 1974; *Kawamura* et al., 1974; *Gilbert* and *Kelly,* 1975; *Updyke,* 1975; *Winfield* et al., 1975), in rat (*Nauta* and *Bucher,* 1954; *Goodman* and *Horel,* 1966; *Montero* and *Guillery,* 1968; *Lund,* 1969; *Gosavi* and *Dubey,* 1972), in oppossum (*Martin,* 1968; *Benevento* and *Ebner,* 1970), in rabbit (*Giolli* and *Guthrie, 197l; Giolli* and *Pope,* 1973; *Ralston* and *Chow,* 1973; *Colwell,* 1975) and in primates (*Beresford,* 1962; *Black* and *Myers,* 1962; *Myers,* 1962; *Compos-Ortega,* 1968; *Wong-Riley,* 1972; *Casagrande,* 1974; *Holländer,* 1974; *Spatz* and *Erdmann,* 1974; *Holländer* and *Martinez-Millán,* 1975; *Lund* et al., 1975; *Benevento* and *Fallon,* 1975).

In certain primates, some authors could not find evidence for cortico geniculate fibers (*Campos-Ortega* et al., 1970; *Spatz* et al., 1970; *Abplanalp,* 1971; *Harting* and *Noback,* 1971; *Tigges* et al., 1973). However, the fibers, at least in the other species mentioned, are fine and dif-

ficult to stain and the negative reports in primates are probably less significant than the positive ones. In cases where degeneration techniques have failed, used of HRP or ARG has usually been successful (*Holländer* and *Martinez-Millán,* 1975; *Lund* et al., 1975; *Updyke,* 1975). Earlier work on efferent pathways from visual cortex to LGNd is summarized by *Shkol'nik-Yarros* (1971); *Freund* (1973) also lists the earlier references.

Simultaneous application of the HRP and ARG techniques by *Colwell* 1975) in the rabbit has shown that there is a very precise reciprocal topography of the geniculocortical and corticogeniculate fibers (see also *Karamanlidis* and *Giolli,* 1976). This confirms earlier suggestions of *Beresford* (1962) and *Niimi* et al. (1971) using degeneration techniques. *Updyke* (1975) has demonstrated by degeneration and ARG techniques in the cat that areas 17 and 18 project to all laminae of the LGNd whereas area 19 projects to laminae C_1 C_2 and C_3. In a yet more precise description, *Gilbert* and *Kelly* (1975) have shown that all the corticogeniculate fibers going to layers A and A_1 of the LGNd originate from cells in layer VI of the ipsilateral cortex (areas 17,18,19 and the posterior suprasylvian gyrus) and are mostly pyramidal cells; *Tömböl* et al. (1975) confirm this observation and, by comparison with Golgi material, show that all the three main efferent neurons of the VC are involved. In addition to the well-known projection of the LGNd to layer IV of the VC there is also a projection to layer VI in the cat (*Rosenquist* et al., 1974; *Gilbert* and *LeVay,* 1975; *Winfield* and *Powell,* 1976), in the rat (*Ribak* and *Peters,* 1975), and in the grey squirrel (*Robson* and *Hall,* 1975). LGNd fibers synapse both with stellate cells and with pyramidal cells, possibly more often with the latter (*Winfield* and *Powell,* 1976). In the macaque monkey also, fibers projecting to the LGNd originate in layer VI of area 17 and are small to medium-sized pyramidal cells; the upper half of layer VI projects to the parvocellular layers of the LGNd and the lower part to the magnocellular layers (*Lund* et al., 1975). Thus there is good evidence for a precise feedback loop between LGNd and layer VI of the VC.

Harvey (1976) found that out of 73 "simple" cells (*Hubel* and *Wiesel.* 1962) in the VC, nine could be activated antidromically from a site near the LGNd and had a mean latency of 9.2 ms, indicative of very fine axons. The corticogeniculate fibers run through all laminae of the LGNd in the cat and synapse mainly in the interstitial zones, not the encapsulated (glomerular) areas; they form terminals with round vesicles (RSD) and it is estimated that these constitute 45% of all terminals in the LGNd (*Guillery,* 1967, 1969a, b). They lie on the dendrites of both relay cells and interneurons in the cat (*Famiglietti* and *Peters,* 1972), but only on the relay cells in the rat (*Lieberman* and *Webster,* 1974). This species dif-

ference may have something to do with the small amount of binocular vision in the rat, since one postulated function of the corticofugal fibers is in the regulation of stereoscopic vision (see above).

Whereas areas 17 and 18 in the cat project to all laminae of the LGNd, area 19 projects specifically to layers C_1, C_2 and C_3 (*Updyke,* 1975). In this region also there appears to be a reciprocal relationship with the cortex because *Maciewicz* (1975) has demonstrated a projection from the C laminae to area 19. As already mentioned the C laminae receive an innervation from the SC. It is possible that extraretinal influences on the C laminae may be more important than they are on laminae A and A_1 but, as yet, there have been few physiological studies on this region (*Cleland* et al., 1975, 1976; *Wilson* and *Stone,* 1975; *Wilson* et al., 1976).

Corticofugal fibers also contribute terminals to the perigeniculate nucleus and these may be collaterals of the corticogeniculate fibers (*Kawamura* et al., 1974; *Updyke,* 1975; *Sanderson* and *Kaas,* 1974 in the mink). Of course, corticofugal fibers could influence the LGNd indirectly via perigeniculate nucleus, SC, RF or other regions.

X. The LGNd and Other Regions of the Brain

As a source of extraretinal inputs to the LGNd other regions of the brain seem to be much less important. However, both the anatomy and physiology of their relationship with the LGNd are as yet poorly understood.

A. Pretectum and Superior Colliculus

Stimulation of SC in cat and rat, by exciting retinal fibers supplying both SC and LGNd (Y fibers in cat, perhaps some W), causes an initial excitation that is then followed by inhibition due to activation of the inhibitory circuits (*Hayashi* et al. 1967; *Singer* and *Bedworth,* 1973; *Hoffmann,* 1973; *Sefton,* 1968, in the rat). This effect is so powerful that it probably masks other direct or indirect effects. These other effects were briefly examined by *Hayashi* et al. (1967) who stimulated the SC in the chronically enucleated cat (in which presumably the retinal fibers had degenerated). They reported a presynaptic and a postsynaptic response of small amplitude. In the squirrel monkey stimulation of the pretectum and the most rostral portion of the SC produced strong enhancement of the OR responses to OT stimulation; stimulation in the caudal region of the SC did not produce consistent effects (*Rapisardi* et al. 1974).

It is well known that the SC projects to the ventral nucleus of the LGN (*Altman* and *Carpenter,* 1961). More recently a projection to the C laminae of the LGNd has been demonstrated (*Niimi* et al., 1970b; *Graybiel* and *Nauta,* 1971), although *Leger* et al. (1975) did not confirm this using the HRP technique. *Robson* and *Hall* (1976) used several methods to demonstrate a topographic projection from the small cells in the outer half of the stratum griseum superficiale in the grey squirrel to lamina 3c of the LGNd. The homologous regions of SC and LGNd in the cat receive W afferents from the retina (*Hoffman,* 1973; *Cleland* et al., 1975, 1976 *Wilson* and *Stone,* 1975). Their cross-linkage by the pathway just described is in contrast to the absence of such a link for Y fibers.

B. Thalamic Nuclei

Okuda (1962) stimulated virtually all the thalamic nuclei in cats either unanesthetized or under light thiopental anesthesia. He found that certain areas had no influence on the LGNd response to OT stimulation. These were: all cortical relay nuclei in the thalamus and all association nuclei projecting to the association areas of the cortex. Effective nuclei were: centrum medianum (CM) and centralis lateralis (CL) (facilitatory, about 70% as effective as MRF); ventralis anterior (VA) was the only inhibitory area found, which *Long* (1959) had found to be only infrequently facilitatory and *Steriade* and *Demetrescu* (1960) ineffective. *Rapisardi* et al. (1974), using squirrel monkeys, found CM, CL, VA, parafascicular nucleus and association nuclei (lateralis dorsalis, medialis dorsalis and pulvinar) to be ineffective. *Skinner* and *Lindsley* (1971) obtained enhancement of transmission through LGNd to OT stimulation by cooling the inferior thalamic peduncle in cats and inferred that the nonspecific thalamocortical system was normally exerting a tonic inhibition at LGNd level. Their cryogenic probe came very close to the OT and a control is needed to show that this also was not blocked, since this could enhance the response in the LGNd (*Hansen* et al., 1967; *Suzuki,* 1967).

Sumitomo 1974) has shown that stimulation of CM in the rat caused excitation of I cells and depression of P cells. This result agrees with the conclusion of *Skinner* and *Lindsley* (1971) but not that of *Okuda* (1962). The excitability decrease in P cells commenced at about 20 ms latency but the excitability increase in I cells at about 5 ms, suggesting that the principal effect of CM on the LGNd was via the I cells.

The specific and association nuclei of the thalamus do not connect with the LGNd. Of the nonspecific nuclei, the CM is known to send fibers to the LGNd (*Totibadze* and *Moniava,* 1969).

C. Vestibular Nuclei

Electric stimulation of the vestibular nuclei (*Papaioannou,* 1969, 1972a; *Jeannerod* and *Putkonen,* 1970, 1971; *Jeannerod,* 1972) has produced increases (and occasional decreases) in the discharge of LGNd neurons. *Papaioannou* (1972a) found that 82% of LGNd neurons were significantly affected. The response of a neuron to stimulation of its receptive field center could be facilitated or inhibited and similarly for the surround. *Papaioannou* made two suggestions, not mutually exclusive, concerning the functional significance of these effects; 1) the level of sensory contrast might be regulated in this way, 2) the inhibitory effects might be responsible for a temporary suppression of vision during movements of the head, so minimizing the effects of retinal blur. Whereas electric stimulation of the brain is useful for determining pathways and mechanisms, physiologic stimulation (e. g., in this case, rotation) is more reliable for inferring function. A direct connection between vestibular nuclei and LGNd has not been detected (*Brodal* and *Pompeiano,* 1957; *Brodal* et al., 1962). It is generally assumed that the effects of stimulation are mediated via the RF.

D. Hypothalamus

Chi and *Flynn* (1968) reported that hypothalamic stimulation increased the response in LGNd to OT stimulation. This effect was not altered by lesions of the RF. *Chi* and *Flynn* suggested that the effect may have been due to stimulation of fibers of the supraoptic commissures which may send fibers to the LGNd (*Knook,* 1965) and which run within the ventral hypothalamus. The supraoptic commissures are implicated in PGO waves (see Section IV); *Laurent* et al. (1974) found that section of the commissures abolished one of the two peaks of the PGO_R wave. Nevertheless, a projection from hypothalamus to the LGNd has recently been demonstrated by *Sakai* et al. (1975) using the HRP method.

XI. Chemical Transmitters and a Model of Extraretinal Input to the LGNd

Electrophoretic application of chemicals has shown that NA has a weak depressant effect on LGNd cells (*Tebĕcis* and *Di Maria,* 1972) but 5-HT has a powerful depressant action affecting over 90% of LGNd cells including all identified relay cells (*Curtis* and *Davis,* 1962; *Phillis* et al., 1967a; *Tebĕcis* and *Di Maria,* 1972) while acetylcholine (ACh) is a strong

excitant of most LGNd neurons including all relay cells tested (*Tebēcis* and *Di Maria,* 1972). Dopamine depressed most LGNd cells, but was less effective than 5-HT (*Tebēcis* and *Di Maria,* 1972). However the histochemical evidence (*Fuxe,* 1965) suggests that dopaminergic fibers do not enter the LGNd, at least in the rat.

In order to explain the facilitatory action of brain stem or cortex it is necessary to have a transmitter that is either excitatory to P cells or inhibitory to I cells. As far as excitation of P cells in concerned, clearly this cannot be NA or 5-HT. Does ACh have a role as a P cell excitant? Synaptic transmission in the LGNd is facilitated by cholinomimetic drugs (*Curtis* and *Davis,* 1963; *David* et al., 1963; *Satinsky,* 1967; *Mészáros,* 1971). *Phillis* et al. (1967b) found that the facilitatory effect of MRF stimulation on the response of cholinoceptive LGNd cells was blocked by benzoquinonium, an ACh antagonist. If there is a direct excitation of P cells it may be via cholinergic fibers from nucleus cuneiformis forming RSD terminals. However, a stronger possibility is that the cholinergic innervation is an inhibitory one to I cells. In the anterior RNT adjacent to the ventrobasal complex almost all cells are inhibited by ACh (*Ben-Ari* et al., 1976). This parallels the inhibition of units in this region by MRF stimulation (*Yingling* and *Skinner,* 1975; *Dingledine* and *Kelly,* 1977; see also *Keene,* 1975, in the rat). Hence it is possible that I cells either intrinsic to the LGNd or extrinsic (perhaps in the perigeniculate nucleus) receive an inhibitory cholinergic innervation, and if so presumably via F_1 terminals. It is also possible that the P cells receive an excitatory cholinergic input from the VC. Possible transmitters released by inhibitory interneurons are 5-HT and GABA.

There is a considerable literature concerning the various chemicals that affect PGO waves (see *Jouvet,* 1969, 1972). However, it is probable that these substances produce their effects outside the LGNd and hence they will not be discussed here.

Figure 9 is a diagram summarizing our present knowledge of LGNd structure and connections. It makes use of the terminology of *Guillery* (1969a) and *Burke* and *Sefton* (1966a) and for anatomic connections relies on the work of several authors (cat – *Peters* and *Palay* (1966), *Szentágothai* et al. (1966), *Guillery* (1969), *Famiglietti* and *Peters* (1972); monkey – *Colonnier* and *Guillery* (1964), *Pecci-Saavedra* and *Vaccarezza* (1968), *LeVay* (1971), *Hámori* et al. (1974); rat – *Lieberman* (1973), *Lieberman* and *Webster* (1974)). Certain connections are inferred mainly from physiologic studies (interaction of P and I cells) and in these cases the nature of the synaptic boutons is not known. The origin of the F_1 terminals on P cells is not known nor is it certain that all RSD terminals are from corticofugal fibers.

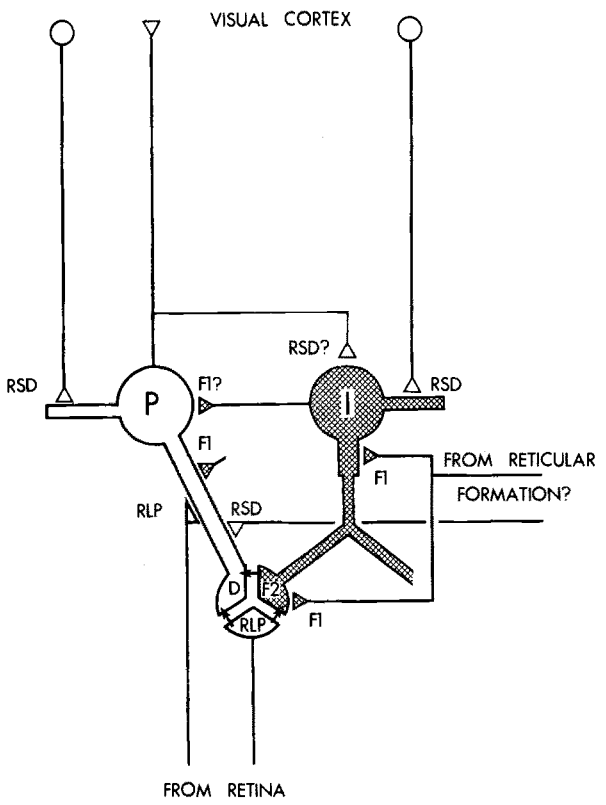

Fig. 9. Diagram of LGNd illustrating interneuronal connections and connections from visual cortex and reticular formation. RSD and RLP terminals contain round vesicles, F_1 and F_2 terminals contain flat (pleomorphic) vesicles presumed to be inhibitory. Axons shown as single lines, dendrites (D) as broad elements, presynaptic dendrites as narrow elements, except that their synaptic contacts are enlarged (F_2). The essential element in the glomerulus is the "triad" (bottom center) in which the optic nerve terminal is presynaptic to both relay cell dendrite and presynaptic dendrite; the latter is also presynaptic to the relay cell dendrite

A model of the extraretinal inputs to the LGNd that will account for most of the observations is as follows. Many parts of the nervous system influencing the LGNd do so via the RF. The facilitatory effect of the RF on the LGNd occurs via ascending fibers that form inhibitory synapses on I-cell dendrites (including presynaptic dendrites) in the LGNd; these may be the F_1 terminals (see Fig. 9). Assuming there is a tonic discharge in I cells, the disinhibition produced by RF activation would lead to facilitation of transmission from OT to OR, increased discharge of P cells on release from inhibition, increased release of potassium as a result of this activity, and increased extracellular potassium, leading to a depolarization of the OT terminals and increased excitability of these terminals

(evidenced by an increased antidromic OT response). However, both PGO wave and EMP commence before the increase in P cell discharge and indeed this discharge reaches its highest rate on the falling phase of both waves (*Bizzi,* 1966b; *Jeannerod,* 1972 – see Fig. 5). Hence, assuming that both waves are due to an elevated extracellular potassium, another source of potassium must be sought. Hyperpolarization of the I cells in the glomerulus (Fig. 9) may depend on an increased potassium permeability leading to a potassium efflux which if it had a prolonged time course could significantly increase extracellular potassium and account for the early part of both waves and possibly for most of the wave.

An alternative model is that the RF effect is via the RNT (Fig. 8a). In this model the RNT exerts an inhibitory effect on P cells in the LGNd presumably via F_1 terminals. RF facilitation would occur by inhibition of the RNT cells. However, it is difficult to account for the PGO wave or EMP on this model, since there is no obvious source of increased extracellular potassium in advance of the increase in P cell firing. Therefore, as far as eye movements are concerned, it is suggested that the former model is better.

A second reason for preferring the first model for eye movements is as follows. It has been proposed on anatomic grounds that only the X cells contribute dendrites to the glomeruli (*Wilson* et al., 1976). Since Y cells are strongly excited during eye movements in a patterned environment and since they are powerful inhibitors of X cells, the most probable route for this inhibition is via the intrinsic interneuron and its presynaptic dendrite. If this is so, RF disinhibition associated with eye movements is probably also via the intrinsic interneuron.

In so far as the RF effect is concerned with arousal, it is likely to be unspecific and could be exerted through both types of interneuron (Fig. 8a). This may be contrasted with the facilitation and lateral inhibition produced by the VC (Fig. 8b). The corticogeniculate projection in the cat is topographically organized and is probably concerned with binocular vision, hence its connections are likely to be more specific and, as far as inhibition is concerned, be mediated via intrinsic interneurons. The corticogeniculate projection in animals with little binocular vision, such as the rat, may be less specific and may supply extrinsic interneurons (Fig. 8a).

Tsumoto and *Suzuki* (1976) found that frontal eye field stimulation facilitated X cells and cells in the ventral nucleus of the LGN but not Y cells, and inhibited RNT cells and intrinsic interneurons along a similar time course. These results imply more specific connections than any considered so far. However, the *encéphale isolé* cats in these experiments were awake and it might be that the Y cells were already fully facilitated (cf. *Eisman* et al., 1967).

XII. The Function of the LGNd and the Role of Nonvisual Influences

There are several theories, not mutually exclusive, of the function of the LGNd. *Singer* and *Wässle* (1971) and *Singer* and *Bedworth* (1974) argue that the inhibition produced by patterns and movement serves to sharpen the tuning curves of LGNd relay cells and is important for simultaneous and successive contrast detection. *Levick* et al. (1969), with particular reference to the rabbit, provided evidence that an LGNd neuron signalling movement direction does so more precisely than its retinal counterpart. These suggestions depend only on intrinsic circuits in the LGNd and not at all on extraretinal influences.

It seems probable that a major function of the LGNd is the filtering of visual input to the visual cortex according to the state of attention, a tonic control being exercised from the RF.

With regard to selective attention, the evidence at present is conflicting. *Kiyono* (1971) found a decrease in transmission in the LGNd in a freely behaving cat during a visually significant stimulus (appearance of a rat) whereas there was no change during an auditorily significant stimulus (a dog's bark). On the other hand, *Horn* and *Wiesenfeld* (1974) concluded that synaptic transmission in the LGNd did not change according to the sensory modality in which the cat was attending (visual or auditory) although there were changes at the cortical level. Eye movements were not monitored in either of these experiments. Eye movements in an alert animal in the light are associated with inhibitory effects in the LGNd, as already discussed, and, if they were present in *Kiyono's* experiments during the visual attention trials, could have accounted for his results.

The essential feature of the reticular influence on the LGNd is disinhibition. *Singer* (1975) and *Singer* and *Schmielau* (1976) have pointed out that all known inhibitory interactions in the LGNd are suppressed by reticular stimulation – inhibition between neurons with the same center characteristics (*on*- or *off*-center), reciprocal inhibition between cells with antagonistic field characteristics (*on*-versus *off*-center and vice versa), inhibition of X cells by Y cells, and binocular inhibition. As a result of reticular activation, there is a loss of surround inhibition, thresholds for on- and off-excitation are reduced and the characteristics of the LGNd in transmitting visual information (simultaneous and successive contrasts) are radically altered. In SW sleep almost no information may reach the visual cortex (*Maffei* et al., 1965). In nonattentiveness the information is rather seriously distorted.

A phasic influence of the RF is specifically concerned with eye movements. During FW sleep there occurs in close temporal relationship a rapid eye movement, a PGO wave, facilitation of synaptic transmission in the LGNd and a burst of firing in LGNd neurons (even in the dark).

This combination of events can be closely mimicked by stimulation of the RF. In the awake animal the combination is less rigid. An EMP always accompanies a saccade, but the burst of firing in LGNd neurons and the facilitation of synaptic transmission in the LGNd are probably absent in the fully alert animal. This observation is not unexpected if the process of alerting involves inhibition of I cells in the LGNd. A phasic hyperpolarization of already silent I cells would not cause firing of P cells nor any further facilitation of synaptic transmission although it could increase extracellular potassium and produce an EMP (see Section XI). It is unlikely that these changes represent a "corollary discharge." *Singer* and *Bedworth* (1974) have suggested that brief disinhibition at the end of a saccade would clear the LGNd from inhibitory gradients generated by the pattern viewed before the eye movement and by moving contrasts during the saccade and this could be necessary for the correct processing of sequentially viewed patterns. This mechanism might well be important in the not fully alert animal. Facilitation following a saccade may also depend on an influence from the frontal eye field, independent of the RF (*Tsumoto* and *Suzuki*, 1976).

Head and body movements produce significant effects on LGNd function. These effects are mediated via the vestibular nuclei and probably via the RF. Usually increases in LGNd discharge have been observed but decreases occur in certain neurons. Most changes are independent of velocity or direction of rotation (*Magnin* et al., 1974) suggesting a nonspecific effect. As in the case of eye movements, these changes are probably not compensatory for the motion but are simply an improvement in transmission through the LGNd at a time the retinal image may be changig and causing inhibitory effects in the LGNd. A probable function of the corticogeniculate fibers is to improve stereoscopic vision by facilitating synapses connected to corresponding points on the retinae when these are simultaneously excited and by lateral inhibition of less strongly excited synapses. However, the presence of corticofugal fibers in species without stereoscopic vision suggests that there must be another function.

In summary, the input from RF to LGNd provides a tonic level of disinhibition which is low in the sleeping animal, so that visual input to the cortex is effectively blocked, and in the alert animal is not so high as to prevent the operation of the various inhibitory circuits that presumably accentuate contrast. A second function of the input is to provide a phasic disinhibition at the end of a saccade to "wipe the slate clean"; this function may also be performed by the frontal eye field. Vestibular stimulation probably acts via the RF with a similar result. The corticogeniculate input may serve stereoscopic vision but is not solely concerned with this.

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