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Dr. Maneli Mozaffarieh
Prof. Josef Flammer

University Hospital Basel
Department of Ophthalmology
Mittlere Strasse 91
4031 Basel
Switzerland
mmozaffarieh@uhbs.ch
jflammer@uhbs.ch

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Author biographies

Maneli Mozaffarieh was born in Tehran, Iran. In 1979, she moved with her family to Hamburg, Germany, where she attended the Hamburg International School. A year later, she continued her education at the International School of Vienna, Austria, and graduated with an International Baccalaureate Diploma in 1990. Following this, she moved to Montreal, Canada, and in 1994 graduated from McGill University with a Bachelors degree in Microbiology and Immunology. That same year, she began studying medicine at the University of Vienna, Austria, from which she graduated successfully in 1999.

Following this, Dr Mozaffarieh began her research at the University Hospital of Vienna publishing several articles on the topic of vitreoretinal surgery. Throughout this time she also worked as a general practitioner at the SMZ Hospital in Vienna, Austria. In 2005, Dr Mozaffarieh had the great fortune to meet Josef Flammer who was to become her mentor, and with whom she has been working ever since.

Josef Flammer, born in 1948, studied medicine in Fribourg and Bern, Switzerland. He wrote his MD thesis in the Department of Neurobiology in the University of Berne on ultrastructure of the sympathetic nervous system. During his residency in neurology he focused on multiple sclerosis, and during his residency in internal medicine he focused on cardiovascular diseases. During his specialization in ophthalmology at the University of Bern, chaired by Peter Niesel, he focused on the development of automated perimetry in the research group of Franz Fankhauser. These studies were continued during a fellowship with Stephen Drance in Vancouver, leading to a PhD thesis on the fluctuation of differential light sensitivity. As chief resident, back in Bern, he became interested in the role of microcirculation in the pathogenesis of eye diseases, in particular glaucoma.

Professor Flammer has held the Chair of the Department of Ophthalmology at the University of Basel since 1987 and has also been Dean of the Faculty of Medicine. In Basel, he continued his research in the fields of automated perimetry, pharmacology, molecular biology and, together with Selim Orgül, microcirculation. He focused on a range of diseases that included multiple

sclerosis, poliarthritis, giant cell arthritis, polymyalgia, Leber's disease, retinal arterial and venous occlusions, and, particularly, glaucoma.

Milestones in his scientific activities have been: the development of visual field indices and the Bebie-curve (together with Hans Bebie); design of the Octopus program G1; recognition of the role of nitric oxide and endothelin in ocular blood flow (together with Ivan Häfliger and Peter Meyer) and the link between vasospasm and glaucoma and other eye diseases; integration of vasospasm in a general primary vascular dysregulation syndrome; description of the clinical correlates of glial cell activation; interpretation of optic disc hemorrhages as part of the dysfunction of the blood retinal barrier; recognition of the role of reperfusion and oxidative stress in the pathogenesis of glaucomatous damage; and altered gene expression of lymphocytes in glaucoma patients (together with Olga Golubnitschaja).

Professor Flammer has published over 500 scientific articles, many of which have been translated into other languages such as Spanish and Russian, has edited nine books, and has written a monograph on glaucoma which has been translated into 20 languages and published in several editions.

He has received a number of national and international awards, including the Chibret, Alcon, Montgomery, William MacKenzie and Alfred–Vogt awards; has received medals such as those from Saudi Arabia, Helsinki and Sicily; has presented many invited lectures, such as the Krushner Lecture in London; has been the guest of many glaucoma societies, including societies in Japan, Brazil, Argentina, England and South Africa; is honorary member of the Czech Glaucoma Society; and has been honorary guest of the meeting of Nobel Prize laureates.

Preface by Maneli Mozaffarieh

For the clinician and researcher, finding a mentor is probably the most crucial and least understood aspect of having a successful career. Unfortunately, in today's self-conscious society it has become even more difficult to find that senior academic and clinician who conducts lectures, carries out advanced research and takes time to teach and train younger academics. This book reflects the depth of care and interest given when teaching a student. It is a summary of the concepts behind glaucoma taught by someone who has devoted a lifetime to carrying out investigations in this field. I have been particularly fortunate to be able to work closely with, and learn from, such a person.

The story of my mentor's work dates back to the end of the 1970s when Josef Flammer first met Goldmann and Fankhauser's research team at the University of Bern with whom he began his investigations. At the beginning of the 1980s he came up with a hypothesis that patients with a general vascular dysregulation (at that time termed vasospastic syndrome), were also likely to have dysregulation in their ocular blood flow and that the resulting fluctuation in oxygen supply may lead to glaucomatous damage. In 1987, Josef Flammer become chairman of the Department of Ophthalmology at the University of Basel. Within his first few years as chairman he promoted and further developed research in glaucoma which eventually, together with other researchers, proved his theories on the role of ocular blood flow in glaucoma, as well as other eye diseases, to be true.

As Professor Flammer's research assistant, I have had the opportunity to discover certain principles about the role of ocular blood flow in glaucoma that simply cannot be learnt from any text book or doctor. My aim is not to reiterate what has already been summarized in other text books, but rather to give the reader a new perspective on the concepts behind glaucoma, namely Flammer's concept on the role of ocular blood flow in glaucoma. I have summarized knowledge gained from my mentor's lectures, books, publications and from observations during my clinical work with him. The chapters in this pocket book include areas that may be overlooked in the management of glaucoma. I hope that the simple format of this book along with the illustrations will encourage the reader to think about glaucoma from a new perspective.

This book is dedicated to my family: Parviz, Mimi, Nazak and Romtin.

Preface by Josef Flammer

In the last decade I have had the privilege to work in the field of glaucoma and ocular blood flow both clinically and scientifically. During this time I have taught many students and fellows, and among these students, Dr Maneli Mozaffarieh showed particular enthusiasm and a distinct aptitude for learning quickly.

Dr Mozaffarieh spared no effort in summarizing the different clinical and scientific aspects of glaucoma, and explaining these new theories in this easily readable pocket book. I am convinced that this book will help many ophthalmologists to understand glaucoma better, and that the reading of this book will not only improve patient care but also give the reader intellectual satisfaction.

Acknowledgements

The illustrations in this book are based on lectures given by Josef Flammer and were all prepared by Daniela Hauenstein. Without talented artists, this book would not have been possible, and we would like to thank Daniela Hauenstein for her support. We would also like to thank Dr Konstantin Gugleta and Dr Asan Kochkorov for proofreading the manuscript.

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Abbreviations

AIDS	acquired immune deficiency syndrome
AION	anterior ischemic optic neuropathy
AMPA	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMP	adenosine monophosphate
ATP	adenosine triphosphate
BBB	blood–brain barrier
BP	blood pressure
C	catechin
CCBs	calcium channel blockers
C/D	cup to disc ratio
cGMP	cyclic guanine monophosphate
CNS	central nervous system
CRA	central retinal artery
CSC	central serous chorioretinopathy
DH	disc hemorrhage
DNA	deoxyribonucleic acid
DLS	differential light sensitivity
EC	epicatechin
EDRF	endothelium derived relaxing factor
EDVF	endothelium derived vasoactive factor
EGC	epigallocatechin
EGFR	epidermal growth factor receptor
eNOS	endothelial nitric oxide synthase
ET	endothelin
ET-1	endothelin-1
ETA	endothelin receptor A
ETB	endothelin receptor B
ETC	electron transport chain
FLA	fluorescence angiography
GON	glaucomatous optic neuropathy
GSH	glutathione
GSSG	oxidized glutathione
HRT	Heidelberg Retinal Tomograph
HSP	heat shock protein
HTG	high tension glaucoma
IOP	intraocular pressure
LCA	lateral ciliary artery

LDF	laser Doppler flowmetry
LDV	laser Doppler velocimetry
MCA	medial ciliary artery
MCP	3-methyl-1,2-cyclopentanedione
MDA	malonyldialdehyde
MDR	multidrug resistance
MMP-9	matrix metalloproteinase-9
MRI	magnetic resonance imaging
MRP	multidrug resistance protein
MS	multiple sclerosis
NADP	nicotinamide adenine dinucleotide phosphate
NADPH	reduced nicotinamide adenine dinucleotide phosphate
NMDA	*N*-methyl-D-aspartate
NO	nitric oxide
NOS	nitric oxide synthase
NOS1	nitric oxide synthase 1
NOS2	nitric oxide synthase 2
NOS3	nitric oxide synthase 3
NTG	normal tension glaucoma
OA	ophthalmic artery
OAG	open-angle glaucoma
OBF	ocular blood flow
OCT	optical coherence tomography
OH^-	hydroxyl ion
OHT	ocular hypertension
ONH	optic nerve head
$ONOO^-$	peroxynitrite
OPA	ocular pulse amplitude
PGI_2	prostacyclin
POAG	primary open-angle glaucoma
PP	perfusion pressure
PVD	primary vascular dysregulation
ROS	reactive oxygen species
RVO	retinal vein occlusion
SVD	secondary vascular dysregulation
TF	theaflavins
TG	thearubigins
t-GSH	total glutathione
TM	trabecular meshwork

TNF	tumor necrosis factor
TNF-α	tumor necrosis factor alpha
TNF-R1	tumor necrosis factor receptor 1
VEC	vascular endothelial cell
VEGF	vascular endothelial growth factor
VSMC	vascular smooth muscle cell

Chapter 1

Introduction

For more than a century it has been suspected that disturbed ocular blood flow (OBF) may play a role in the pathogenesis of glaucoma. Reduced OBF has been measured in glaucoma patients by numerous medical centers using a variety of techniques in different tissues of the eye. A decrease in OBF has been proven to be of prognostic value, however the implementation of this knowledge into clinical practice has been very slow. There are still some physicians who do not diagnose the vascular problems of their patients and who do not consider them when treating patients. There are a number of reasons for this.

As human beings we tend to prefer simple relationships, for example one factor causes one type of damage. If this single factor can be easily measured and influenced, as in the case of intraocular pressure (IOP) it is even more accepted. However, nature is not so simple. Even when considering the regulation survival of a single cell we realize that a complex network of information is involved. Any event in biology is regulated by the interplay of many different factors. Accordingly, the question is not whether glaucomatous damage is due to ocular pressure or vascular factors but rather a question of how these, and other factors, interact together in an individual patient.

Let us explain this using an analogy, with an analysis of the causes of car accidents. There is little doubt that driving speed is a proven risk factor for car accidents and that reducing driving speed can significantly reduce the likelihood of a car accident. Nevertheless, there is general agreement that other factors like driving performance, the condition of the road and the vehicle also play a role. These factors are not independent risk factors; for example, a given driving speed may be safe on one road but dangerous on another. Likewise, a given IOP may be well tolerated by one patient with excellent OBF, but may be too high for another patient with compromised regulation of ocular perfusion. It is important, therefore, not to look at individual factors in isolation.

Therapy also needs to be adapted to the individual situation. A car driver, for example, would need to reduce their speed on a hazardous road in order to reduce the risk of an accident. However, reducing the speed limit alone would not be sufficient to prevent a car accident. Society would also advocate the need to improve both the condition of the road and the car. Likewise, we cannot treat all patients with glaucoma simply by reducing IOP.

While we know that OBF is reduced in glaucoma there is still some discussion as to why this is the case. Some experts focus on perfusion pressure (PP) as the reason. While there is little doubt that low PP is a risk factor for low OBF and also, statistically speaking, a risk factor for glaucomatous optic neuropathy (GON), OBF is not simply a function of PP. If this were the case, patients with systemic hypertension would have a better OBF than healthy subjects, and treatment of systemic hypertension would reduce OBF; however this is, normally, not the case. Like the perfusion of any organ, OBF is the result of the relationship between PP and local resistance to blood flow. This explains why not all patients with high IOP or low blood pressure (BP) have low OBF. When treating systemic hypertension it is important to also remember that most drugs reduce BP by reducing peripheral resistance. Therefore, on average, OBF will improve even when the BP of patients with systemic hypertension is reduced.

The situation is very different when BP is low in a patient with primary vascular dysregulation (PVD). Such subjects not only tend to have low PP but very often have a defective autoregulation and may therefore insufficiently adapt to low PP. The BP of patients with PVD tends to be low due to reduced sodium reabsorption in the proximal tubules of the kidney.

Patients with PVD often suffer from migraine. A statistical association between GON and migraine might indicate that a migraine attack may damage the optic nerve head (ONH). However, such a relationship may also result from the fact that patients with migraine frequently have PVD which (through the altered regulation of OBF) is a risk factor for GON. These examples indicate that a statistical relationship does not necessarily imply a causal relationship.

Some experts believe that mechanical factors (distinguishing between vascular and biochemical) are the reason why OBF is reduced in glaucoma. Again, as with PP there are several strong interactions to consider. For example, stretching a cell will change the biochemistry in and around the cell. These chemical changes, including a change in oxygen supply, alter the mechanical properties of the cell, and the extracellular matrix.

Another cause for the slow implementation of measuring OBF in clinical practice is the issue of methodology. It is far more challenging to calculate OBF than it is to simply measure IOP. Nevertheless, it is possible to do so and

methods are continually improving. While such measurements are crucial for research and can be carried out in a medical center, a great deal of information can be obained from the patient's history.

Why then is OBF so relevant in the role of glaucoma? There are a number of conditions which reduce OBF without being major risks factor for GON. Some patients with severe arteriosclerosis or with reduced OBF due to high levels of endothelin (eg, autoimmune diseases) often have a normal or slightly pale, but not excavated, ONH. We also know that biological tissue can adapt to relatively low levels of oxygen and a constant reduction of OBF within a certain limit can be tolerated by the eye. If OBF is markedly reduced an infarction will result. If, however, the oxygen supply is unstable (eg, due to a fluctuation in PP, especially when autoregulation is defective) oxidative stress will occur. This, in turn, contributes to the activation of glial cells and damages the mitochondria of the axons, especially in the ONH. In other words, a constant reduction of OBF (eg, as a result of atherosclerosis) is a risk factor for an infarction; unstable OBF, however, is a risk factor for GON.

Although an unstable perfusion may occur often (and early in life), fortunately, only a small percentage of these subjects will develop GON and usually only later in life. Nature has developed a number of mechanisms to prevent disease and this is also true for glaucoma. If an unstable oxygen supply leads to an increase in free radicals, the human body has a number of ways to eliminate them. If the production of reactive oxygen species (ROS), however, is bigger than the coping capacity of the cell the resulting oxidative stress damages macromolecules and cell structures. Mechanisms also exist to either repair or eliminate and replace damaged molecules. If, however, this capacity is also exhausted the damage accumulates over time resulting in a disease that becomes detectable.

In order to understand these mechanisms, it is essential to have some understanding of OBF measurements, its regulation and dysregulation. It is also important to further our understanding of the beneficial yet potentially harmful effects of oxygen.

The goal of this pocket book is not to provide a scientific discussion on the role of ocular blood flow and glaucoma, and its therapeutic consequences. This would take too much space in the book and time from the reader. We have included aspects that are scientifically reasonable but have yet to be proven by large rigorous scale studies. In our experience, science develops in three stages. If a brand new theory is introduced, some experts state that it is not true because it is not proven. If, with time, more and more observations support the new theory the same experts state that the theory might be true but of no relevance.

If, finally, the relevance becomes obvious, the same experts state that the theory is correct but far from new!

This book is written for clinicians who would like to learn from the scientific and clinical experience of others. For the scientific debate we refer to the original publications, in the further reading section, at the end of the book.

Chapter 2

Basic sciences relevant to glaucoma

In order to understand the role of ocular blood flow (OBF) in glaucoma better, it is worth going over some of the basics.

One of the main molecules to play a role in the destructive processes of the body is, paradoxically, the oxygen molecule. This molecule can be induced to form deleterious types of oxygen molecule or reactive oxygen species (ROS) when provided with an additional electron or energy. In the context of ophthalmology, light also has a paradoxal role; it is on one hand a prerequisite for our sight, and on the other hand it is a risk factor for molecular damage. Before we probe further into the field of glaucoma, it is worth briefly outlining:

- the basic chemistry of the oxygen molecule
- the physical properties of light, and
- the concept of cellular stress.

What is a redox reaction?

Figure 2.1a shows a reduction/oxidation (or redox) reaction. The term oxidation refers to the loss of electrons and reduction refers to the gain of electrons. Pro-oxidants are molecules which can oxidize other molecules (ie, they are molecules with an oxidizing potential that is stronger than the oxidizing potential of the molecule they react with). Pro-oxidants can be in the form of free radicals as in the case of superoxide, $O_2^{\cdot -}$ (Figure 2.1b) or non-radical species (eg, hydrogen peroxide H_2O_2, or ozone O_3). Free radicals are molecules that contain one or more unpaired electrons. Although the chemical reactivity of free radicals varies, the need of the molecule to pair-up the unpaired electron can make free radicals highly reactive.

The eye is a unique organ and although biologically part of the brain it is directly exposed to the environment. In addition, exposure to light is necessary for it to function. The concentration of oxygen is high, both in

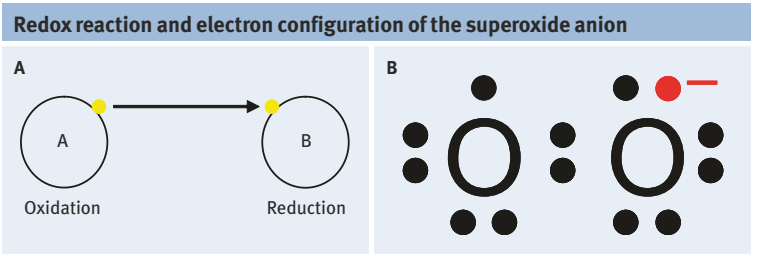

Figure 2.1 Redox reaction and electron configuration of the superoxide anion. **A,** redox reaction. Oxidation describes the loss of electrons by a molecule, atom or ion. Reduction describes the gain of electrons by a molecule, atom or ion. **B,** electron configuration of the superoxide anion. The unpaired electron is shown in the upper left and the additional electron conferring a negative charge is shown in red.

the anterior part of the eye which is exposed to the environment, and in the choroids with its dense network of blood vessels. Energy metabolism involving oxygen can generate a potentially damaging, ROS (ie, pro-oxidants) as illustrated in Figure 2.2. Under optimal conditions the formation of ROS is balanced by the rate of oxidant elimination by available the antioxidants. An antioxidant is by definition any substance, that when present at a lower concentration than an oxidizable substrate, significantly delays or prevents oxidation of that substrate. In very simple words, an antioxidant can neutralize pro-oxidants. However, even in healthy subjects ROS may cause some macromolecular damage. An imbalance between pro-oxidants and antioxidants, in favour of the former, results in oxidative stress which damages molecules and leads to up-regulation of the antioxidative system. This will be discussed in more detail in Chapter 3.

What is the role of light?

Light comprises a very small section of the broad electromagnetic field spectrum that can be perceived by our eyes and consequently interpreted by our brain as light. For example, we can see a star because it emits light which eventually finds its way into our eyes. A book placed on a table is visible because it absorbs, scatters, and also reflects the light differently than the table. While the effect of light can easily be recognized it remains difficult to understand the nature of light. From a physical point of view, light can be described either as a small corpuscle (photon) or as an electromagnetic wave (Figure 2.3). Light is the basis for vision and also provides energy, through photosynthesis, to plants. Unfortunately, light can also have a detrimental effect. The following sections discuss how energy from light can transform

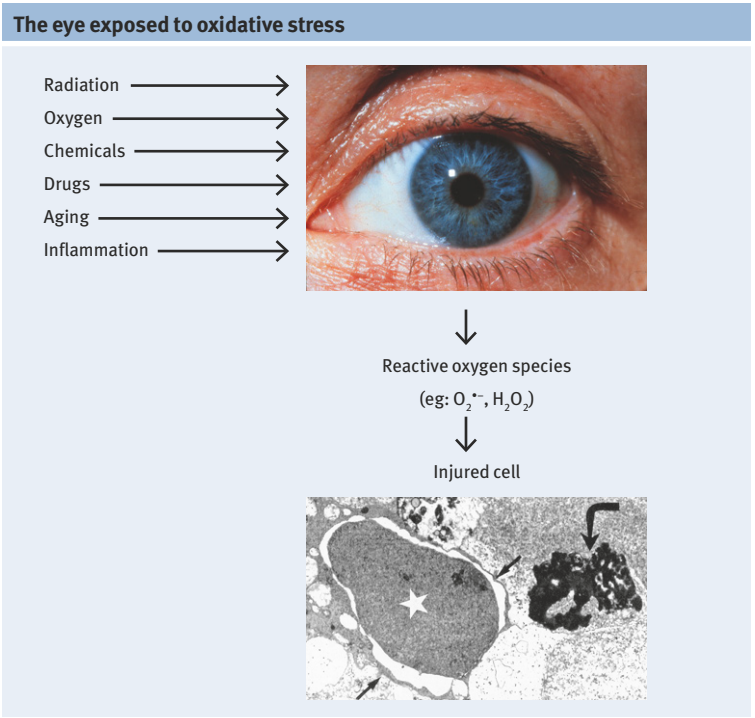

Figure 2.2 The eye exposed to oxidative stress. The eye is an organ that is predisposed to great levels of oxidative stress. The eye is constantly exposed to factors which induce the formation of reactive oxygen species that can ultimately damage cells.

relatively inert molecules or atoms into damaging ROS. The specific role of free radicals in the pathogenesis of glaucomatous optic neuropathy (GON) will also be discussed.

The oxygen molecule

The oxygen molecule in the earth's atmosphere

The oxygen in the earth's atmosphere increased as it was released from water by cyanobacteria (blue-green algae) more than a billion years ago. Cyanobacteria are photosynthetic and aquatic. These bacteria used light as an energy source for their metabolism (ie, photosynthesis) and in order to gain protons (eg, for the synthesis of carbon-hydrates) they split water molecules, and thereby released tonnes of oxygen into the atmosphere (Figure 2.4).

When living organisms first appeared on earth, they did so under an atmosphere containing very little oxygen (ie, they were anaerobes). Anaerobic

Light: an electromagnetic wave

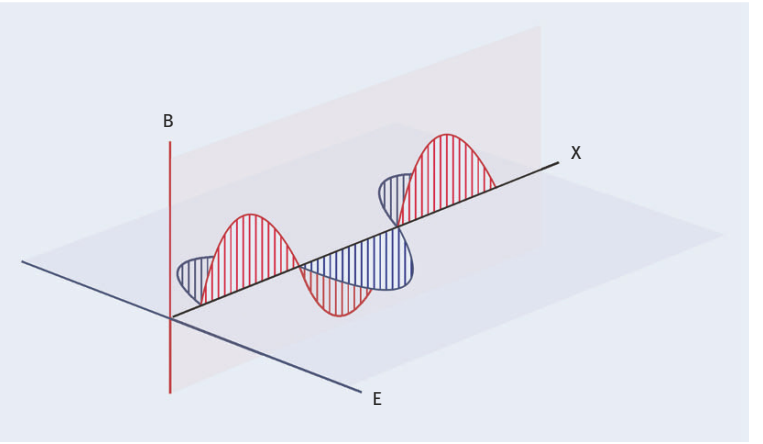

Figure 2.3 Light: an electromagnetic wave. Electromagnetic waves are formed when an electric field (shown as blue) couples with a magnetic field (shown as red).

Photosynthesis and respiration create a balance

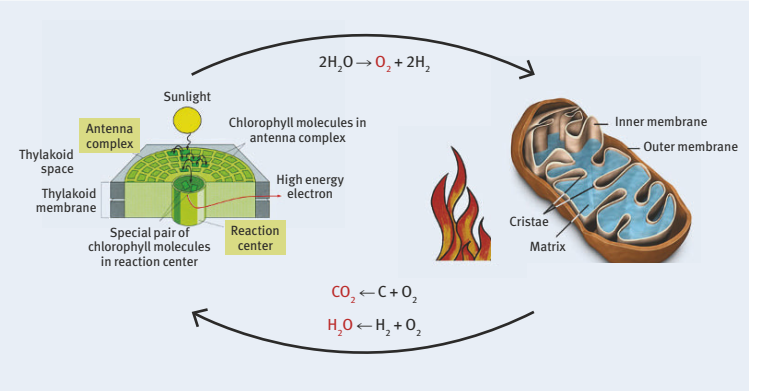

Figure 2.4 Photosynthesis and respiration create a balance. Photosynthesis uses energy from sunlight to liberate oxygen (O_2) whereas respiration gains energy by using O_2.

organisms still exist today but their growth is limited and they can be killed by the current atmospheric level of 21% oxygen. The damaging effect of oxygen on strict anaerobes seems to be due to the oxidation of essential cellular compounds. As the oxygen content of the atmosphere increased, many primitive organisms must have died out. Eukaryotic organisms began

the evolutionary process of using both oxygen, and adapting to higher oxygen concentrations in the atmosphere. Oxygen is used to gain energy by oxidizing other molecules such as protons, however this resulted in the production of some unwanted ROS. Organisms that tolerated the presence of oxygen were naturally selected over others; these organisms developed antioxidant defenses and developed through the evolution of the electron transport chain (ETC). For energy production they developed the electron transport chain where oxygen was used as the terminal electron acceptor, enabling the oxidation of 'food' more efficiently. The development of the ETC first developed in bacteria and then evolved further by endosymbiosis of bacteria (mitochondria are descendants of bacteria) in eukaryotic host cells (Figure 2.5). In a similar way, the chloroplasts of plants originated by endosymbiosis of bacteria.

When is the oxygen molecule beneficial and when is it harmful?

The oxygen molecule is considered to be the elixir of life and this benign image is well deserved, as long as the molecule remains in its electronic ground state. By adding energy to this molecule, the electron configuration is changed to an electronically excited state. As this molecule is now more reactive it belongs to the ROS and becomes damaging, particularly if it is in excess of the cellular antioxidant balance. The oxygen molecule in its ground state however is quite inert although it is a (di-)radical.

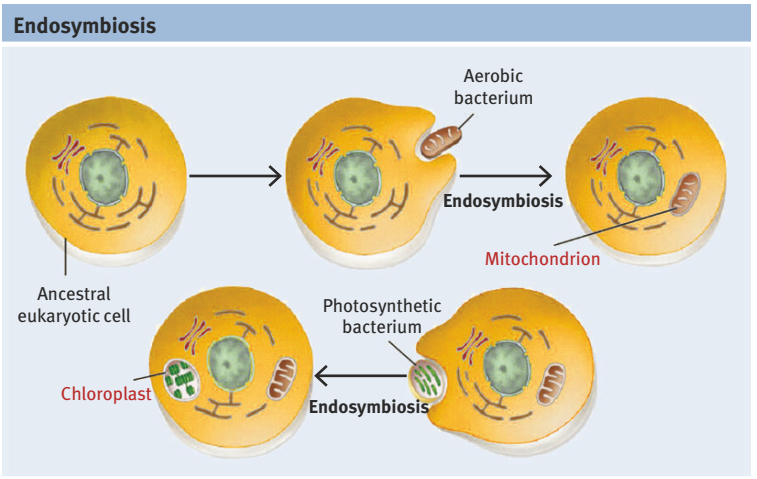

Figure 2.5 Endosymbiosis. The mitochondria and chloroplasts of eukaryotes evolved from ancient endosymbiosis of aerobic bacteria by the ancestral eukaryotic cell.

As mentioned earlier, a free radical is an atom or a molecule with an unpaired electron in its outer shell. By gaining or losing an electron, free radicals maintain a much more stable electronic configuration which explains their reactivity. The question therefore arises as to why 'normal' atmospheric oxygen is only minimally reactive? In its ground state the oxygen molecule has two unpaired electrons, each of which are located in a different pi antibonding orbital (Figure 2.6). These two electrons rotate about their own axis in the same direction (ie, they have the same or parallel spin). When two free radicals fuse to form a new molecule, the electrons of the two molecules that will pair together have a different (anti-parallel) spin. The oxygen molecule would need another molecule with two electrons with the same spin, which are both anti-parallel to the spin of the electrons of the oxygen molecule. This, however, is very rare and it is this spin restriction which makes molecular oxygen in its ground state normally non-reactive. Oxygen molecules become reactive if they gain either energy (eg, in the case of a fire) or if they accept one individual electron (eg, as in the production of superoxide).

How can oxygen be activated in biological tissues?

Oxygen can be activated by two different mechanisms: through the absorption of sufficient energy to reverse the spin on one of the unpaired electrons, or through monovalent reduction (Figure 2.7).

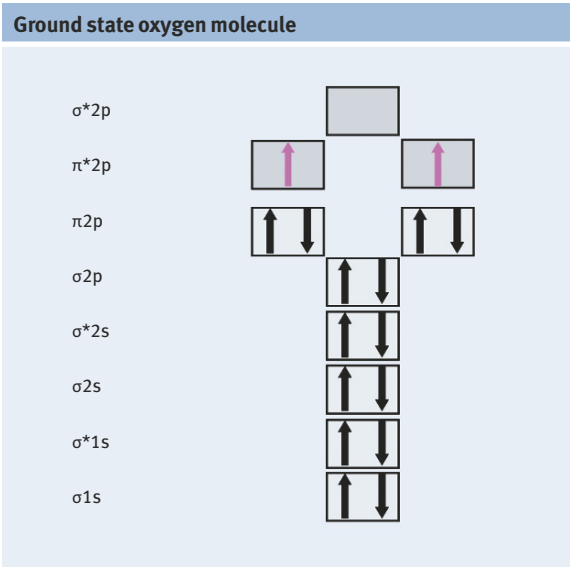

Figure 2.6 Ground state oxygen molecule. In ground-state, the last two unpaired electrons have the same quantum spin number (ie, they have parallel spins).

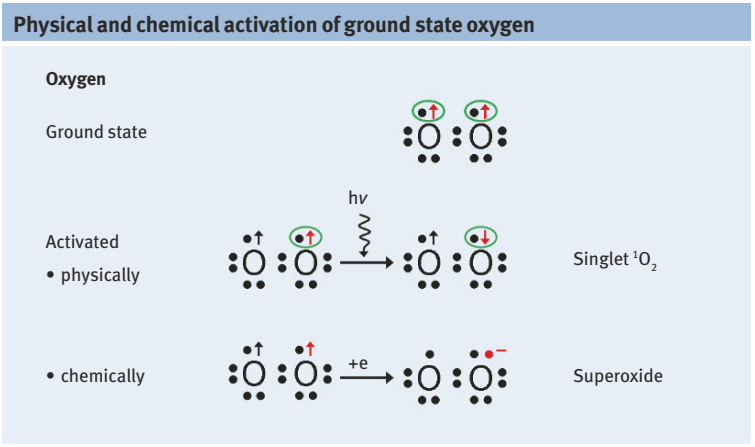

Figure 2.7 Physical and chemical activation of ground state oxygen. The oxygen molecule can be activated physically (eg, direct excitation by light) to form the singlet oxygen, or it can be activated chemically by a reduction reaction (eg, one electron reduction of dioxygen) to form the superoxide anion.

If ground state oxygen absorbs sufficient energy to reverse the spin of one of its unpaired electrons, the two unpaired electrons now have opposite spins. This activated form of oxygen, known as singlet oxygen ($^1O_2$) is much more reactive than ground state oxygen and reacts, for example, with molecules which have double bonds. The damaging effect of ROS can also be used in a beneficial manner. For example, in photodynamic therapy the photosensitizer, activated by light, can deliver its energy to a nearby oxygen molecule in ground state, converting it to the reactive singlet oxygen. This in turn destroys unwanted neovascularizations (Figure 2.8).

The second mechanism of activation is by the stepwise monovalent reduction of oxygen which gives rise to the superoxide anion radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and finally to water (H_2O) as shown in Figure 2.9.

Hydrogen peroxide is a molecule with a high oxidizing capacity. Many transformations are also facilitated by enzymes or by metal atoms.

The concept of cellular stress

What happens when a cell is subjected to stress?

All diseases, including glaucoma, are based on the functional or structural damage of cells. Damaging factors include hypoxia, toxins, ionizing radiation, viruses, and bacteria or immune processes. Cells respond to stress in a limited number of ways as shown in Figure 2.10. If the stress induced is high, the cell

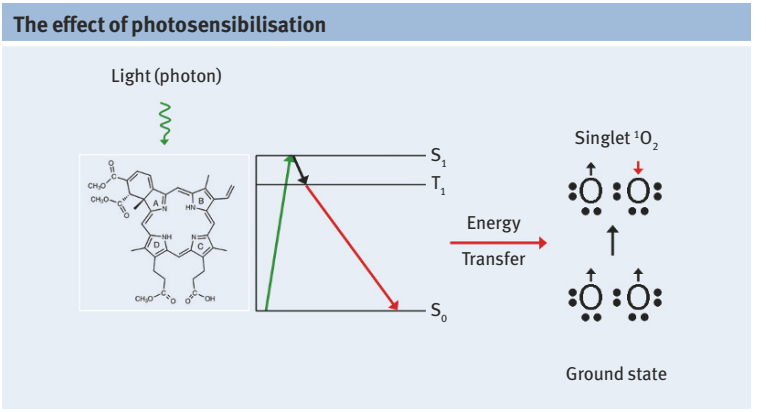

Figure 2.8 The effect of photosensibilisation. A photosensitizer (eg, verteporfin) can absorb light. Once activated, the photosensitizer can transfer a part of this energy to an oxygen molecule to produce singlet oxygen.

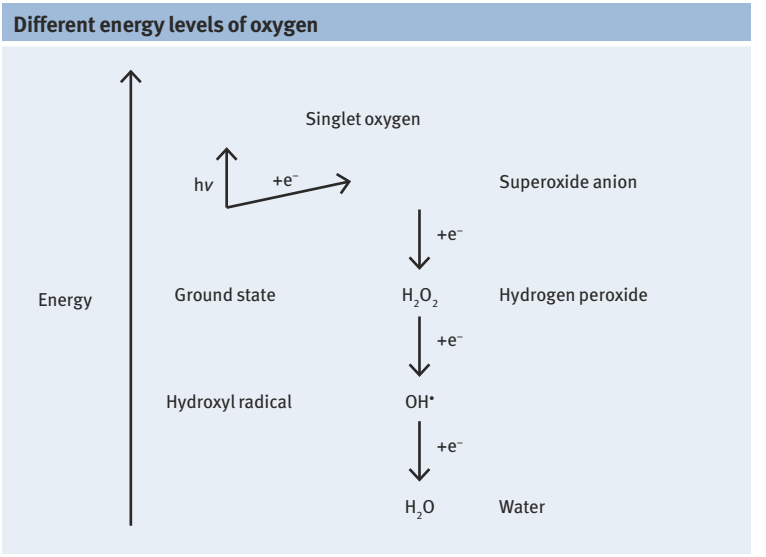

Figure 2.9 Different energy levels of oxygen. Activation of oxygen occurs either through absorption of light or through reduction.

dies either by necrosis or apoptosis. If the stress is moderate, the cell survives but it temporarily changes its gene expression, and may lead, for example, to an increased production of heat shock proteins. These proteins act as molecu-

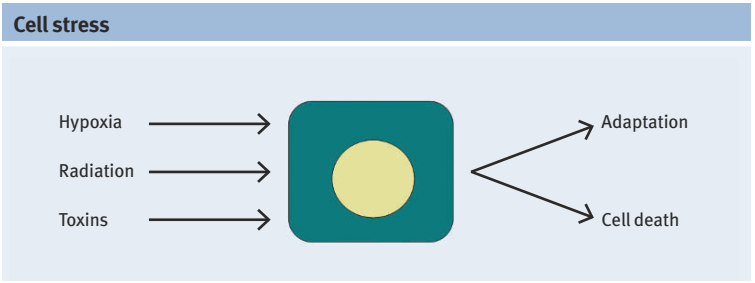

Figure 2.10 Cell stress. In response to stress a cell can either adapt or die.

lar chaperones protecting the three-dimensional structure of other proteins. Repeated or chronic cellular stress leads to a chronic 'response to injury' and according to the level of stress, can lead to preconditioning, metaplasia, dysplasia or even apoptosis. Depending on the tissue the extracellular matrix is also involved leading to tissue remodeling. These tissue changes are often accompanied by cellular loss. In vitro, it has been shown that mechanical, biochemical or ischemic stress induces a similar cellular response. Such changes can also be observed in human GON, especially in the glial cells.

Oxidative stress

What is oxidative stress?

The eye is exposed to light, a high concentration of oxygen, environmental chemicals and physical abrasion. As mentioned previously, the metabolism of oxygen by cells generates potentially harmful ROS. Under optimal conditions the rate and magnitude of oxidant formation is balanced by the rate of oxidant elimination through the action of antioxidants. Nature has therefore provided us with mechanisms to help us cope with pro-oxidants. If ROS production exceeds this capacity, however, oxidative stress will occur (Figure 2.11).

As long as nature is capable of repairing damaged molecules (eg, DNA) or eliminating damaged molecules (eg, proteins via proteasomes) no major structural damage will occur. If, however, oxidative stress exceeds the capacity of repair mechanisms, structural damage will accumulate and result in damage that is ultimately clinically relevant and which we term a disease, as illustrated in Figure 2.12.

There are several ways to gain information about oxidative stress (eg, quantifying the amount of antioxidants or indirectly measuring the oxidation of certain molecules such as lipids), we will focus here on the comet assay methodology, which looks at the number of breaks in DNA.

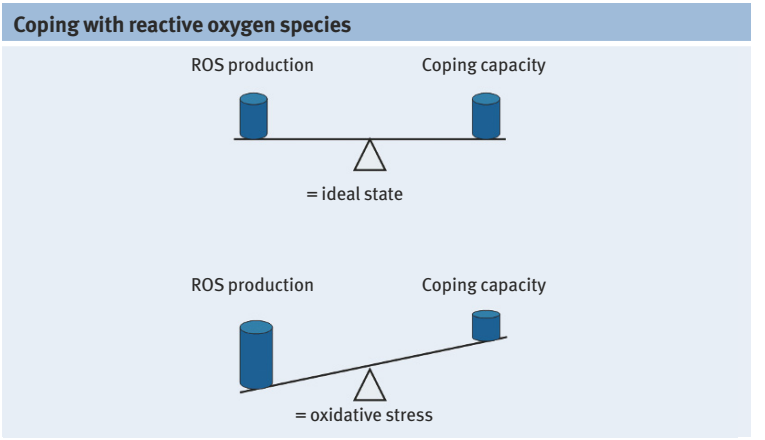

Figure 2.11 Coping with reactive oxygen species. ROS, reactive oxygen species. Nature has provided us with mechanisms to cope with ROS. If ROS production, however, exceeds this capacity, oxidative stress results.

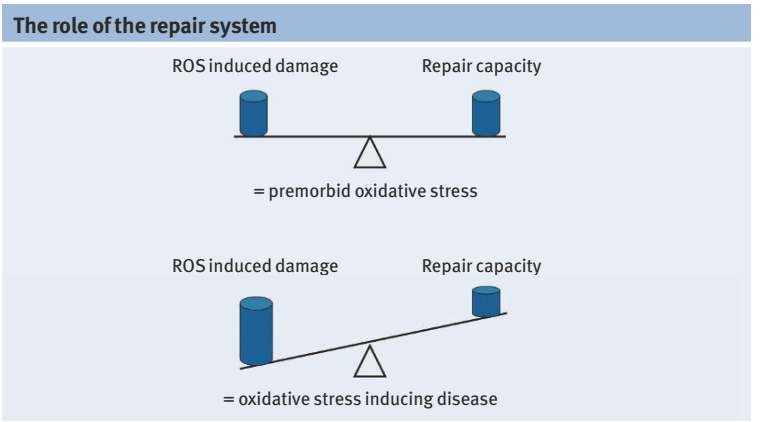

Figure 2.12 The role of the repair system. ROS, reactive oxygen species. As long as the repair capacity exceeds the induced damage no disease will develop. If oxidative-induced damage exceeds the repair capacity a disease may develop.

Comet assay

Comet assay, also known as single cell gel electrophoresis, allows measurement of DNA breaks, induced by different factors such as radiation and oxidative stress. If these factors known to cause DNA damage are weak and kept constant, the amount of DNA damage measured by comet assay is a good parameter of oxidative stress.

The principle is simple and relies on the fact that DNA molecules are negatively charged. An intact DNA molecule has such a large size that it does not migrate towards the anode in electrophoresis. However, if breaks are present in the DNA the resulting smaller fragments move in the electrical field towards the anode; the smaller the fragment, the faster the migration. As the fragments have different sizes the final result of the electrophoresis is not a distinct line but rather a continuum with the shape of a comet. This method allows the resulting 'comet' to be measured and assessed.

How is comet assay performed practically?

A sample of blood is drawn from the subject by venipuncture, and leukocytes are isolated from the sample. The cells under study are embedded in agarose on a slide and subjected to lysis, followed by electrophoresis. Finally, to visualize DNA, the slides are stained with propidium bromide and examined by fluorescence microscopy equipped with a computer-based analysis system which accurately enables the number of DNA breaks to be counted (Figure 2.13).

Assessment of ocular blood flow

While it is possible to measure OBF, it is not simple. Methods used today are based on a number of physical principles and measure different aspects of OBF in various tissues of the eye. The discussion here will be limited to some of the more commonly used methods.

When performing measurements of blood flow in the eye, we can take advantage of optical phenomena. Measurements behind the eye rely on ultrasound. Most methods primarily calculate blood flow velocity and, although the relationship is not always strictly proportional, in general lower blood flow velocity indicates a lower blood flow (Figure 2.14).

There is still debate over which vascular bed is most relevant in the case of glaucoma although it is most likely to be the optic nerve head (ONH). Given that OBF outcome measures in different parts of the eye correlate quite well with each other, the selection of the measuring field may not be that relevant. There is even a correlation between OBF and blood flow in the fingers, at least in subjects with vascular dysregulation. Although OBF in patients with glaucoma is, on average, different from healthy subjects at baseline, these differences become much more evident when OBF is challenged in a provocation test.

Measuring temperature

The temperature of any organ, particularly in the extremities, is related to a number of factors including blood flow.

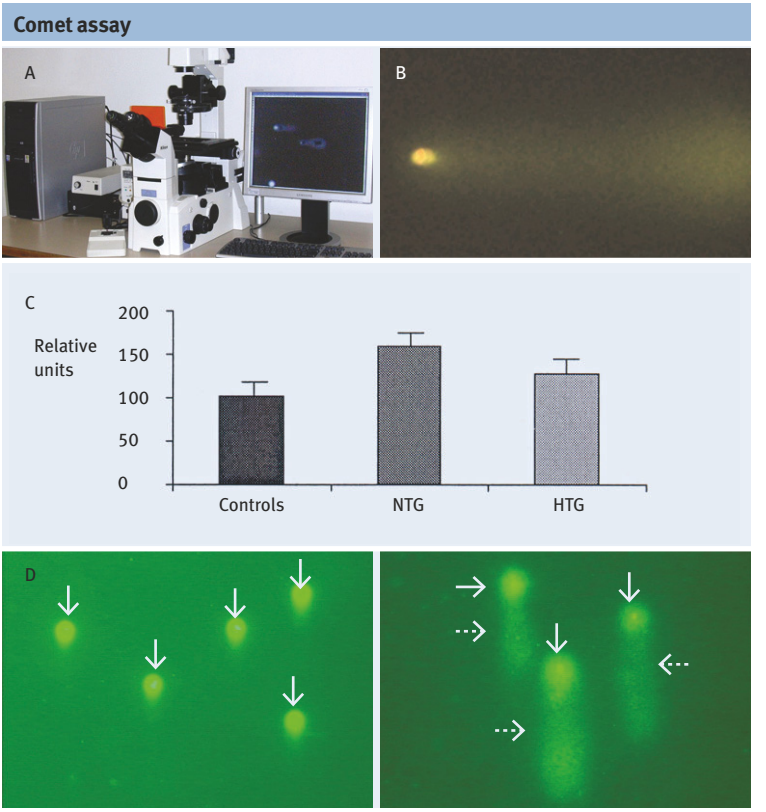

Figure 2.13 Comet assay. HTG, high tension glaucoma; NTG, normal tension glaucoma. Comet assay is a micro gel electrophoresis technique which allows quantification of DNA breaks. **A**, equipment. **B**, an example. **C**, increased DNA breaks in the lymphocytes of patients with glaucoma. **D**, photo showing the lymphocytes of a control patient (left), and a patient with glaucoma (right). The dashed arrows show the resulting 'comets' in the DNA of the patient with glaucoma which is a good indicator of oxidative stress.

Reports indicate that the eyes of patients with glaucoma, particularly patients with normal tension glaucoma (NTG), are cooler that those of patients without glaucoma (Figure 2.15). Furthermore, reports also show that an increase in OBF, induced directly pharmacologically or by decreasing IOP, increases corneal temperature.

Fluorescence angiography

Fluorescence angiography (FLA) is normally used to determine if there is an alteration in any of the ocular vessels (eg, occlusions, decrease in barrier func-

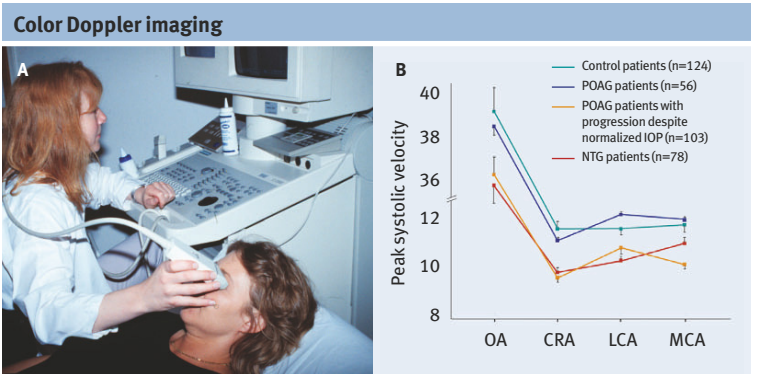

Figure 2.14 Color Doppler imaging. CRA, central retinal artery; LCA, lateral ciliary artery; MCA, medial ciliary artery; OA, ophthalmic artery. **A**, representation of its clinical use. **B**, peak systolic velocity in the different retroocular vessels. The graphs represent the mean (±SD) of the different groups.

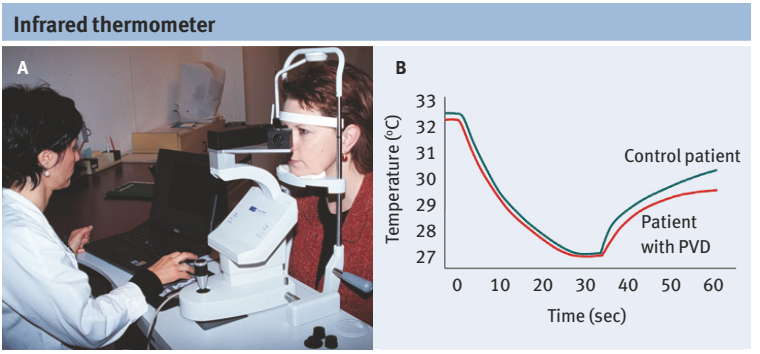

Figure 2.15 Infrared thermometer. PVD, primary vascular dysregulation. **A**, representation of its clinical use. **B**, after cooling the cornea with an air stream, patients with PVD have a prolonged re-warming time.

tion, microaneurysma). FLA is not used routinely for patients with glaucoma, nevertheless studies with FLA have demonstrated the following alterations in these patients:

- a reduction in blood flow velocity as demonstrated by delayed filling (ie, prolonged arm retina time) and as prolonged arteriovenous passage time;
- filling defects in, and around, the ONH in the juxtapapillary area (Figure 2.16); and
- increased leakage impairment of the blood–brain and blood–retinal barrier (Figure 2.17)

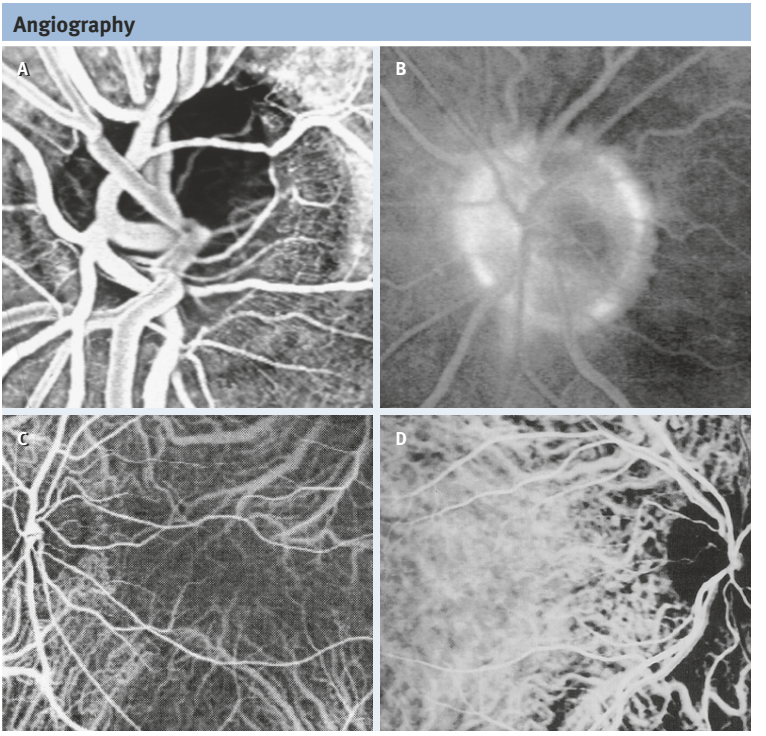

Figure 2.16 Angiography. A, fluorescence angiography of a glaucomatous papilla, parts of the optic nerve head are not perfused. **B**, mild diffuse staining. **C**, **D**, indocyanine angiography in a glaucomatous eye, delayed filling of some of the choriocapillaries.

Retinal vessel analyzer

The retinal vessel analyzer is an instrument that quantifies the size of retinal arteries and veins along a selected segment over a period of time (Figure 2.18). The vessel diameter provides only indirect information about OBF. Nevertheless, the size of arteries and veins, and their spatial and temporal variation, provides very useful information which can be used to study in depth whether a provocation or treatment dilates or constricts a vessel.

The Doppler effect

The Doppler effect was first described by the Austrian scientist Christian Doppler in 1842. Doppler observed the fact that the light emitted from a star moving away from us would have a slightly longer wavelength (ie, a shift towards the red end of the electromagnetic spectrum) than the light of one moving towards us (ie,

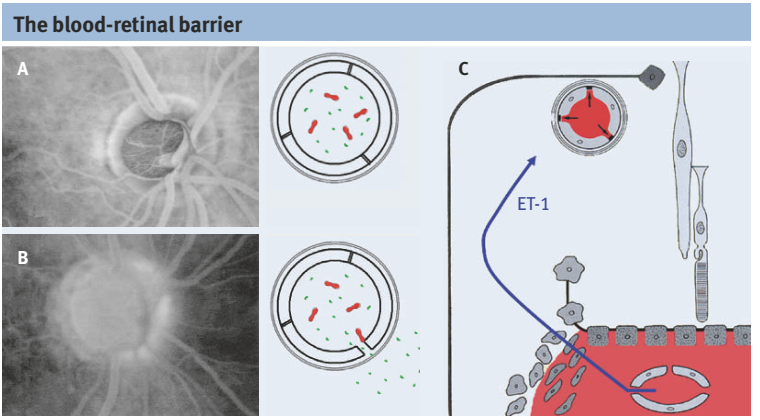

Figure 2.17 The blood-retinal barrier. ET-1, endothelin-1. **A,** in contrast to the choriocapillaries, retinal vessels have a dense blood retinal barrier. **B,** in glaucoma the barrier is partially opened as a result of diffusion of ET-1 into this area. **C,** line drawing of B.

a shift towards the blue end of the electromagnetic spectrum). The 'Doppler-effect', describes a frequency shift of waves of any nature (eg, light, acoustic, water) emitted from an object which is moving away or towards an observer (Figure 2.19). Today, with lasers providing optical light waves of extreme purity, it is possible to detect Doppler shifts with very high resolution. A continuous laser light is projected into a tissue and the backscattered light is analyzed; this light contains two components:

- shifted light – scattered by moving particles such as blood cells
- unshifted light – scattered by relatively stationary structures (eg, vessel walls).

Most of the light is backscattered without a shift in frequency. Moving particles, however, cause a Doppler shift on scattered light. The relative number of shifted photons depends on the number of moving particles (in our case blood cells) whereas the size of the shift depends on the velocity of these particles. The laser Doppler principle can therefore be used to measure blood flow velocity and to some extent OBF itself.

Laser Doppler velocimetry

Laser Doppler velocimetry (LDV) is a technique that measures blood flow velocity by directing a laser beam at a selected blood vessel. The blood flowing through the vessel causes a Doppler shift, or change in wavelength, allowing the speed of the blood to be measured. If in addition, the diameter of the vessels is measured, blood flow can be calculated.

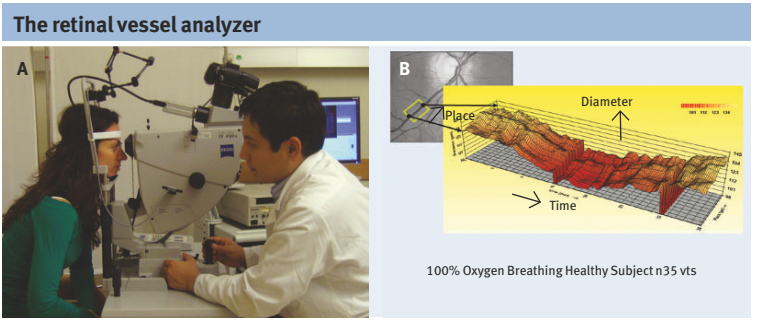

Figure 2.18 The retinal vessel analyzer. A, representation of its clinical use. **B,** the retinal vessel diameter of a vascular section using the retinal vessel analyzer. Constriction resulted after the patient breathed in 100% oxygen.

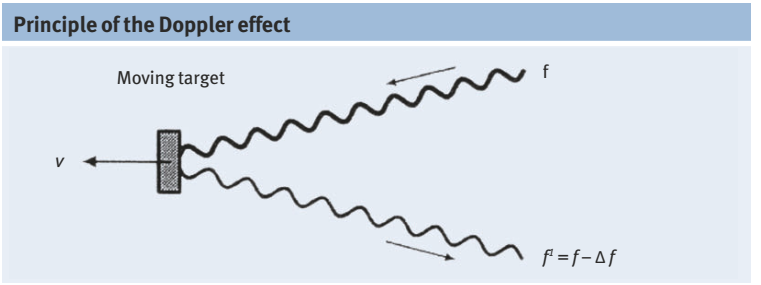

Figure 2.19 Principle of the Doppler effect. A laser beam reflected by a moving object returns with a frequency change, Δ f, known as Doppler shift. The velocity, *v*, can be determined from Δ f.

Laser Doppler flowmeter

Laser Doppler flowmetry (LDF) is based on the fact that the size of the shift depends on the velocity of the moving particles and the relative amount of light that is shifted depends on the number of moving particles (Figure 2.20). Based on these two variables, a third variable, the so-called "flux" can be calculated. Unlike velocimetry, flowmetry measures the blood flow in capillary beds. The laser is directed to the areas between larger vessels and can be applied either to the ONH or to the retina. Blood flow in the choroid, however, can be measured only in the subfoveal area and by using a longer wavelength. LDF is best suited for intra-individual comparisons.

Color Doppler imaging

As light cannot reach the tissues behind the eye, ultrasound is used to measure blood flow velocity in the retroocular vessels (Figure 2.21).

Laser Doppler flowmetry

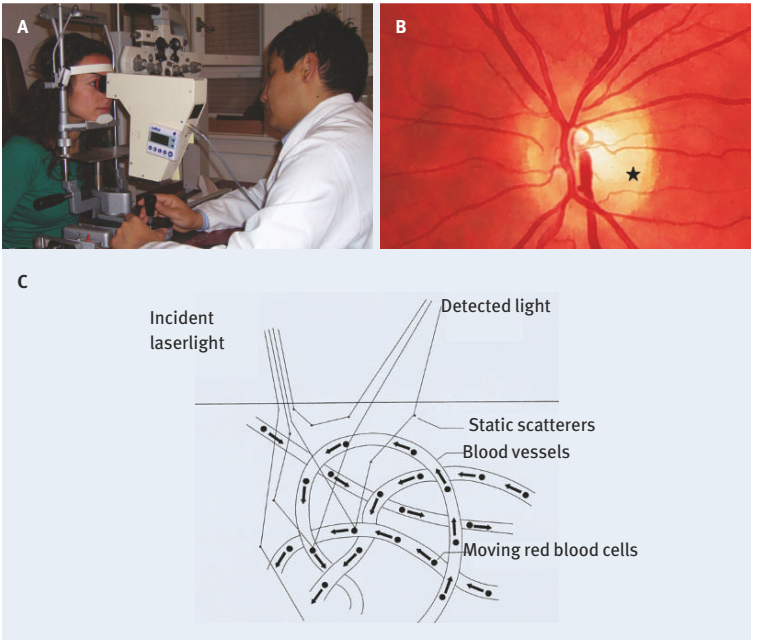

Figure 2.20 Laser Doppler flowmetry. **A**, representation of its clinical use. **B**, laser Doppler flowmetry measures flow at a selected point (*). **C**, a portion of the scattered light eventually leaves the tissue in the direction of the detector.

Color Doppler imaging

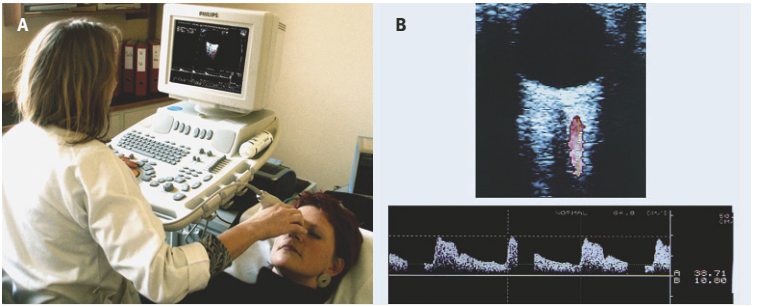

Figure 2.21 Color Doppler imaging. **A**, representation of its clinical use. **B**, color coding of flow direction superimposed on a B-scan (top). Blood flow velocity in a selected vessel (bottom).

A color Doppler is a device that combines imaging with a B-scan, localization of blood flow based on a Doppler principle, and quantification of blood flow velocity with pulsed Doppler technique. This is the technique used most often today for patients with glaucoma.

Ocular pulse amplitude

Ocular pulse amplitude (OPA) can be measured using a variety of methods (Figure 2.22). These techniques, however, will not be discussed any further here as, at present, there is limited information concerning the relationship between OPA and OBF.

Nailfold capillaromicroscopy

Capillaromicroscopy is an old technique used by angiologists to quantify the blood flow velocity in the capillaries of the extremities (Figure 2.23).

The fact that the capillaries in the nailfold are arranged in a unilayer and are parallel to the surface and therefore perpendicular to the observer makes it very easy to measure blood flow in this area. How then is blood flow in the nailfold capillaries related to OBF? In the past, before OBF measurements were possible, the relationship between nailfold capillary blood flow and visual field had already been observed in certain patients. It was assumed, therefore, that there could be a relationship between blood flow in the fingers and in the eye. Today, we know that such a relationship does indeed exist in patients with primary vascular dysregulation (PVD). Nailfold capillaromicroscopy can be used, therefore, to some extent as a substitute for OBF. However, it is most often used to test for dysregulation.

Visual field changes as a parameter for ocular blood flow

It may seem strange for perimetry to be listed among the methods used to quantify OBF. Perimetry is used to measure differential light sensitivity (DLS)

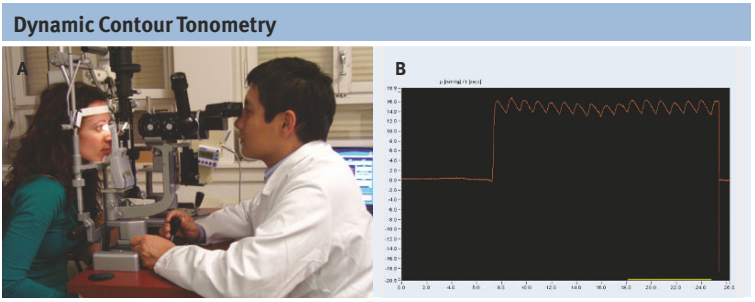

Figure 2.22 Dynamic Contour Tonometry. A, representation of its clinical use. **B,** output depicting ocular pulse amplitude.

Nailfold capillaromicroscopy

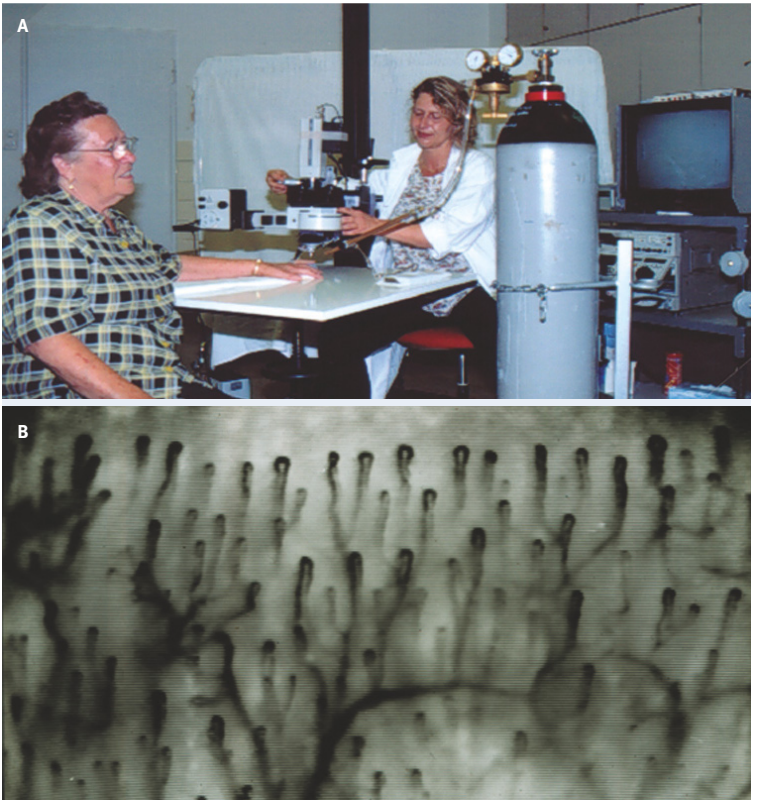

Figure 2.23 Nailfold capillaromicroscopy. A, representation of its clinical use. A microscope coupled to a television monitor, allows the observed blood flow to be videotaped and to be analyzed off-line. Cold compression is induced by decompression of gas. **B,** a closure of one or more of the visible capillaries can be detected on the video screen.

in the visual field (Figure 2.24). The outcome fluctuates in normal eyes and this fluctuation is amplified in patients with glaucoma. The fluctuation has both a short- and long-term component. The short term component depends on factors such as damage of the visual field or cooperation of the patient, whilst the long-term component seems to be, among other factors, influenced by ocular circulation. DLS is also strongly related to blood-oxygen tension and the DLS threshold fluctuates in parallel with IOP. In patients with vascular dysregulation, the improvement observed in the visual field while undergoing vasoactive

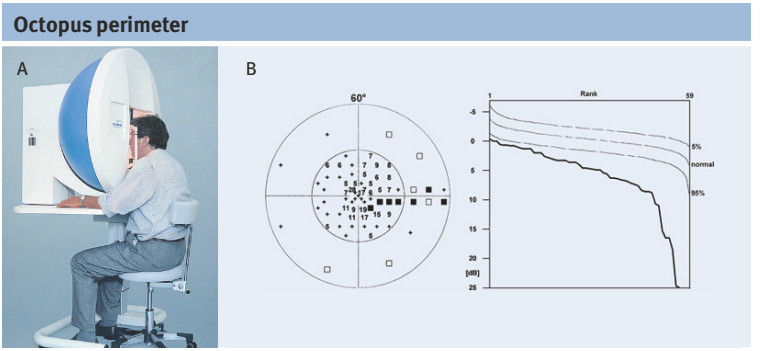

Figure 2.24 Octopus perimeter. A, representation of its clinical use. **B**, output of an Octopus G1 program (left) and representation of the same visual field by the Bebie curve (right).

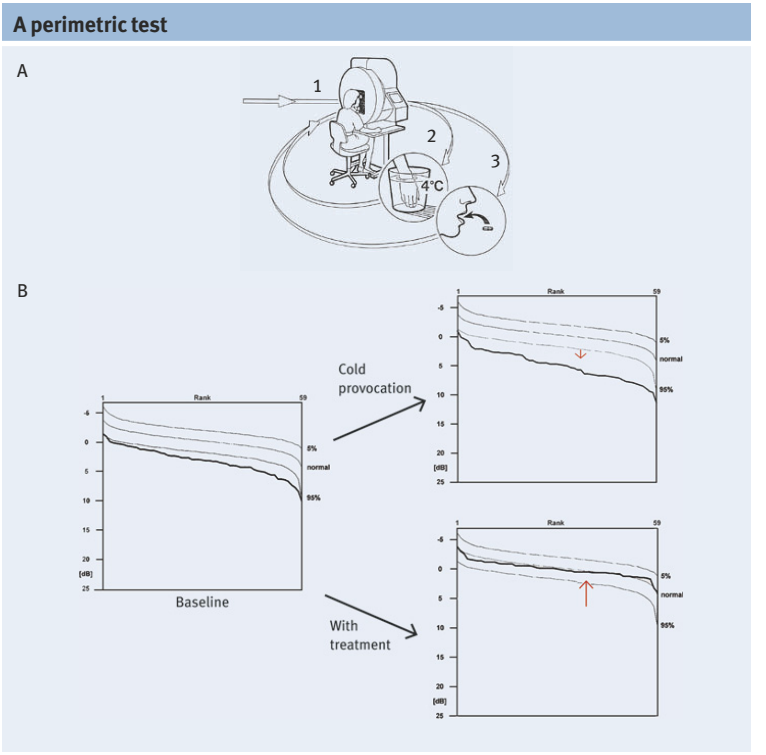

Figure 2.25 A perimetric test. The visual field is represented by a Bebie curve of a patient with primary vascular dysregulation. **A**, schematic representation of a perimetric test (1) after cold provocation (2) and after treatment (3). **B**, baseline values (left), deterioration after cold provocation (top right), improvement after treatment with calcium channel blockers (bottom right).

treatment (eg, with calcium channel blockers) correlates to the changes in blood flow observed in the periphery. Likewise, deterioration of the visual field after cold provocation also correlates with blood flow deterioration. A relatively quick (reversible) change in the visual field can therefore be an indirect sign of a change in ocular perfusion (Figure 2.25).

Provocation tests

When measuring blood flow it is important to be aware that blood flow in a given tissue is not constant. OBF depends upon a number of factors such as exposure to light, environmental temperature, physical activity or emotional status. In certain patients blood flow may be within normal limits when measured under baseline conditions. If blood flow is measured under challenging conditions the regulation may not be to the same extent as in healthy controls. Provocation tests, therefore, provide other and more relevant information than baseline measurements. The various provocation tests used to challenge blood flow include:

- artificially increasing IOP (induced by a suction cup)
- stimulating the autonomic nerves with a hand grip (Figure 2.26)
- coldness (eg, by blowing cold air) which is a standard procedure in nailfold capillaromicroscopy (Figure 2.27).

In patients with vascular dysregulation blood flow in the fingers correlates very well with OBF and consequently can be used as a proxy measurement.

The handgrip test

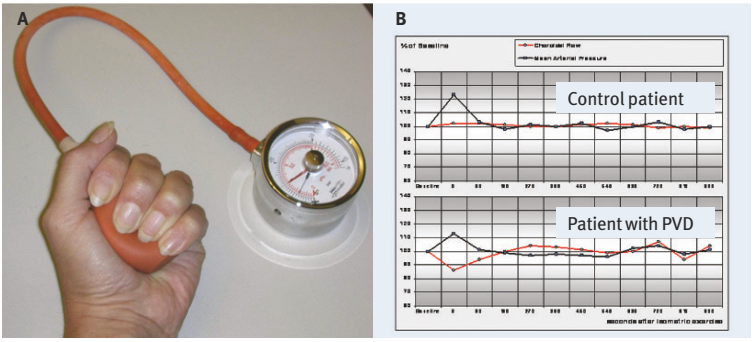

Figure 2.26 The handgrip test. A, an increased sympathetic tonus is induced by hand grip. **B,** choroidal blood flow (measured with laser Doppler flowmetry) remains stable (red line) in non-PVD controls despite an increase in blood pressure (blue line) (top right). There is a temporary reduction in ocular blood flow in subjects with PVD (bottom right).

Cold provocation test in nailfold capillaromicroscopy

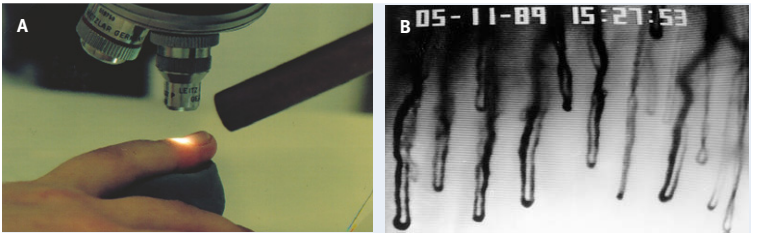

Figure 2.27 Cold provocation test in nailfold capillaromicroscopy. A, the nailfold is made transparent with a drop of oil. **B,** picture of the nailfold capillaries, taken from the monitor of the video nailfold capillaromicroscopy.

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2.5: Mozaffarieh M, Grieshaber MC, Orgül S, Flammer J (2008). Surv Ophthalmol 53(5):479-505
2.13A/B, 2.15A, 2.18, 2.20A, 2.22, 2.26A: Photo collection Dept. of Ophthalmology, University of Basel
2.13C: Moenkemann H, Flammer J, Wunderlich K, Breipohl W, Schild HH, Golubnitschaja O (2005). Amino Acids 28(2):199-205
2.13D/E: Golubnitschaja O, Flammer J (2007). Surv Ophthalmol 52: Suppl 2: S155-S161
2.17: Modified from: Grieshaber MC, Flammer J (2007). Surv Ophthalmol 52: Suppl 2: S115-S121
2.19: Kaiser HJ, Flammer J, Hendrickson Ph (Eds.): Ocular Blood Flow: New Insights into the Pathogenesis of Ocular Diseases. Karger Basel (1996) (reproduced with permission from H. Bebie)
2.20: Kaiser HJ, Flammer J, Hendrickson Ph (Eds.): Ocular Blood Flow: New Insights into the Pathogenesis of Ocular Diseases. Karger Basel (1996) (adapted from Bonner and Nosal with permission)

Chapter 3

What is glaucoma?

Glaucomatous optic neuropathy

The term glaucoma originates from the ancient Greek word, *glaukos*, meaning gray-blue. In glaucoma, retinal ganglion cells and their axons progressively die (Figure 3.1). Consequently, the connection between the eye and brain, so crucial for vision, is gradually severed. The eye still sees the light because the rods and cones are still working, but the transmission of visual information to the brain is interrupted. This is the core of the problem and is called

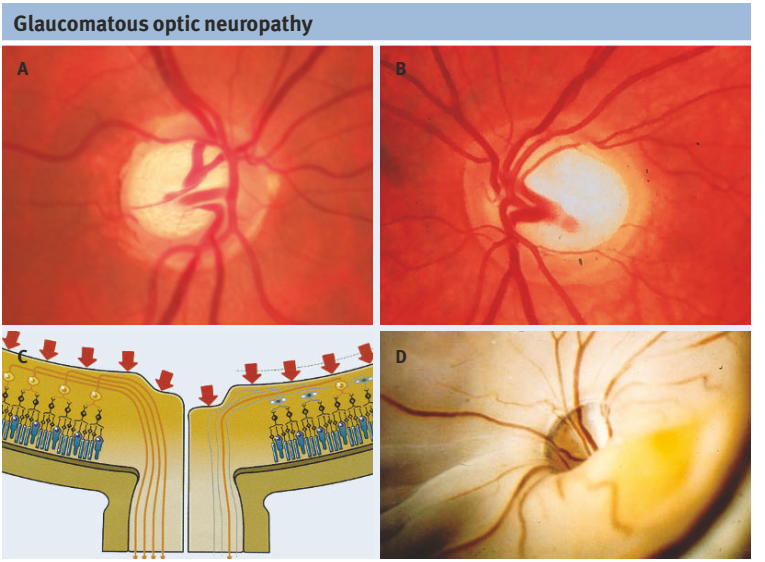

Figure 3.1 Glaucomatous optic neuropathy. A, B, clinical observation of an excavated optic nerve head. **C,** glaucomatous optic neuropathy (GON) implies loss of retinal ganglion cells and axons. **D,** GON combined with major tissue remodeling as seen in a cadaver eye.

glaucomatous damage or glaucomatous optic neuropathy (GON). The loss of nerve fibers is morphologically seen in the retina and in the optic disc with the help of red-free photographs. The optic nerve also gets thinner which can be observed with ultrasound, and the ganglion geniculate nucleus shrinks which can be seen histologically, but also using magnetic resonance imaging (MRI). In addition to the loss of nerve fibers GON implies an activation of glial cells and tissue remodelling leading to the characteristic excavation of the optic nerve (Figure 3.2). Blood flow is also reduced. These changes are discussed in more detail below.

Epidemiology of glaucoma

It is estimated that around 70 million people worldwide have some form of glaucomatous damage (Figure 3.3), with only half of them being aware of the fact, and an even smaller percentage receiving adequate treatment. The number of people afflicted with glaucoma is significantly higher when older populations are considered. The proportion of patients with GON, despite having a normal IOP (ie, normal tension glaucoma), seems to be on the increase and varies considerably from one part of the world to another.

Symptoms of chronic glaucoma

In glaucoma, as the retinal nerve cells and their fibers slowly die, the connection between the retina and the brain is diminished. A healthy eye is connected to the brain by approximately one million nerve fibers. These fibers fan out throughout the innermost layer of the retina, come together at the optic disc and leave the back part of the eye in bundles, the optic nerve head. Throughout the course of our life we all lose some of our nerve fibers, this is part of the natural aging process, unfortunately for glaucoma patients the loss of nerve fibers occurs at a faster rate.

Histological cross-sections of a normal and glaucomatous optic nerve

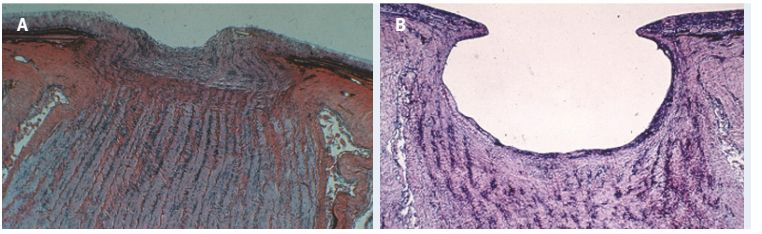

Figure 3.2 Histological cross-sections of a normal and glaucomatous optic nerve.
A, normal optic nerve. **B,** glaucomatous optic nerve.

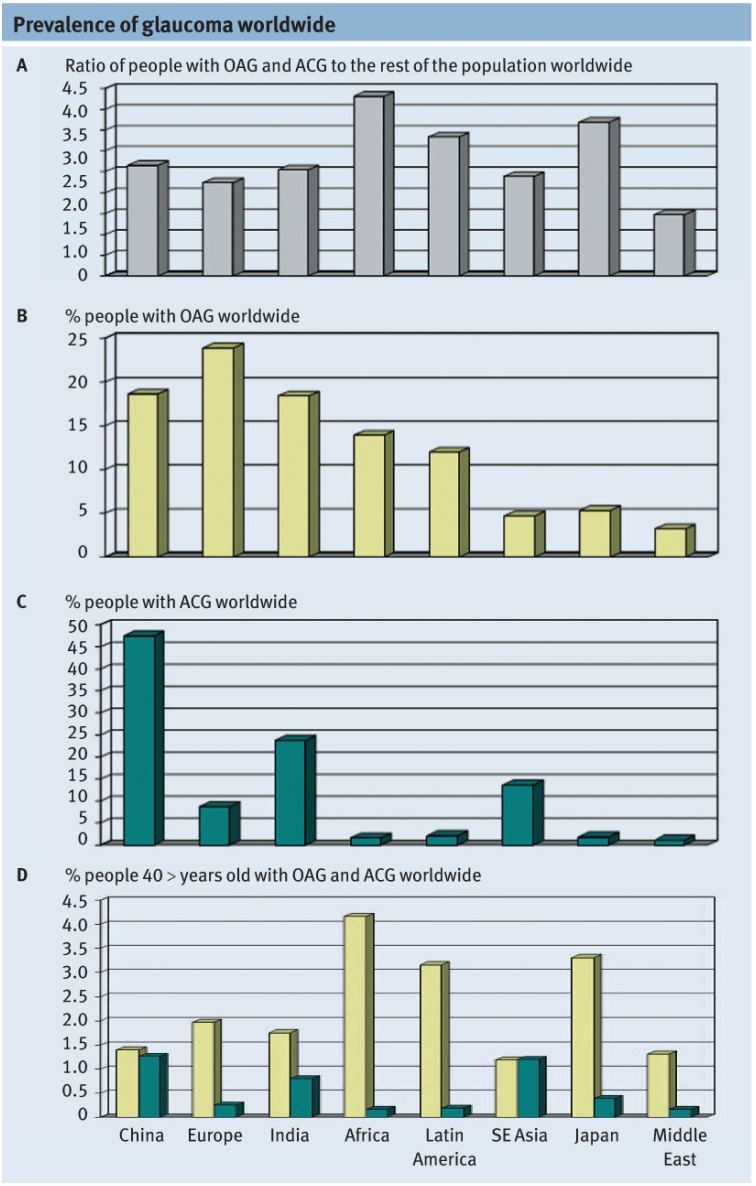

Figure 3.3 Prevalence of glaucoma worldwide. ACG, angle closure glaucoma; OAG, open angle glaucoma. **A**, ratio of people worldwide with OAG and ACG as compared to the rest of the population. **B**, distribution of people with OAG worldwide. **C**, distribution of people with ACG worldwide. **D**, percentage of people over 40 years old with OAG and ACG worldwide.

One might expect a loss of nerve fibers to manifest symptoms but this is only the case in later stages of the disease. By contrast, other diseases may cause symptoms which subjects recognize immediately (eg, metamorphopsia caused by macular degeneration). However, in the case of glaucoma, although less information reaches the brain, this missing information is 'filled in' by the brain which extrapolates information from the surrounding area as illustrated in Figure 3.4. The leading 'symptoms' are visual field defects which are not consciously perceived by the patient. In addition to visual field loss, some patients suffer from a number of other visual symptoms, such as a loss in both chromatic and achromatic sensitivity, reduced contrast sensitivity, and increased blur.

Signs of glaucomatous optic neuropathy

The following are often occurring signs of glaucomatous optic neuropathy:

- optic nerve excavation
- palor of the neuroretinal rim
- peripapillary atrophy
- hemorrhages

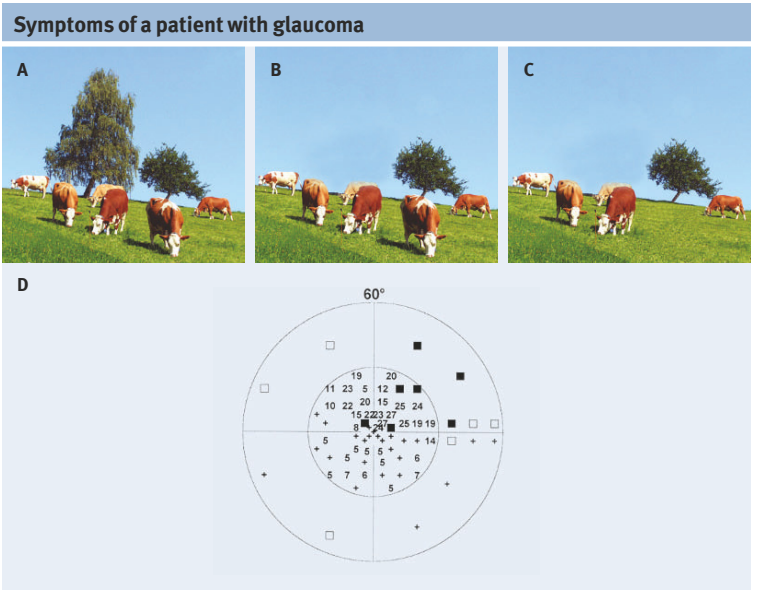

Figure 3.4 Symptoms of a patient with glaucoma. A, picture perceived by a control patient (no defects). **B**, picture perceived by a glaucoma patient with a small scotoma. **C**, picture perceived by a glaucoma patient with more advanced damage. **D**, even if a glaucoma patient has a measurable visual field defect he/she may not realize it.

- thinning of the retina
- glial activation
- thinning of the optic nerve and shrinking of the ganglion geniculate nucleus (as diagnosed by ultrasound or MRI).

Optic nerve excavation can be measured using laser scan imaging (ie, Heidelberg Retinal Tomograph) as shown in Figure 3.5 or by stereo photography (Figure 3.6). Nerve fiber layer loss is a sign which can be observed clinically (Figure 3.7) and measured using the GDx laser scanning device. Optical coherence tomography (OCT) can be used to measure retinal thickness (Figure 3.8).

Peripapillary atrophy (Figure 3.9) is not specific for glaucoma yet it is more often observed in patients with glaucoma when compared to healthy subjects. The topography of the peripapillary atrophy correlates with the topography of glaucomatous damage. Histologically, there are also signs of glial cell activation both in the optic nerve head and in the retina, which can be observed clinically (Figure 3.10). Fluorescence angiography reveals increased leakage of fluorescein as a sign of a malfunctioning blood–brain and blood–retinal barrier. The peripapillary vessels may constrict, which again, is not specific for glaucoma but occurs more often in patients with glaucoma in comparison to healthy subjects.

Finally, optic disc hemorrhages occur more often in patients with glaucoma than in those without glaucoma and within the former it is more prevalent in patients with normal tension glaucoma (NTG) than in patients with high tension glaucoma (HTG). It also occurs more often in women than in men. A hypothetical explanation for the occurrence of these hemorrhages has been given in Figure 3.11. Haemorrhaging in the optic disc very often precedes nerve fiber loss in the

Heidelberg retinal tomography

Figure 3.5 Heidelberg retinal tomography. Laser scan imaging with Heidelberg retinal tomography. Representation of its clinical use.

Stereophotography

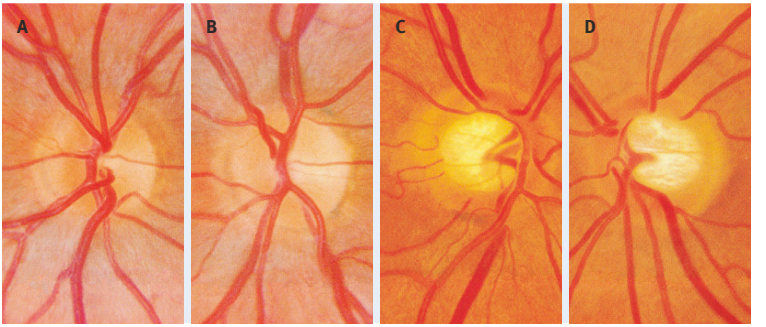

Figure 3.6 Stereophotography. A, B, both optic discs of this patient are normal, being symmetrical in appearance, shape, color, contour, and having a clearly visible and full retinal nerve fiber layer. **C, D,** in this patient, again, the optic discs and cups are large but normal.

Fundus photograph depicting nerve fiber layer loss

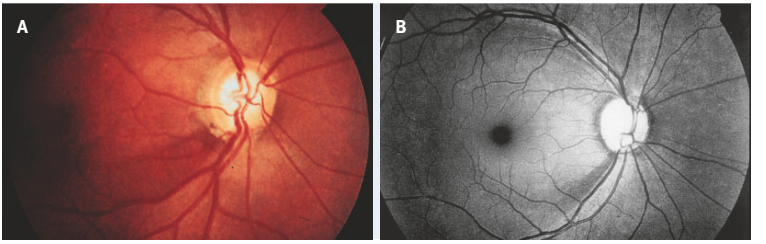

Figure 3.7 Fundus photograph depicting nerve fiber layer loss. A, color photograph. **B,** red-free photograph.

Optical coherence tomography

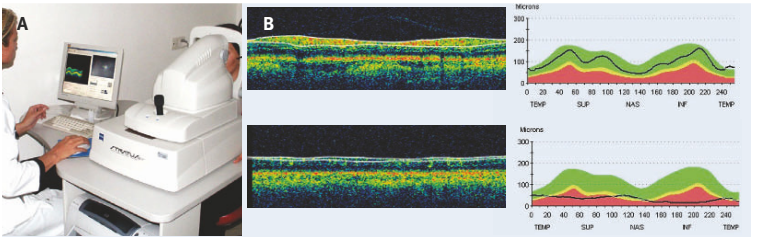

Figure 3.8 Optical coherence tomography. A, clinical use of optical coherence tomography (OCT). **B,** output from the OCT, showing a patient without glaucoma (top), and a patient with glaucoma (bottom).

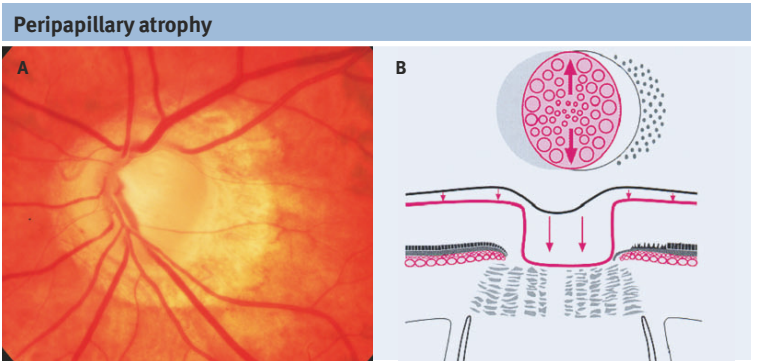

Figure 3.9 Peripapillary atrophy. A, clinical picture of a peripapillary atrophy. **B,** schematic illustration. A peripapillary atrophy is a zone in which the pigment epithelial layer and the layer of choriocapillaries is partially or totally absent giving direct view through the transparent retina to the sclera. Reproduced with permission from Naumann GOH. Pathologie des Auges. Berlin: Springer, 1997.

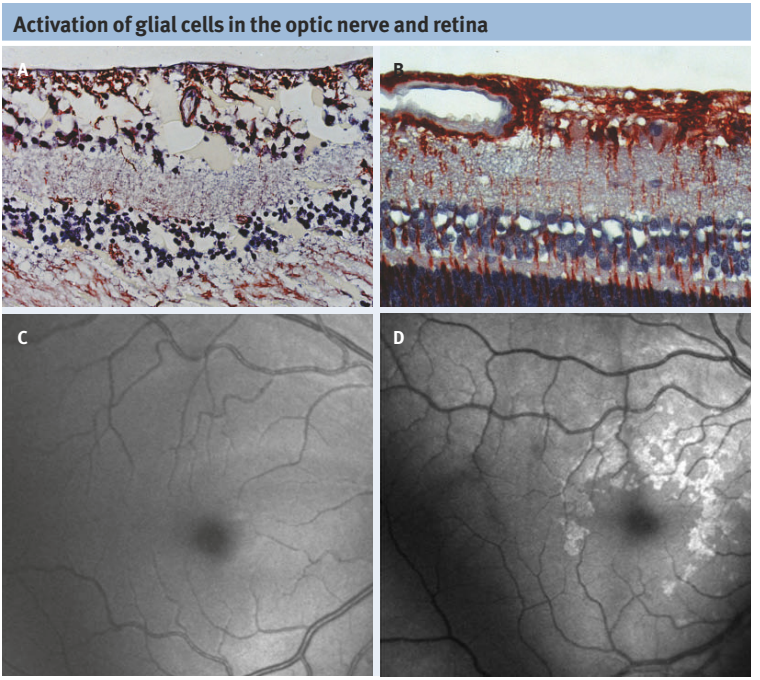

Figure 3.10 Activation of glial cells in the optic nerve and retina. A, glaucoma leads to activation of glial cells in the optic nerve head. **B,** activation of glial cells in the retina. **C,** red-free photograph showing a patient without glaucoma. **D,** red-free photograph showing a patient with glaucoma.

corresponding area. Histochemically, there are signs of ischemia both in the optic nerve head and in the retina which can be demonstrated, for example, with an increased concentration of hypoxia inducible factor-1 alpha.

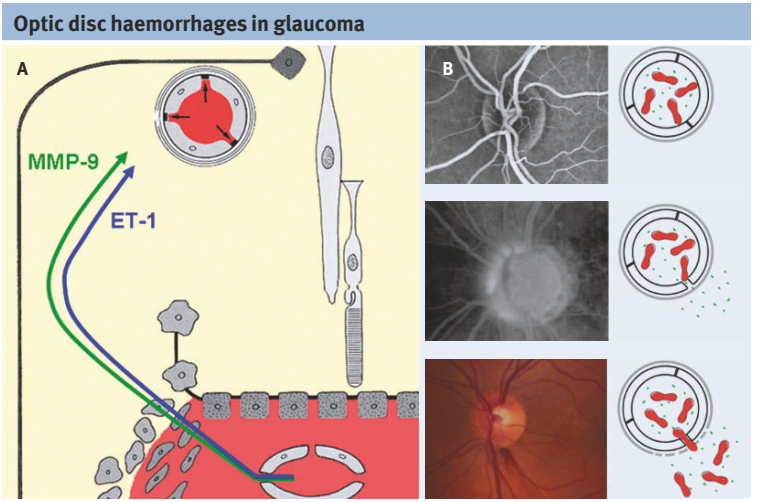

Figure 3.11 Optic disc haemorrhages in glaucoma. ET-1, endothelin 1; MMP-9, matrix metalloproteinase-9. **A**, schematic representation. **B**, top: depicts a physiological condition and minimal leakage of fluorescein; middle: a glaucomatous optic nerve head with a disturbed blood–brain barrier as a result of an increase in ET-1 leading to dysfunction of tight junctions. Clinically, a marked increase in fluorescein leakage is detected; bottom: if the basement membrane is simultaneously digested by MMP-9, erythrocytes may escape the vascular lumen leading to what is clinically observed as 'optic disc haemorrhages'.

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3.5, 3.6: Photo collection Dept. of Ophthalmology, University of Basel
3.9B: Reproduced with permission from Naumann GOH. Pathologie des Auges. Springer, Berlin (1997)
3.10: Flammer J, Mozaffarieh M (2007). Surv Ophthalmol 52: Suppl 2: S162-S173
3.11: Grieshaber MC, Flammer J (2007). Surv Ophthalmol 52: Suppl 2: S115-S121

Chapter 4

Risk factors for glaucoma

In discussing risk factors for glaucoma, we need to distinguish between risk factors which cause an increase in intraocular pressure (IOP), and those which lead to glaucomatous damage.

Risk factors for an increase in intraocular pressure

IOP increase in glaucoma (except primary open-angle glaucoma)

In addition to primary open-angle glaucoma (POAG), there are several other types of glaucoma marked by an increased in IOP (Figure 4.1). These will not be discussed here in any further detail, but include:

- congenital glaucoma
- juvenile glaucoma
- angle closure glaucoma
- pseudoexfoliation glaucoma
- pigmentary dispertion glaucoma
- secondary glaucoma due to uveiitis, rubeosis, lens luxation, hemorrhages.

IOP increase in primary open-angle glaucoma

In POAG the risk factors for an increase in IOP include:

- age, which increases with age
- a positive family history of POAG
- race, with people of African descent being at a greater risk.

In addition, atherosclerosis (and its associated risk factors) is also a risk factor for an increase in IOP. For example, smokers, patients with dyslipidemia, systemic hypertension or diabetes have a higher IOP, on average, than healthy individuals. Interventional studies also appear to support this relationship as treating dyslipidemia also leads to a slight reduction in IOP.

There is still debate as to why these risk factors are associated with an increase in IOP. On one hand, ischemia can damage the outflow system,

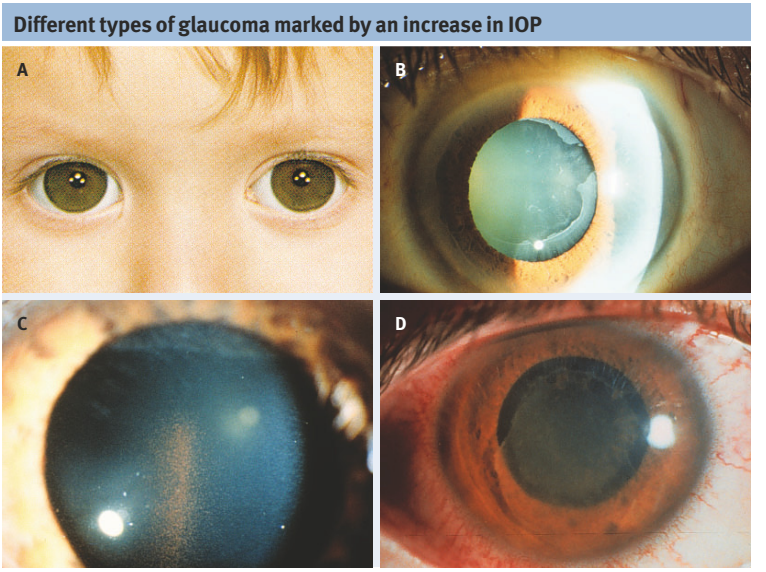

Figure 4.1 Different types of glaucoma marked by an increase in IOP. IOP, intraocular pressure. **A,** congenital glaucoma. **B,** pseudoexfoliation glaucoma. **C,** pigmentary dispersion glaucoma. **D,** fibrin in the anterior chamber of an eye afflicted with iritis.

particularly the trabecular meshwork, and thereby increase IOP. However, changes brought about at the molecular level in the trabecular meshwork of patients with glaucoma have similarities in the vessel walls of patients with atherosclerosis. This signifies that the two pathologies (of the trabecular meshwork and the arterial wall) may have common causes and similar pathomechanisms. We would like to mention, however, that although these relationships are highly significant, their association is relatively weak. When considering individual cases, for example, a patient with, let us say, systemic hypertension may not automatically have an increase in IOP.

Risk factors for glaucomatous optic neuropathy

The main and well-known risk factor for glaucomatous optic neuropathy (GON) is IOP; the higher the IOP, the higher the probability of developing GON. The relationship is not linear but rather curvilinear. Such a relationship seems to exist, to some extent, even in the normal range of IOP, which indicates that IOP may also be a risk factor for normal tension glaucoma (NTG).

In addition to the level of IOP, the fluctuation of IOP also seems to be relevant (Figure 4.2). This includes not only the circadian fluctuation but also

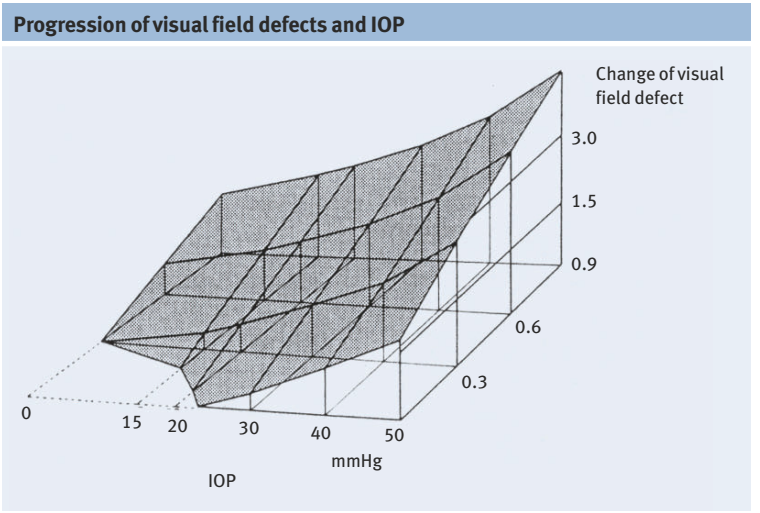

Figure 4.2 Progression of visual field defects and IOP. IOP, intraocular pressure. Progression of glaucomatous visual field defects (scotomas) depends weakly on the mean IOP and strongly on IOP fluctuation.

the fluctuation from one day to another. The relationship between IOP and GON, although highly significant, is astonishingly weak especially in the lower end of the IOP spectrum (ie, NTG), which indicates that other risk factors are involved as well.

In this context we would like to define these additional risk factors for GON, as those factors associated with an increase in the prevalence of GON when comparing patients with the same IOP. Risk factors are qualitatively but not quantitatively the same across different parts of the IOP spectrum. The lower the IOP at which damage occurs or progresses, the higher the probability that vascular factors are involved (Figure 4.3).

In terms of progression, a reduced OBF has a similar effect as an increased IOP. It is worth noting that risk factors for GON in patients with NTG are not identical to risk factors for the conversion of ocular hypertension (OHT) to POAG. This can be explained by the fact that patients with OHT at the beginning of an observation period had not developed GON despite a high IOP, whereas, in contrast, patients with NTG had already developed GON despite a normal IOP. The risk profile situation for the two populations, therefore, must be different.

For teaching purposes the risk factors for patients with OHT to develop to POAG will be discussed separately from those risk factors for NTG. However, all risk factors for NTG are also, to some extent, risk factors for GON in POAG.

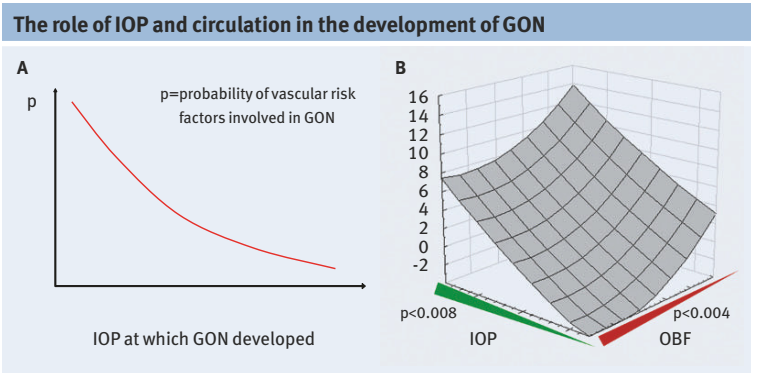

Figure 4.3 The role of IOP and circulation in the development of GON.
GON, glaucomatous optic neuropathy; IOP, intraocular pressure; OBF, ocular blood flow. **A**, the lower the IOP at which damage occurs or progresses, the higher the probability that vascular factors are involved. **B**, the higher the IOP and the lower the OBF, the higher the chance of progression of GON.

Risk factors for the conversion of ocular hypertension to primary open-angle glaucoma

Besides IOP, other factors which may lead to the development of POAG from OHT are:

- age
- corneal thickness
- cup to disc ratio
- disc hemorrhage.

Age

With age we have to bear in mind that any damage will accumulate over time. Naturally, the older the patient the higher the chance that this slowly increasing damage will reach the threshold of detection. It is possible that this damage progresses faster in older people however this needs further study. Other potentially important factors have not yet been studied in this context.

Corneal thickness

Corneal thickness influences IOP measurements, whether it is meaningful to correct the outcome of the IOP measurement by a correcting factor, based on corneal thickness, is still a debate as the correlation between corneal thickness and IOP, although significant, is very weak. Consequently, any correction may possibly cause further error. Corneal thickness may also be an independent risk factor for GON, however this requires further validation.

Cup/disc ratio

Additional risk factors described in the literature include the cup/disc (C/D) ratio and the C/D ratio asymmetry or pattern standard deviation. This is not surprising, as it indicates that patients with slight changes in morphology or visual function are already closer to the defined threshold of pathology. In other words, a patient who has reached subthreshold damage in the past, obviously has a higher chance of further damage in the future. Interestingly, studies have shown that patients with diabetes have a significantly lower risk of conversion from OHT to POAG. This observation challenged the old view that diabetes was a major risk factor for GON.

Risk factors for normal tension glaucoma

Most studies tend to focus on patients with NTG when analyzing risk factors for GON. In NTG the role of IOP is minimal, however other risk factors have a more clearly defined role. The focus of this chapter will be confined to NTG, as the profile of non-IOP risk factors can be demonstrated best in this group of patients. However, we would like to repeat that the risk factors for GON in NTG are also risk factors for GON in HTG, but less frequent. As a general rule, the lower the IOP at which damage occurs or progresses, the higher the chance of additional risk factors being involved. Known risk factors for NTG are:

- vascular dysregulation
- arterial hypotension
- ethnic origin
- gender, with women being most at risk.

Other risk factors include disc hemorrhages and gliosis-like alterations. Statistically, age is also a risk factor for NTG as the latter is almost never seen in young children or young adults. However, it is also a fact that patients with NTG often stabilize as they get older and rarely become totally blind. The reason for this might be the fact that major risk factors such as vascular dysregulation tend to mitigate as people get older.

Myopia is also reported to be a risk factor according to some studies, however other studies suggest that this may not be the case. Likewise, it is not clear whether peripapillary atrophy predisposes for damage, although the location of the damage often corresponds to the area of atrophy.

The prevalence of NTG also varies from one country to another; the proportion of patients with NTG among those with POAG is approximately 20% in Western countries, and up to 90% in countries such as Japan and Korea. This may be a result of both genetic and environmental factors. Women have double the risk for NTG than men, which may suggest that hormones are also involved.

The role of ocular blood flow

Atherosclerosis

As mentioned earlier, atherosclerosis and its risk factors are risk factors for an increase in IOP. However, at a given level of IOP patients with atherosclerosis (and its risk factors) have only a mildly increased risk for GON (Figure 4.4). Even patients with a carotid stenosis have a mildly increased risk for GON. Similarly, patients with decreased OBF due to high levels of endothelin have an increased risk for bland atrophy but not for GON.

Endothelin levels increase in many different inflammatory diseases (eg, multiple sclerosis, polyarthritis, AIDS), and in all these diseases the optic nerve head, while it sometimes becomes pale, does not excavate to the extent that it is defined as glaucoma (Figure 4.5). An exception to this rule is giant cell arteritis which, in contrast to non-arteritic anterior ischemic optic neuropathy (AION), leads to some excavation of the optic nerve head. This can be explained by the instability of blood flow in the early phase of this disease leading to reperfusion injury, which is discussed further in Chapter 7. However, low blood pressure and vascular dysregulation are relatively strong risk factors for GON and therefore worth discussing further.

Blood pressure

Arterial hypotension is a risk factor for GON. This includes low blood pressure (BP), orthostatic hypotension, and nocturnal dips (Figure 4.6). Similar to an

GON and atherosclerosis

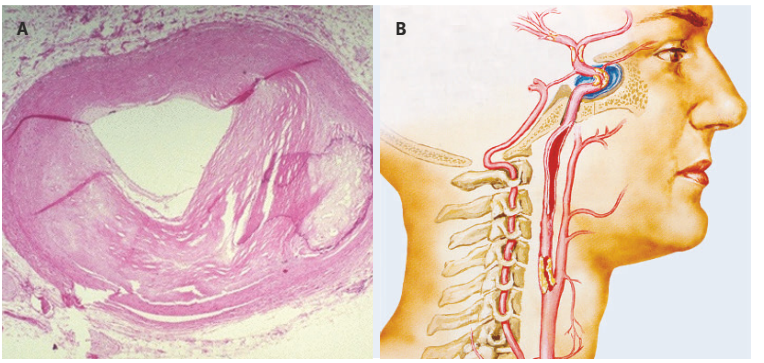

Figure 4.4 GON and atherosclerosis. GON, glaucomatous optic neuropathy. **A,** patients with atherosclerosis have only a mildly increased chance for GON. **B,** patients with a carotid stenosis rarely develop GON. Reproduced with permission from Netter, FH. Farbatlanten der Medizin. Band 6: Nervensystem II – Klinische Neurologie. Stuttgart: Thieme Verlag, 1989.

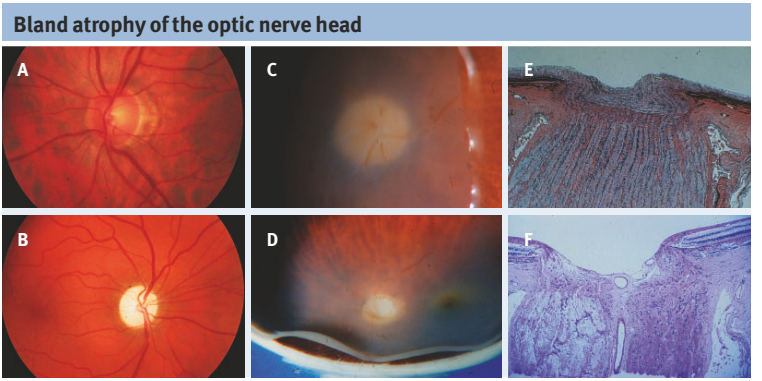

Figure 4.5 Bland atrophy of the optic nerve head. Both atherosclerosis and a high level of endothelin may lead to some optic nerve head atrophy as depicted in the bottom figures. **A,** clinical picture of a normal optic nerve head. **B,** clinical picture of an atrophic optic nerve head. **C,** photograph of a normal optic nerve head in a cadaver eye. **D,** photograph of an atrophic optic nerve head in a cadaver eye. **E,** histology of a normal optic nerve head. **F,** histology of an atrophic nerve head.

elevated IOP, low BP does not necessarily lead to GON in all patients. In order to better understand the role of BP in GON it is worth going over some of the basics concerning the physiology of circulation.

The total circulation in our body is equal to the cardiac output. However, this cardiac output is not simply a function of BP; people with high BP, on average, do not have a higher cardiac output than those with low BP. Perfusion pressure is, therefore, not the main factor affecting overall perfusion. The distribution of cardiac output to different organs and tissues is a function of local resistance to blood flow relative to other vascular beds in the body. Local resistance, however, may also depend on blood pressure. For example, if blood pressure drops, vasodilation occurs more intensively in vital organs (eg, the brain) and to a lesser extent in less vital organs (eg, the hands). The crucial issue, therefore, is not so much the level of blood pressure in itself, but rather how the eye adapts to high or low blood pressure. In a similar way, it is less relevant whether a drug reduces blood pressure but, rather, whether this drug reduces local resistance in the eye more or less than in other parts of the body. This explains why reducing blood pressure pharmacologically tends to cause an increase in OBF rather than a reduction.

On average, patients with NTG or POAG with disease progression (despite a normal IOP) tend to have lower blood pressure than non-progressive patients. However, not all patients with low blood pressure will progress. Whether low blood pressure is damaging depends on the role of autoregulation. As long as

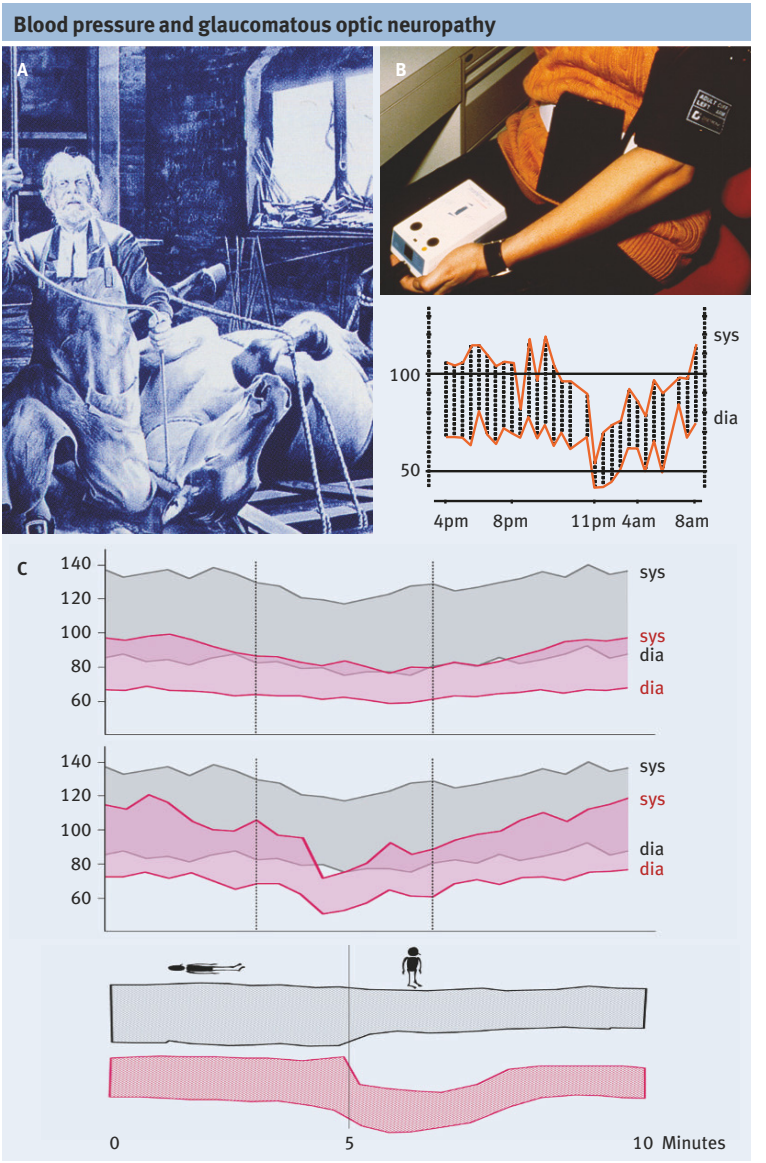

Figure 4.6 Blood pressure and glaucomatous optic neuropathy. A, it was not until 1733 that blood pressure was first directly measured in an artery of a mare, by the English naturalist, the Reverend Stephen Hales. **B**, today it is possible to register blood pressure over 24 hours. **C**, general low blood pressure, nocturnal dips and orthostatic hypotension are all risk factors for GON. Red: glaucoma patients, Gray: healthy control patients.

autoregulation functions well, low blood pressure can be tolerated. However, in patients with vascular dysregulation autoregulation is disturbed (Figure 4.7). The role of low blood pressure should therefore be discussed in the context of vascular dysregulation.

Vascular dysregulation

Studies indicate that both migraine and low blood pressure are significant risk factors for GON. Interestingly, both migraine and low blood pressure occur more often in patients with vascular dysregulation. The statistical relationship between low blood pressure and GON might be due, in part, to the relationship between vascular dysregulation and GON. These rather complex interrelationships and the fact that physicians are better trained to diagnose atherosclerosis and its risk factors rather than vascular dysregulation, will be discussed in more detail in Chapter 6. Before this, however, it is worth going over some of the basics concerning the regulation of OBF and this is discussed in the next chapter.

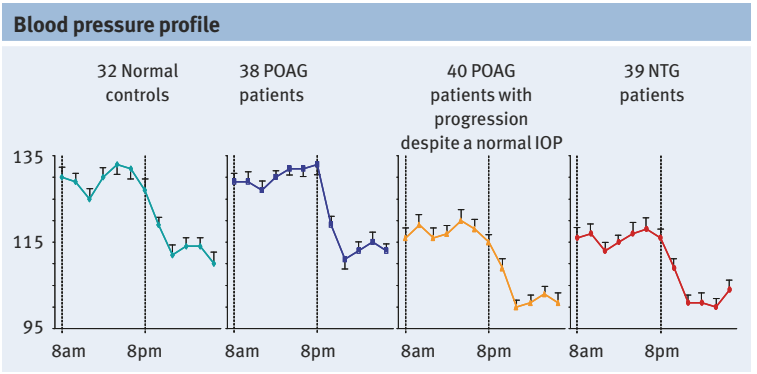

Figure 4.7 Blood pressure profile. IOP, intraocular pressure; NTG, normal tension glaucoma; POAG, primary-open angle glaucoma. Patients with NTG and POAG that progress despite having a normal or normalized IOP have a lower systolic blood pressure than control patients or stable POAG patients.

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Chapter 5

Regulation of ocular blood flow

The blood circulation provides transport for a large variety of molecules including oxygen, cells such as leukocytes, and heat. Regulation of blood flow is necessary to adapt to the varying internal and external demands of the body. During exercise, for example, the oxygen demand of the muscles increases dramatically. When we move quickly from a warm to a cold environment, our circulation adapts to redistribute body temperature in order to avoid too much heat loss.

Overall blood flow is regulated by cardiac output (Figure 5.1) which is controlled mainly by the autonomic nervous system and circulating hormones. The distribution of this cardiac output (minute volume) to different organs, or organ parts, is regulated by the relative local resistance to flow. A small vasoconstriction leads to a marked reduction in blood flow.

The regulation of ocular blood flow (OBF) shares common aspects known from general vascular physiology. The discussion here, however, shall be limited to those aspects specific to the eye.

Regulation of blood flow in the eye

The retina

The regulation of blood flow in the eye varies in different tissues (Figure 5.2). The regulation of retinal blood flow is very similar to the regulation of blood flow in the brain, with the exception that retinal blood flow has no autonomic innervation and therefore its regulation depends even more on the activity of endothelial cells. These cells release a number of vasoactive factors to regulate the size of the vessels by influencing both vascular smooth muscle cells (VSMC) and pericytes locally. These factors are also released, to a lesser extent, intraluminally to influence platelet aggregation and blood rheology and, when released, influence the size of vessels in remote organs of the body. Endothelial-derived vasoactive factors (EDVFs) will be covered in more detail later on in the chapter.

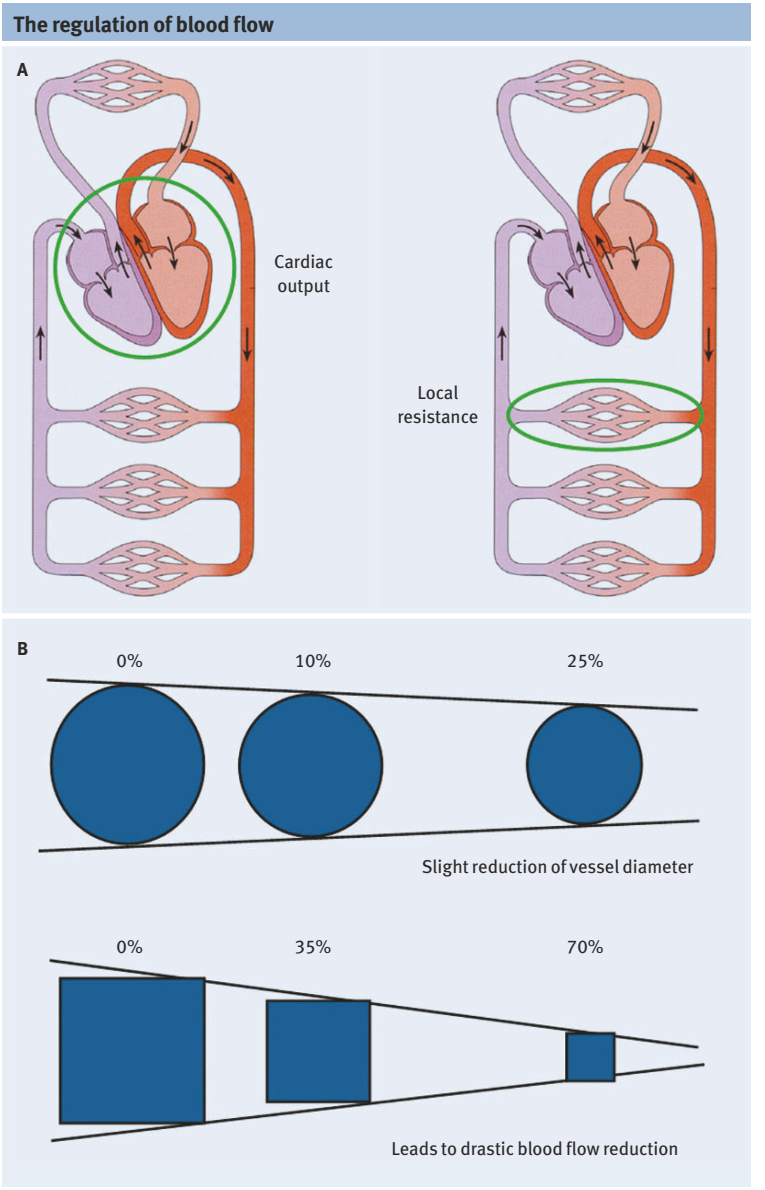

Figure 5.1 The regulation of blood flow. A, overall perfusion is identical to the cardiac output (left). The distribution to the different organs is regulated by the relative local resistance (right). **B,** a slight reduction of the vessel diameter leads to a marked reduction of blood flow.

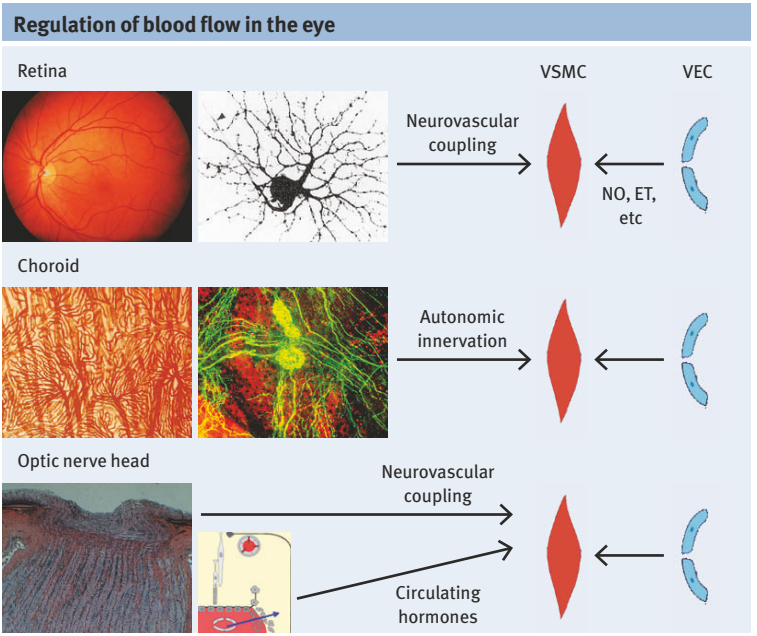

Figure 5.2 Regulation of blood flow in the eye. ET, endothelin; NO, nitric oxide; VEC, vascular endothelial cell; VSMC, vascular smooth muscle cell. While all vessels are under the influence of the vascular endothelial cells, the vessels of the retina and the optic nerve head (ONH) are also regulated by neural and glial cells whereas the choroidal vessels are autonomically innervated. The ONH is additionally influenced by the circulating hormones.

This regulation by the endothelial cells is crucial to the cell's ability to adapt to changes in perfusion pressure, which is known as autoregulation.

Neural and glial cells also influence the size of the retinal vessels – this is known as neurovascular coupling. For example, if flickering light hits the eyes the blood vessels dilate within seconds. The exact mechanism of this regulation remains unknown, however it is known that the production of nitric oxide (NO) is involved.

The choroid

The regulation of blood flow in the choroid is different from that of blood flow in the retina. The choroidal vessels are extensively autonomically innervated, and the capillaries are fenestrated. In addition to providing oxygen and other molecules to the retina, the choroid regulates the temperature of the back of the eye and most likely contributes to the fine tuning of accommodation by regulation

of volume. For example, the regulation of temperature can be demonstrated by the fact that moving from a warm to a cold environment causes the choroidal blood flow to increase within seconds. If one drop of a local anaesthetic is put into one eye, but not the other, the eye with the local anaesthetic will adapt much less as there are local cold sensors in the anterior part of the sclera.

The optic nerve head

Blood flow in the optic nerve head is regulated in a similar way to that in the retina with the important exception that no efficient blood–brain barrier (BBB) exists in the optic nerve head. Consequently circulating molecules such as vasoactive hormones, enzymes, or even drugs, have direct access to the smooth muscle cells and pericytes of the vessels in the optic nerve head (Figure 5.3). The impact of hormones on the ONH circulation is discussed in more detail on page 50.

Endothelial-derived vasoactive factors

Vascular endothelium is a confluent monolayer of flattened cells that line the inner surface of the vasculature. This layer is not just a barrier but also an active regulator of vascular tone. Endothelial cells function like a relay station receiving physical information (eg, sheer stress), chemical information (eg, oxygen tension) and biological information (eg, hormones). All of this information is integrated which leads to the production and release of endothelial-derived vasoactive factors (EDVF) as shown in Figure 5.4. EDVFs work in conjunction with other systems such as the autonomic nervous system. Among the EDVFs, the most important are: NO, endothelin-1 (ET-1), and prostacyclin (PGI_2).

Nitric oxide

NO has long been known by many to be a constituent of air pollution originating from exhaust emissions. Over the past few decades it has been recognised that NO also plays a very crucial role in biology. NO is a very small, liposoluble, free radical and, in contrast to NO in the atmosphere, in biological tissues has a short half life of only a few seconds. Although it diffuses easily, its action is limited to neighbouring cells. According to the very different biological functions of NO, three different enzymes known as nitric oxide synthase 1 (NOS1), nitric oxide synthase 2 (NOS2) and nitric oxide synthase 3 (NOS3) are involved in the production of NO (Figure 5.5).

NOS3, also known as endothelial nitric oxide synthase (eNOS), plays an important role in vascular regulation. This enzyme is expressed constitutively and its activity is regulated by calcium. In addition to the basal production of NO, the

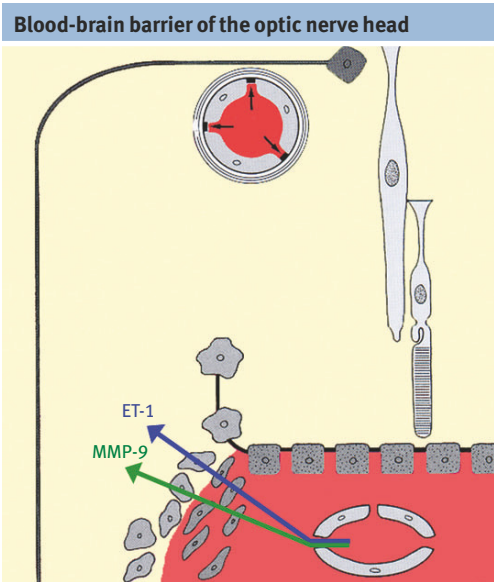

Figure 5.3 Blood-brain barrier of the optic nerve head. ET-1, endothelin-1; MMP-9, matrix metalloproteinase-9. Although the optic nerve head belongs to the central nervous system the blood–brain barrier is insufficient as molecules such as ET-1 or MMP-9 can diffuse from the choroid into the optic nerve head.

latter can be enhanced by tissue hormones such as bradykinin or acetylcholine. This stimulated production is temporarily disturbed by local anaesthetics (Figure 5.6). In order to avoid a reduction in blood flow in the back of the eye during glaucoma surgery, local anaesthetics are given subconjunctivally and not retrobulbarly. If NO diffuses into the neighbouring smooth muscle cells or pericytes it stimulates the enzyme guanylate cyclase, which increases the production of cyclic guanine monophosphate (cGMP) (Figure 5.7). This leads to the relaxation of smooth muscle cells and pericytes which, in turn, leads to vasodilation. We will encounter the NO molecule in Chapter 8 when discussing the pathophysiology of GON. It is often confusing whether NO is a good or bad molecule, however as will be discussed later, this depends on the location, concentration, and more importantly the presence of other molecules.

Endothelin

ET is a 21-amino acid peptide (Figure 5.8) that is secreted mostly abluminally, but also intraluminally, leading to a concentration of endothelin-1 (ET-1) in the blood. Three different isoforms of ET exist, namely:

- ET-1
- ET-2
- ET-3

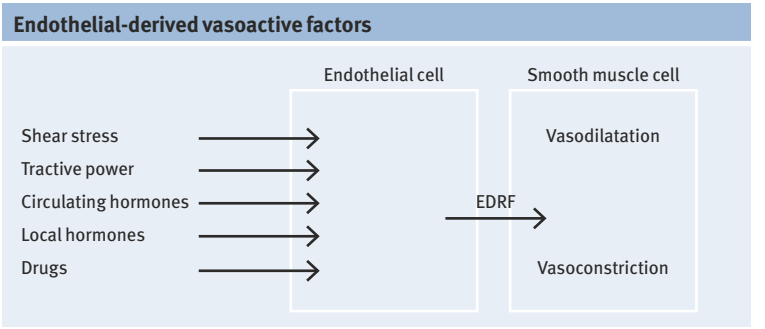

Figure 5.4 Endothelial-derived vasoactive factors. EDRF, endothelium-derived relaxing factor. Endothelial cells receive physical, chemical and biological information. All this information is integrated which leads to the production and release of endothelial-derived vasoactive factors and either to vasodilatation or vasoconstriction.

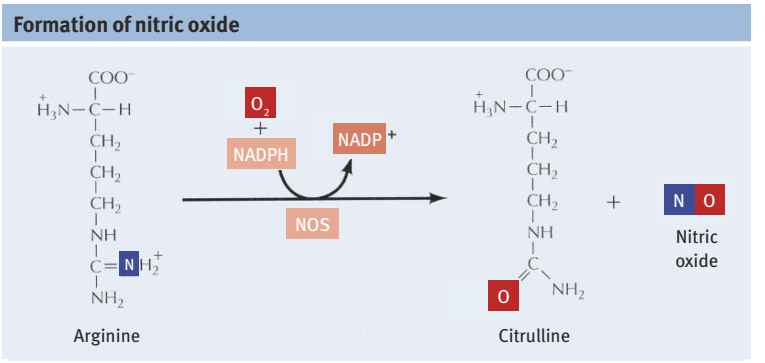

Figure 5.5 Formation of nitric oxide. $NADP^+$, nicotinamide adenine dinucleotide phosphate (reduced form); NADPH, nicotinamide adenine dinucleotide phosphate (oxidized form); NO, nitric oxide; NOS nitric oxide synthase; O_2, oxygen. L-arginine is converted into citrulline and nitric oxide in the presence of NADPH (reducing factor) and oxygen by the enzyme NOS.

The most important vasoconstrictive factor is ET-1. There are also two different ET receptors: endothelin A (ETA) receptor and endothelin B (ETB) receptor. The stimulation of ET receptors on smooth muscle cells or pericytes increases the influx of cytoplasmic calcium into the cell and liberates calcium from the internal storage supply which leads to constriction of the vessels (Figure 5.9). If the concentration of ET-1 is sufficiently high, vasospasm can occur.

Circulating hormones

As mentioned earlier, vasoactive molecules are secreted intraluminally and are therefore present in the circulating blood. A clinically important example is

Nitric oxide and local anaesthetics

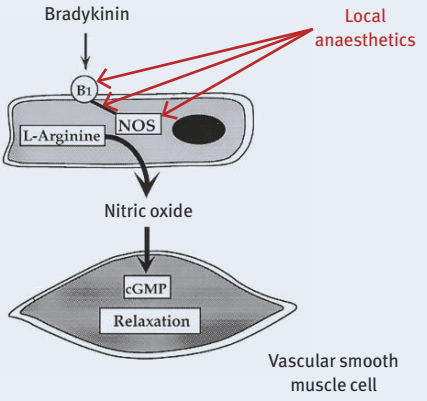

Figure 5.6 Nitric oxide and local anaesthetics. B_1, bradykinin receptor; cGMP, cyclic guanosine monophosphate; NOS, nitric oxide synthase. Nitric oxide production is temporarily disturbed by local anaesthetics.

The effect of nitric oxide diffusing into neighbouring cells

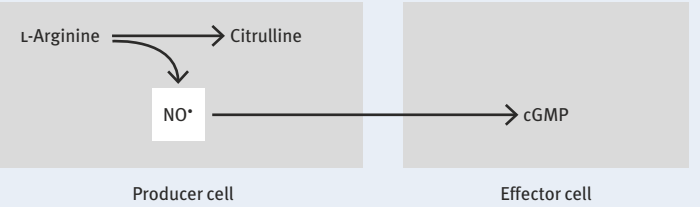

Figure 5.7 The effect of nitric oxide diffusing into neighbouring cells. cGMP, cyclic guanosine monophosphate; NO, nitric oxide. NO leads to an increase in cGMP in neighbouring cells. The effect of cGMP depends upon cell type. In smooth muscle cells it leads to relaxation.

Endothelin-1

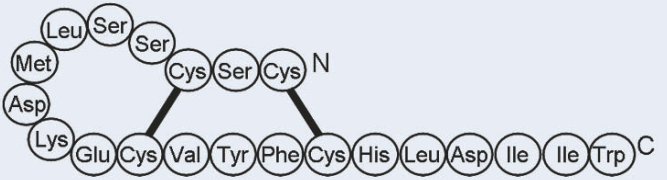

Figure 5.8 Endothelin-1. There are three isoforms of endothelin. This figure shows the amino acid sequence of endothelin-1.

The effect of endothelin-1 in circulating blood

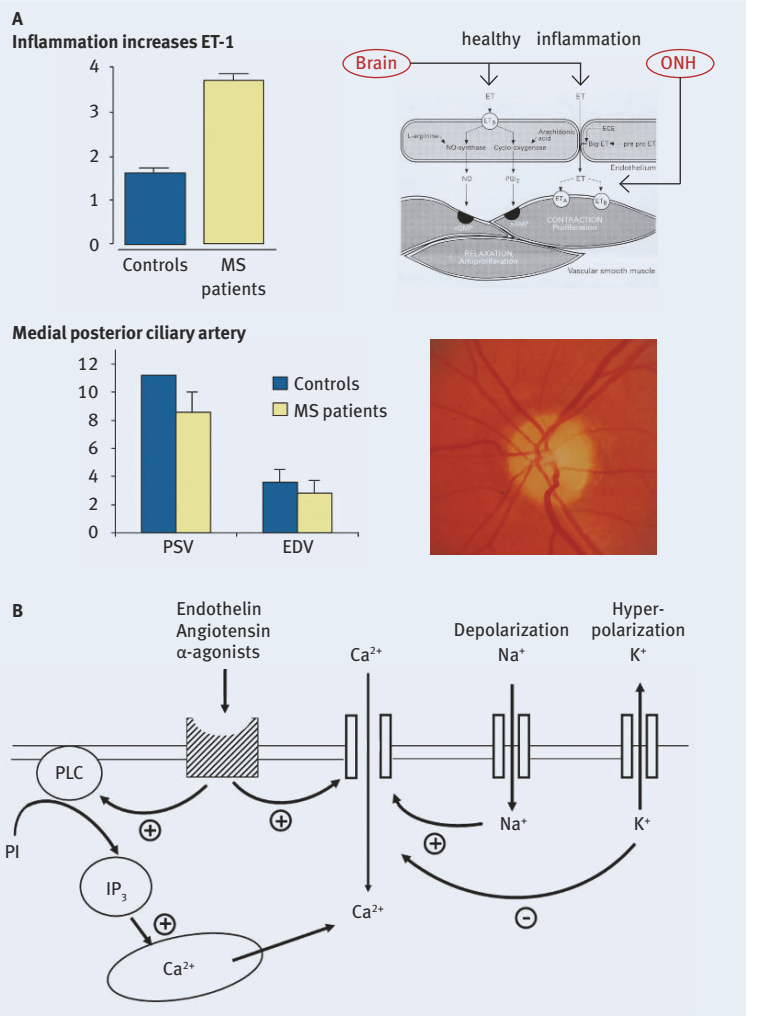

Figure 5.9 The effect of endothelin-1 in circulating blood. Ca^{2+}, calcium; EDV, end-diastolic velocity; ET-1, endothelin-1; IP_3, inositol triphosphate; K^+, potassium; MS, multiple sclerosis; Na^+, sodium; ONH, optic nerve head; PI, inorganic phosphate; PSV, peak systolic velocity; PLC, phospholipase C. **A**, ET-1 is increased in many inflammatory diseases such as MS (upper left). This has little impact on brain or retinal circulation as long as the barrier is intact (upper right). However, it reduces blood flow in the choroid and ONH and thereby also in the retroocular vessels as depicted for MS patients (lower left). This sometimes leads to a slight paleness of the optic nerve head (lower right). **B**, stimulation of ET receptors: endothelin similar to angiotensin-2 and α-agonists leads to an increase of intracellular calcium both by opening the calcium channels and by liberation from internal storages.

angiotensin-I which is converted locally by the membrane-bound angiotensin-converting enzyme to angiotensin-II. The effect of these molecules depends on the site of action. For example, serotonin dilates vessels by stimulating the S1-receptors on endothelial cells, whereas it constricts vessels when it has access to S2-receptors on smooth muscle cells.

In a similar way, ET-1 has a minimal effect on brain circulation as due to the BBB, it only has access to the B-receptors on endothelial cells. However, vasoconstriction occurs if, as in the case of a disturbed blood–retinal barrier, ET-1 has direct access to the ETA or ETB receptors of smooth muscle cells. An intravenous infusion of ET-1 in young healthy subjects therefore leads to a major reduction in choroidal blood flow (ie, due to fenestrated capillaries), but does not significantly change the brain circulation (Figure 5.9). In conditions where the BBB is disturbed (eg, in an acute plaque of a patient with multiple sclerosis [MS]) circulating ET-1 leads to vasoconstriction. Interestingly, the ONH although anatomically part of the central nervous system (CNS), is not fully protected by a barrier. This is due to diffusion from the surrounding choroid and the fact that blood vessels in the ONH, originating from the ciliary circulation, have a weaker barrier function than the capillaries in other parts of the CNS or the retina. This explains why circulating hormones such as angiotensin-II or ET-1 have a major impact on the blood circulation in the ONH and have no impact in the retina or in the CNS while the vessels there remain intact. ET levels are raised in many clinical conditions, such as MS. This explains why MS patients have perfusion in the choroid and ONH, and often have a slightly pale ONH.

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5.2, 5.9A: Flammer J, Mozaffarieh M (2008) Can J Ophthalmol 43(3): 317-321
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5.4: Copyright Flammer; used with permission from Flammer: Glaucoma. A Guide for Patients. An Introduction for Care-Providers. A Quick Reference. Hans Huber, Hogrefe, Bern (2006)
5.6, 5.9B: Grieshaber MC, Mozaffarieh M, Flammer J (2007) Surv Ophthalmol 52: Suppl 2: S144-S154

Chapter 6

Vascular dysregulation syndrome

What is vascular dysregulation?

In biology any cell, organ or living organism must constantly adapt to different environments, and only organisms which have adapted well to their environment have survived evolution. Several systems are normally involved in the regulation of a given parameter, and these work in such a way that if one system fails, the other systems compensate. The regulation of different parameters is often interrelated, for example, regulation of blood flow also controls oxygen supply, temperature and volume. This explains why a basic dysregulation, such as vascular dysregulation, leads to many different and seemingly unrelated symptoms. Vascular tone is regulated on different levels of the vascular bed (eg, large or small arteries, capillaries, veins). Dysregulation means that this local regulation is not carried out in accordance with the needs of the organ or its corresponding tissues. This may be due to damage of local endothelial cells caused by rupture of an atherosclerotic plaque or by mechanical irritation of a vessel. In addition, inflammation of the vessel walls may also hinder effective local regulation.

The discussion here will be confined to the more systemic type of dysregulation which can be grouped into: primary vascular dysregulation (PVD) and secondary vascular dysregulation (SVD). In the context of glaucoma, PVD is more important than SVD therefore most of the discussion in this chapter will center on PVD, however some aspects of SVD will also be discussed.

Primary vascular dysregulation

PVD refers to a genetic predisposition to respond differently to a number of discrete stimuli such as cold, mechanical or emotional stress. The fact that it largely involves, but is not exclusively confined to, the vascular system has led to it being known as vascular dysregulation syndrome. Moreover, because it is not caused by another disease, and has a hereditary component it is called primary vascular dysregulation. Vasospasm is a prominent, but

not exclusive, sign and in the past this syndrome was known as primary vasospastic syndrome (Figure 6.1).

What are the underlying mechanisms of PVD?

The causes and basic mechanisms underlying PVD at the molecular level are still unknown. It is difficult to distinguish between patients with PVD and those without PVD under baseline conditions, however, the former do respond differently to stimuli such as cold, mechanical or psychological stress.

Subjects with PVD have a normal capacity to produce adenosine triphosphate (ATP) and therefore appear to be as strong as those without PVD. Under certain circumstances, however (eg, sitting quietly in a cold environment), they are not able to produce as much ATP-independent heat as those without PVD (Figure 6.2). This is one of the reasons why subjects with PVD become cold (Figure 6.3), and why, in an attempt to reduce heat loss, vessels in their extremities constrict. The ocular vessels behave very similarly to skin vessels. This is why measurements of blood flow in the nailfold capillaries provides indirect information on OBF.

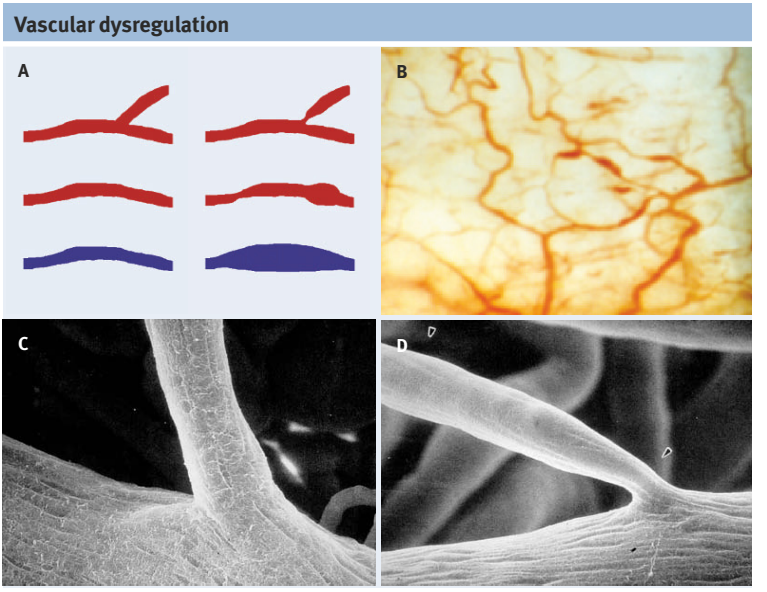

Figure 6.1 Vascular dysregulation. A, vascular dysregulation can lead to vasoconstriction (vasospasm) as well as to vasodilation. **B,** dysregulation can also be observed in conjunctival vessels. **C, D,** spasms occur often at the ramifications. Reproduced with permission from Cioffi et al. Curr Eye Res 1995; 14: 1147–53.

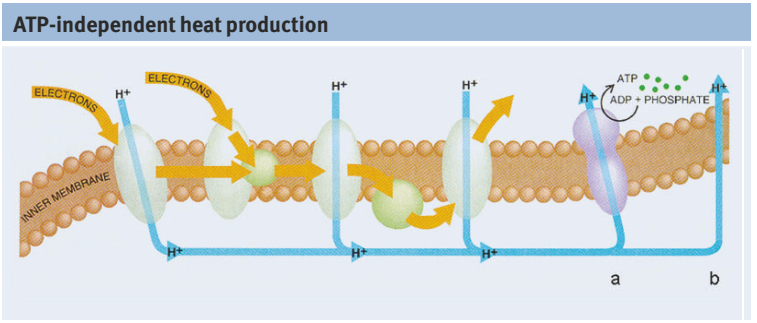

Figure 6.2 ATP-independent heat production. ADP, adenosine diphosphate; ATP, adenosine triphosphate. The energy of the electrons in the respiratory chain pumps protons (H^+) into the matrix of the mitochondria. The resulting proton gradient is used either to drive the enzyme ATPase for ATP production (a) or to produce heat directly (b).

PVD syndrome occurs more frequently in women than in men, in Japanese populations than in Caucasian populations, and in academics than in blue collar workers. The symptoms normally manifest initially in puberty and mitigate with age which indicates that sexual hormones may play a role.

Clinical signs and symptoms of PVD

There is no real gold standard for the diagnosis of PVD although cold provocation in nailfold capillaromicroscopy is the most often used diagnostic test by angiologists. In clinical practice, however, tests are not always necessary as there are certain signs and symptoms that clearly point toward PVD, these include:

- low blood pressure, especially when younger
- cold extremities (eg, cold hands or feet)
- a reduced feeling of thirst (ie, drinking because they recognize they should drink and not because they are thirsty)
- often a low body mass index
- frequent migraines, although PVD and migraine are two distinct entities, people with PVD more often suffer from migraines than people without PVD
- altered drug sensitivity.

Altered drug sensitivity may be partially due to these subjects having an altered expression of ABC transport proteins, which are also involved in the transport of drugs (Figure 6.4). The sensitivity for certain groups of drugs (eg, calcium channel blockers, systemic β-blockers) is increased and this explains why these subjects do not appear to tolerate certain drugs well. In reality, it is simply a question of dosage; this means that by lowering the dose of these drugs a good effect can still be achieved but the side-effects

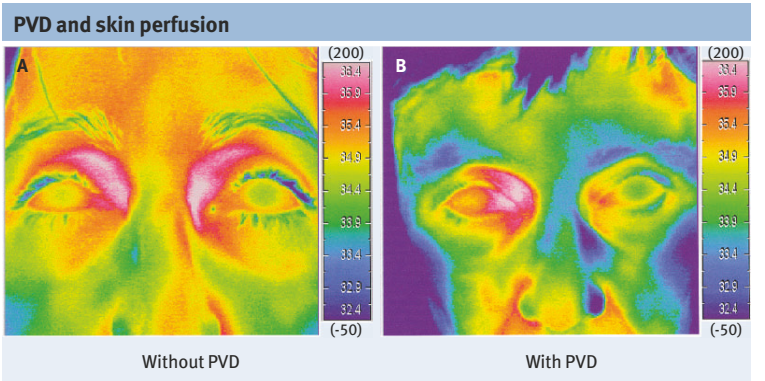

Figure 6.3 PVD and skin perfusion. PVD, primary vascular dysregulation. **A**, patient without PVD. **B**, patient with PVD. Patients with PVD often have cold extremities as a result of reduced skin perfusion.

can be significantly reduced. For certain drugs (eg, pain killers) sensitivity is normal or even decreased.

Subjects with PVD have a good sense of smell. They also have, on average, a longer sleep onset time especially when they are cold and warming up the feet becomes a strict prerequisite to falling asleep (Figure 6.5). They often have a meticulous personality and are therefore very successful in their professions. In daily practice, some of these symptoms are not mentioned spontaneously by the patient but can be pinpointed by specifically asking. Some of the clinical symptoms and signs of PVD are described, in more detail, next.

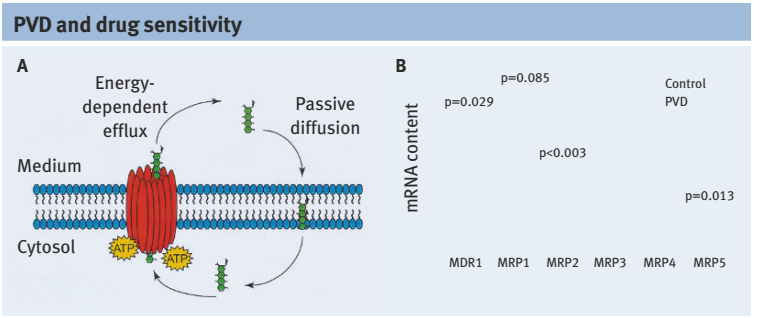

Figure 6.4 PVD and drug sensitivity. ATP, adenosine triphosphate; MDR, multidrug resistance; MRP, multidrug resistance protein; PVD, primary vascular dysregulation. **A**, drugs diffuse passively into the cells. They are pushed out of the cell actively by ATP consumption. As these proteins have an ATP binding cassette they are called ABC transport proteins. Reproduced with permission from Johnstone et al. Trends Biochem Sci 2000; 25:1–6. **B**, subjects with primary vascular dysregulation have some ATP proteins upregulated and others down-regulated.

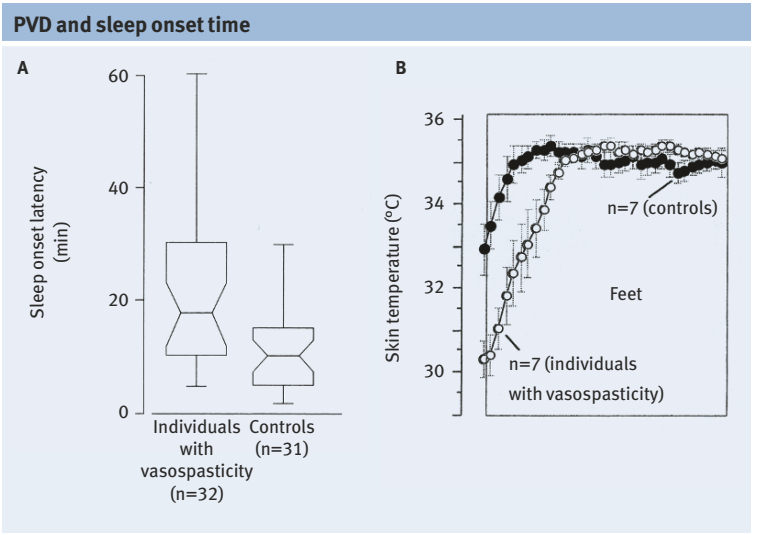

Figure 6.5 PVD and sleep onset time. PVD, primary vascular dysregulation. **A,** subjects with PVD have a prolonged sleep onset time. **B,** this is because subjects with PVD have cooler feet and it takes a longer time to warm them up.

PVD and blood pressure

Patients with PVD tend, on average, to have low blood pressure, especially at night and particularly when they are young. Some patients also suffer from orthostatic hypotension. The major cause for systemic hypotension in these patients is reduced reabsorption of sodium in the proximal tubule of the kidneys (ie, subjects with PVD lose more salt in the kidneys) as shown in Figure 6.6. This dysfunctional management of sodium in the kidney, which is partially dependent on ET-1, has also been seen in patients with normal tension glaucoma (NTG). Nocturnal non-dipping and over-dipping, however, are not only a consequence of PVD but rather a manifestation of a dysfunctional autonomic nervous system.

PVD and temperature

Cold hands are a leading symptom of PVD, however people with cold hands may not realize this as they are accustomed to having cold hands. Shaking a patient's hand can often provide the doctor with sufficient information. When temperature is measured (eg, using thermography), temperature differences are even more striking in the legs. However, this information is not as easy to use in daily clinical practice.

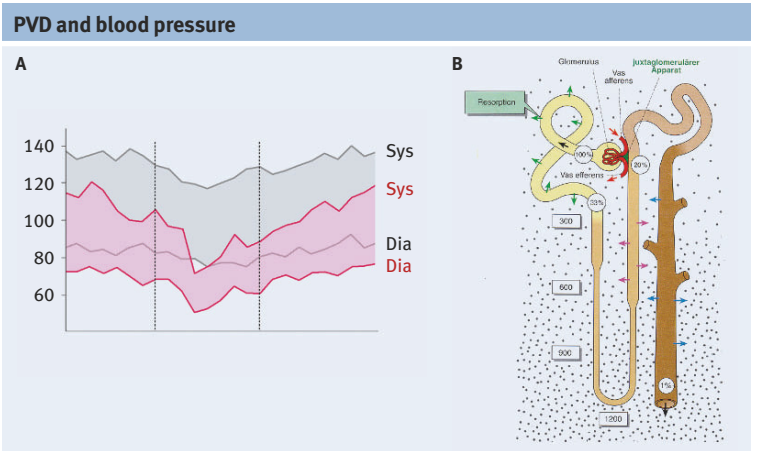

Figure 6.6 PVD and blood pressure. Dia, diastole; PVD, primary vascular dysregulation; Sys, systole. **A,** PVD subjects tend to have a low blood pressure. **B,** the major cause for systemic hypotension in these patients is reduced reabsorption of sodium in the proximal tubule of the kidneys.

PVD and thirst

Patients with PVD often have a reduced feeling of thirst which is not specific for PVD as it also occurs in other diseases where there is an increased level of ET-1 (eg, multiple sclerosis). This phenomenon can be explained by a mild increase in ET-1 which exerts, via prostaglandin-E2, an antidipsogenic effect on the hypothalamus which regulates thirst (Figure 6.7). The average daily intake of fluid of patients with PVD is only slightly less than patients without PVD. However, this is due to the fact that the former drink because they recognize the need, rather than having a desire, to do so.

PVD and mechanical stress

Subjects with PVD respond with vasoconstriction in areas which are exposed to mechanical stress. This is why, for example, people with PVD cannot work on vibrating instruments such as compressors. This is also the reason why after a whiplash trauma, subjects with PVD have more extensive and longer-lasting symptoms.

PVD and emotional stress

It is well known that certain subjects when under stress respond with red or white spots on their face or throat. Although this change is impressive, it is harmless. However, if a similar type of dysregulation occurs in a vital organ, (eg, the eye) it is feasible that this may cause long-term damage. Patients frequently indicate that they have cold hands when under stress.

PVD and thirst

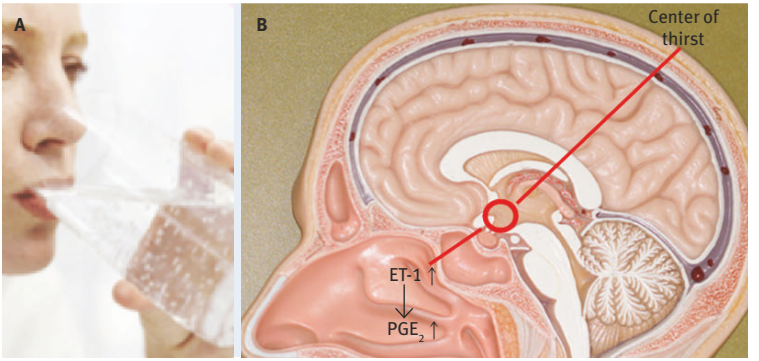

Figure 6.7 PVD and thirst. ET-1, endothelin-1; PGE_2, prostaglandin-E2; PVD, primary vascular dysregulation. **A**, PVD subjects, on average, have less thirst than others. **B**, this is due to a mild increase in ET-1 which, via PGE_2, exerts an antidipsogenic effect on the hypothalamus.

PVD and migraine

Although there is a relationship between migraine and PVD, the two conditions should not be confused. Migraine is essentially a spreading depression of neural cell activity in the brain causing transient vasoconstriction and vasodilation. As subjects with PVD are more sensitive to different kinds of stimuli, it is not surprising that they also respond stronger to this spreading depression. Patients with migraine, on average, suffer more often from PVD and vice-versa. However, there are some people with PVD who have never had a migraine, and people with migraine who have had no symptoms of PVD. If, however, a patient with PVD does suffer from a migraine, the probability of having aura-type symptoms is higher. These can be in the form of visual symptoms but can also sometimes lead to a unilateral attack of cold hands and, in extremely rare cases, a transient paralysis. A migraine attack can be triggered by emotional or physical stress and also from an orgasm. The migraine-induced vasospasms that lead to visual symptoms are normally located in the area of the visual cortex and rarely in the retina. Spasms, however, can occur in any vessel including the mid-basilar artery (Figure 6.8).

Primary vascular dysregulation and other risk factors for glaucomatous optic neuropathy

PVD as a risk factor for GON explains the following:

- women suffer more often from NTG than men (women are more likely to be disposed to PVD)

Vasospasms during a migraine attack

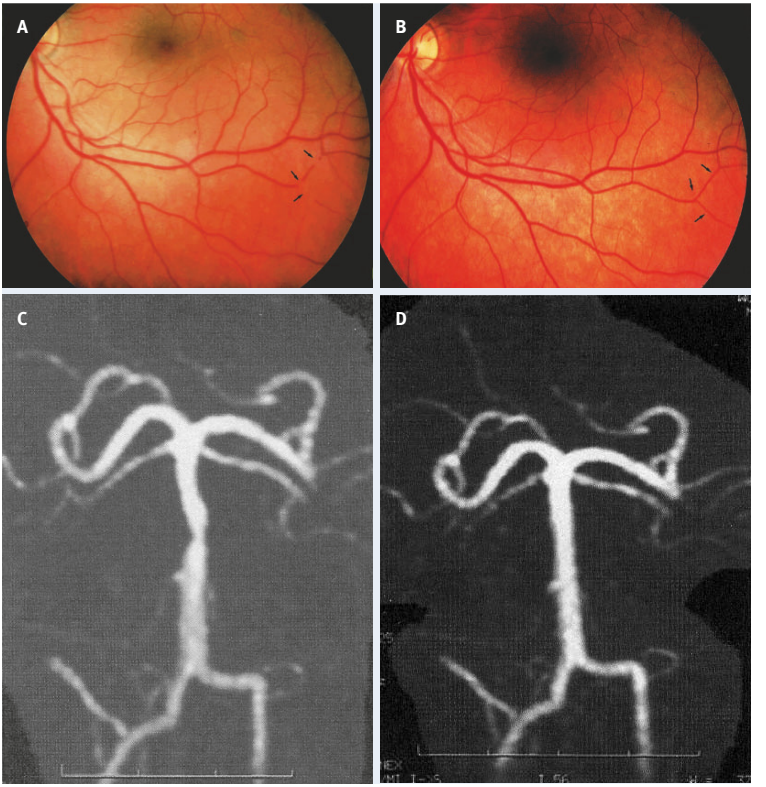

Figure 6.8 Vasospasms during a migraine attack. A, fundoscopy reveals vasospasms during a migraine attack (arrows). **B,** shortly after the attack the vasospastic reaction has normalized (arrows). **C,** local constriction in the mid-basilar artery in an orgasmic headache. **D,** the same vessels six months later. Figures C & D reproduced with permission from Schlegel et al. Headache 2004; 44:710–2.

- migraine is a risk factor for GON (patients suffering from migraines frequently suffer from PVD)
- people of Japanese or Korean descent frequently suffer from NTG, (this population group also suffers significantly from PVD).

Moreover, the fact that PVD is also a main cause of splinter hemorrhages at the border of the ONH explains why:

- ONH hemorrhages occur more often in patients with NTG than in patients with high tension glaucoma (HTG)
- hemorrhages occur more often in women than men
- hemorrhages can occur in the very early stages of the disease.

The involvement of PVD also explains why certain patients with a low blood pressure (BP) acquire glaucomatous damage whereas other patients do not. If systemic hypotension is due to failure of the autonomic nervous system (eg, patients with Shy–Drager syndrome or diabetic autonomic neuropathy), autoregulation of OBF will still function and therefore will compensate for low perfusion pressure. If however, low BP is due to PVD, the same subjects will also suffer from disturbed autoregulation making them susceptible to a drop in perfusion pressure.

PVD as a risk factor for GON also explains why patients with glaucoma, particularly NTG, suffer from silent myocardial ischemia more often than healthy control patients. This can be explained by the fact that PVD can also cause silent myocardial ischemia.

Primary vascular dysregulation and eye circulation

The regulation of OBF in patients with PVD is different in a number of ways:

- Morphologically the retinal vessels demonstrate a high level of irregularity (Figure 6.9).
- When stimulated by flicker light, the retinal vessels respond with a small vasodilation.
- When stimulated with a hand-grip test (ie, to stimulate the sympathetic nervous system) the choroidal vessels in the retina constrict more than normal.

This complex dysfunction in regulation, leads to an incomplete adaptation to stimuli and, in turn, to an unstable ocular perfusion. This instability of blood flow leads to repeated but very mild reperfusion which contributes, via oxida-

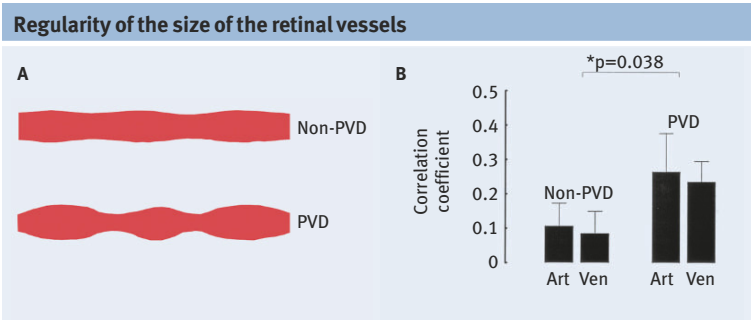

Figure 6.9 Regularity of the size of the retinal vessels . Art, artery; Ven, vein; PVD, primary vascular dysregulation. **A, B,** the retinal vessels of PVD subjects show higher irregularities than non-PVD subjects indicating that the dysregulation is not homogenous along the vessel.

tive stress, to glaucomatous damage (Figure 6.10). Reperfusion appears to be a key factor and is discussed further in Chapter 7.

Primary vascular dysregulation and other eye diseases

In addition to glaucoma PVD is also a risk factor for the following diseases:

- Anterior ischemic optic neuropathy (AION) – this normally occurs in elderly patients with risk factors for atherosclerosis. Sometimes it can be observed in younger patients without any risk factors for atherosclerosis (Figure 6.11). These type of patients normally suffer from PVD. In these patients AION occurs after major emotional stress.
- Retinal vein occlusion (RVO) – this also occurs more often in elderly subjects and, interestingly, risk factors for atherosclerosis are also risk factors for RVO. Occasionally, RVO occurs without any recognizable risk factors and such patients usually also suffer from PVD (Figure 6.12).

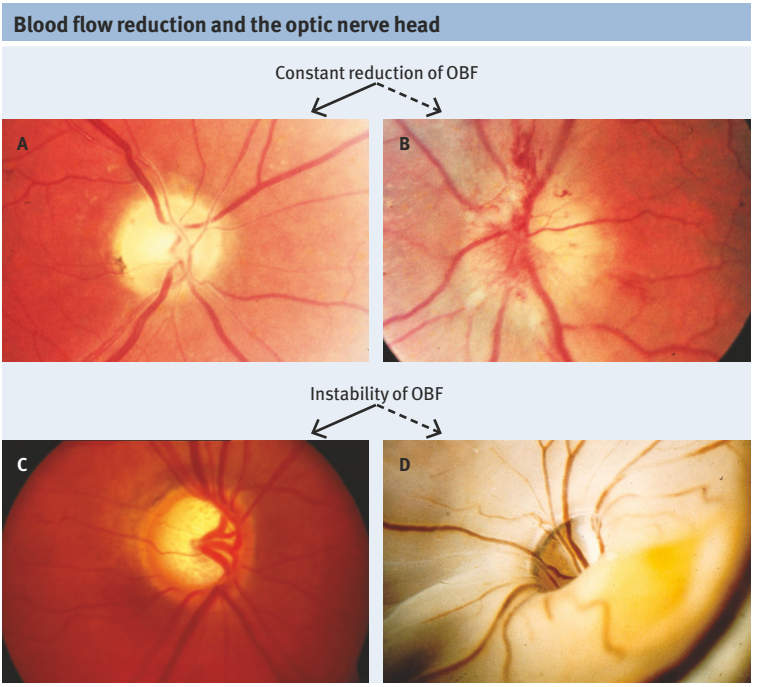

Figure 6.10 Blood flow reduction and the optic nerve head. OBF, ocular blood flow. **A,** a constant reduction of OBF leads to bland atrophy. **B,** in extreme cases even to infarction. **C, D,** the instability of blood flow leads to glaucomatous optic neuropathy.

AION with the corresponding visual field defect

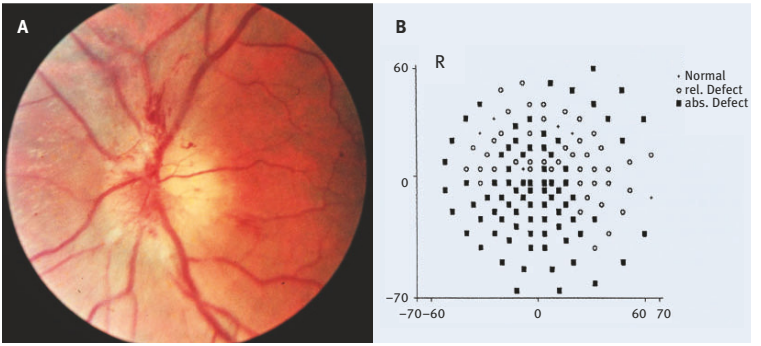

Figure 6.11 AION with the corresponding visual field defect. AION, anterior ischemic optic neuropathy. AION in a young, healthy individual without atherosclerosis but marked primary vascular dysregulation syndrome.

- Retinal arterial branch occlusion – this is sometimes a consequence of PVD (Figure 6.13).
- Susac syndrome – this is defined as a circulatory disturbance in the eye, ear and brain. It is often assumed that this might be a consequence of an inflammatory process. It has been observed that these subjects often suffer from a PVD (Figure 6.14).
- Central serous chorioretinopathy (CSC) – this is a reversible disturbance of the outer retinal blood–brain barrier. As discussed previously, a

Retinal vein occlusion

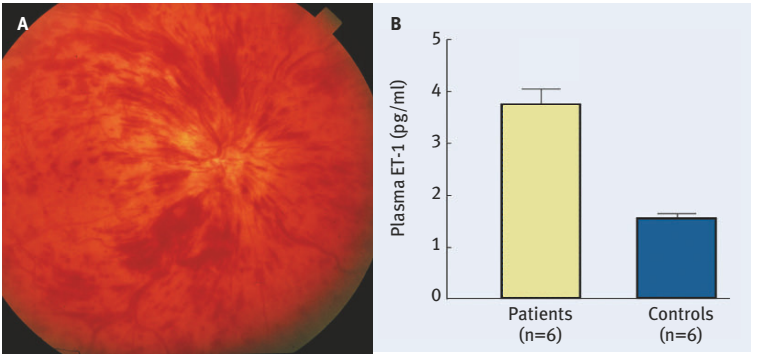

Figure 6.12 Retinal vein occlusion. ET-1, endothelin-1. **A**, retinal vein occlusions in young subjects without classical risk factors but with primary vascular dysregulation. **B**, increased levels of endothelin in the circulating blood.

Retinal arterial branch occlusion

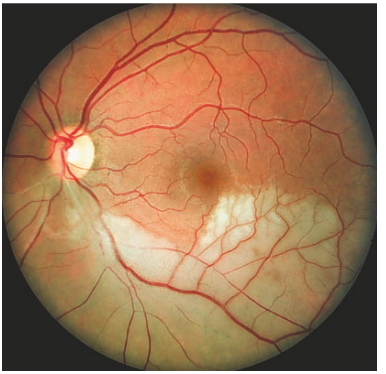

Figure 6.13 Retinal arterial branch occlusion. This young banker with primary vascular dysregulation had an arterial occlusion as the shares dropped.

dysregulation of the size of the blood vessels is often paralleled by a dysregulation of the barrier function. Patients with CSC normally suffer from PVD syndrome and their endothelin levels are very high for a few days or weeks (Figure 6.15).

Secondary vascular dysregulation

Secondary vascular dysregulation (SVD) refers to a situation where dysregulation occurs as a consequence of another disease. As a result of an underlying disease the levels of vasoactive substances in the blood, particularly endothelin, are high. As mentioned, under physiological conditions endothelin is produced by endothelial cells which release vasoactive substances abluminally and also intraluminally. Under pathological conditions, however, any cell under marked stress can produce vasoactive molecules such as endothelin and thereby contribute to the level of endothelin in the blood. For example, in patients with arthritis the synovial cells produce endothelin, in multiple sclerosis it is the lymphocyte cells, and in AIDS the macrophages produce endothelin. The consequence of such a high level of endothelin varies from one organ to the other as vasoactive substances only have access to smooth muscle cells and pericytes in organs that have fenestrated capillaries (eg, the choroid). If the blood–retinal barrier is intact, the effect is minimal. If, however, the blood–retinal barrier is disturbed, endothelin leads to vasoconstriction and in the choroid it leads to a reduction of blood flow. Endothelin can also diffuse into the optic nerve head.

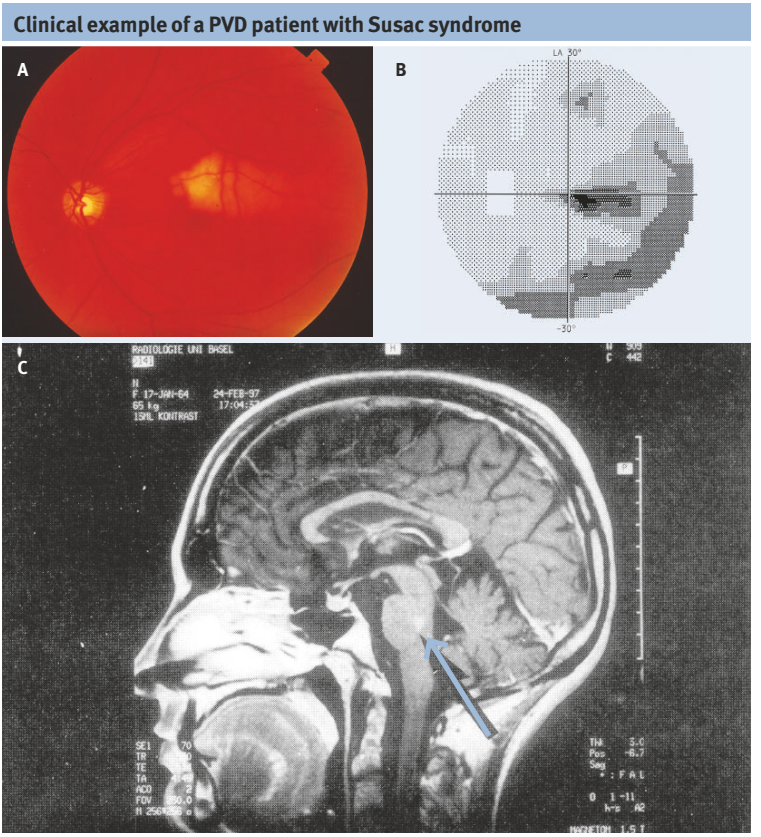

Figure 6.14 Clinical example of a PVD patient with Susac syndrome. PVD, primary vascular dysregulation. **A,** retinal arterial branch occlusion. **B,** corresponding visual field defects. **C,** an additional ischemic lesion in the brain (arrow). The patient also suffered from hearing problems.

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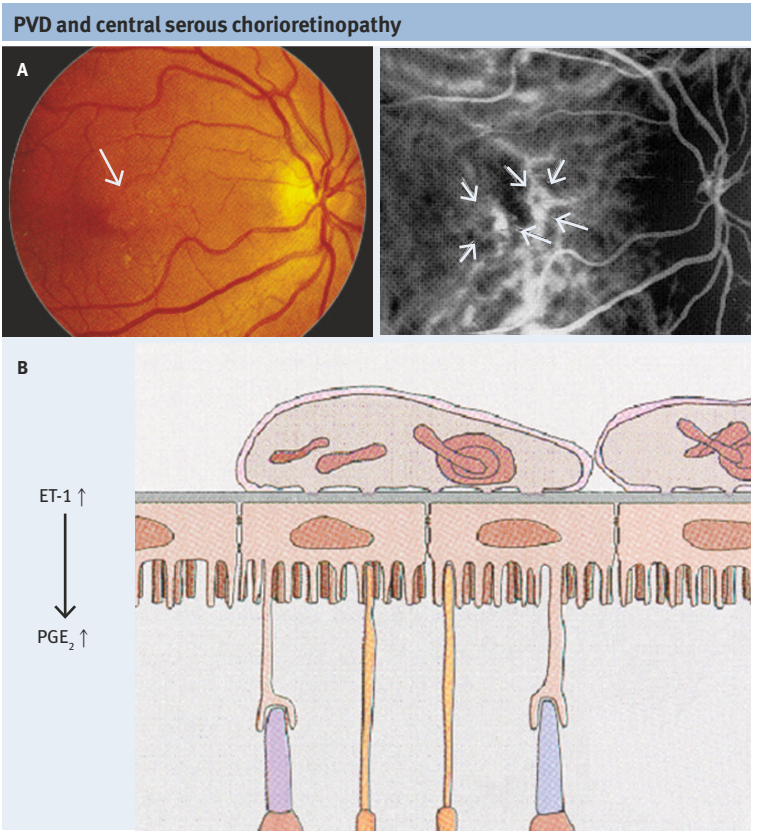

Figure 6.15 PVD and central serous chorioretinopathy. ET-1, endothelin-1; PGE_2, prostaglandin E2; PVD, primary vascular dysregulation. **A,** clinical picture of the fundus showing the chorioretinopathy (left). Dysregulation of the choroidal vessels in indocyanin green angiography (right). **B,** dysregulation of the vessel size is often accompanied with a dysregulation of the barrier function. The increased level of ET-1 opens the barrier by upregulating PGE_2.

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6.12A, 6.13: Photo collection Dept. of Ophthalmology, University of Basel
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Chapter 7

Reperfusion damage

What is reperfusion injury?

Reperfusion injury refers to tissue damage caused by the blood supply returning to the tissue after a period of ischemia. The absence of oxygen and nutrients from the blood creates a condition in which the restoration of the blood circulation results in inflammation and oxidative damage through the induction of oxidative stress, rather than restoration of normal function.

Reperfusion injury is best known from organ transplantation. The transplanted organ is not perfused for minutes or hours until it gets blood supply from the recipient. Experimentally, reperfusion injury to the retina is caused when retinal circulation is reversibly blocked due to a marked increase in intraocular pressure (IOP). This type of reperfusion damage is clearly different from what happens in the human eye with primary open-angle glaucoma (POAG). In people with POAG, the oxygen tension in the tissue falls temporarily; this drop is very mild but recurs over years. Such a drop in oxygen leads to what is known as preconditioning, which makes the cell more resistant to a further decrease in oxygen. If the oxygen amount exceeds a certain limit, the result is reperfusion damage. If this amount is even larger or lasts longer, a tissue infarction occurs (Figure 7.1).

It is our assumption that POAG occasionally leads to a bigger decrease in oxygen than the drop in oxygen which leads to preconditioning, but less than the decrease that results in an infarction.

What leads to reperfusion injury?

As mentioned earlier, ocular blood flow (OBF) is highly regulated to adapt to different functions or compensate for any changes in perfusion pressure. The oxygen supply is inadequate when a challenge exceeds the physiological capacity of regulation (eg, in cases with a marked increase in IOP) or when autoregulation is disturbed. Autoregulation of OBF is disturbed in cases with

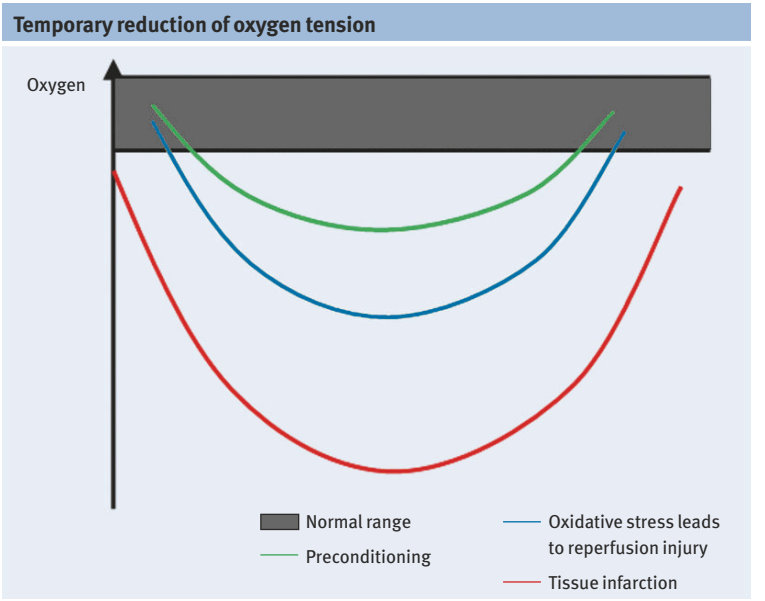

Figure 7.1 Temporary reduction of oxygen tension Under physiological conditions the oxygen tension in the brain or in the eye varies only slightly. Nature has the capacity to adapt to slight drops of oxygen (preconditioning). If the reversible drop is larger, tissue damage results (reperfusion injury). If the drop is even larger, an infarction occurs.

PVD. In other words, in patients with PVD, reperfusion injury occurs where there is:

- either a high IOP or very low blood pressure (despite normal autoregulation)
- a normal or mildly increased IOP
- a normal or mildly decreased blood pressure.

Reperfusion also occurs in patients with sleep apnea, shock-like states, orthostatic hypotension and, to a lesser extent, in patients with giant cell arteritis.

Why does reperfusion injury induce glaucomatous optic neuropathy?

Recurrent, mild reperfusion leads to chronic oxidative stress (Figure 7.2), especially in mitochondria which, due to the high energy consumption in this active area, are abundant in the unmyelinated optic nerve head. The mitochondria become increasingly damaged, which means that energy production becomes progressively inefficient. This in turn causes all cellular compartments

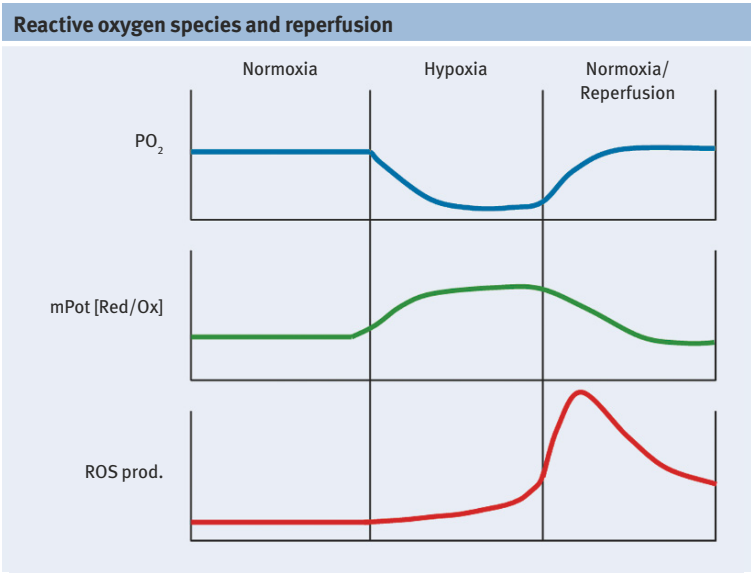

Figure 7.2 Reactive oxygen species and reperfusion ROS, reactive oxygen species. ROS production increases with increasing membrane potential but decreases with low partial oxygen tension (PO_2). This explains a mild increase during hypoxia and a marked increase in the beginning of the reperfusion stage.

to suffer and effectively undergo an accelerated aging process. Astrocytes are simultaneously activated which causes a change in the microenvironment of the optic nerve head and, together with reperfusion, causes glaucomatous optic neuropathy (GON).

Are there signs of oxidative stress in human glaucoma?

Various signs indicate that oxidative stress plays an important role in the pathogenesis of GON. Based on experience with reperfusion in other organs, we would expect an increased number of DNA breaks, an upregulation of endothelin-1 (ET-1) and matrix metalloproteinase-9 (MMP-9), and an increase in activity of proteasomes. Indeed, this is the case in patients with glaucoma.

Analysis of DNA breaks by means of comet assay in the circulating lymphocytes reveals an increase in DNA breaks in patients with glaucoma when compared to (age-matched) control patients (Figure 7.3).

Oxidative stress also leads to an increase in ET-1 and various studies have demonstrated this, particularly in patients with progression of glaucoma disease, despite having a normalized IOP.

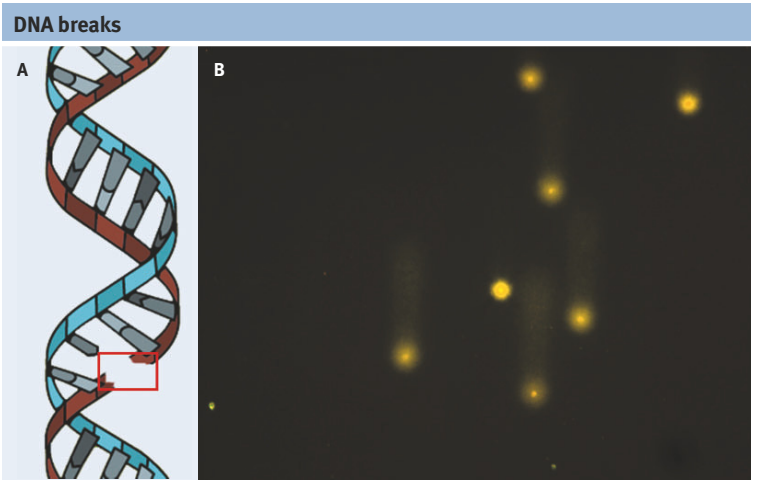

Figure 7.3 DNA breaks. DNA, deoxyribonucleic acid. **A,** schematic illustration of a single-strand DNA break. **B,** comet assay showing DNA breaks ('comets') in a patient with glaucoma.

Metalloproteinases (eg, MMP-2, MMP-9) are upregulated in the optic nerve head of patients with glaucoma.Upregulation of MMP-9 has been demonstrated in lymphocytes which, not only fits the hypothesis of reperfusion injury, but also explains various steps in the pathogenesis of GON.

Oxidative stress damages a variety of molecules including proteins, however, unlike damaged DNA, damaged proteins cannot be repaired. Nature has developed sophisticated methods to eliminate damaged intracellular proteins; they are first marked by ubiquitin and pulled electrostatically into proteasomes, where they are cut into pieces which are then recycled. The activity of proteasomes therefore gives us an indirect measure of protein damaged, and indeed the upregulation of the 20S proteasome alpha subunit into the lymphocytes of patients with glaucoma, further supports the hypothesis of oxidative stress (Figure 7.4).

Additional support for the role of oxidative stress also comes from analyzing the end products of lipid peroxidation. Malonyldialdehyde (MDA) is one example of a lipid peroxidation end product which can be used as a marker of oxidative stress. The serum of patients with POAG has been shown to contain more than twice the level of MDA, when compared with healthy control patients.

A weak antioxidant defense system in patients with POAG also supports the role of oxidative stress. For example, the enzyme glutathione is critical in combating oxidative stress. It is known that glutathione levels are influenced by age and sex. Elderly people have low levels of the reduced form of glutathione

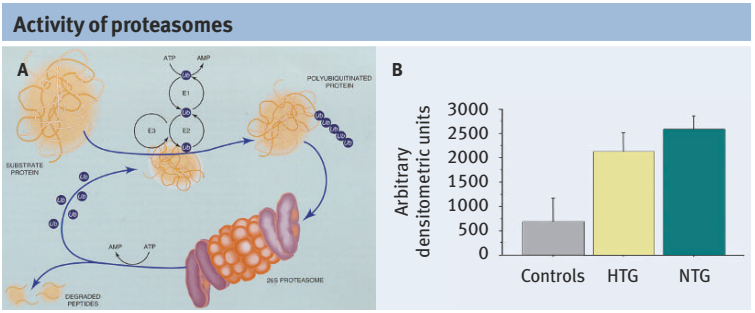

Figure 7.4 Activity of proteasomes. AMP, adenosine monophosphate; ATP, adenosine triphosphate; HTG, high tension glaucoma; NTG, normal tension glaucoma. **A,** damaged intracellular proteins cannot be repaired. They are marked with ubiquitin, pulled electrostatically into the proteasomes and cut into pieces. **B,** upregulation of the proteasome subunit 20S in the lymphocytes of patients without glaucoma and with normal- and high-tension glaucoma indicating increased protein damage.

(GSH) and a decreased ability to recycle the oxidized form of glutathione (GSSG) to GSH. It is also known that men have higher levels of GSH than women. Nevertheless, even when such age- and sex-based differences are taken into account, patients with glaucoma have lower serum GSH and total glutathione (t-GSH) levels when compared to age-matched control patients. Low levels of circulating GSH enhances oxidative stress and also reduces the availability of nitric oxide (NO) which, in turn, may inhibit relaxation of the ciliary muscle and trabecular meshwork causing an increase in IOP.

Support for the role of oxidative stress in GON also comes from other molecular studies which indicate the involvement of tumor necrosis factor (TNF) death receptor signaling. TNF-α induced cell injury is most likely mediated through its ability to promote oxidative stress. TNF-α is a proinflammatory cytokine that, when bound to its receptor, can induce apoptosis through a caspase-mediated pathway. Normal tissue shows constitutive expression of TNF receptor 1 (TNF-R1) in the vasculature of the optic nerve head but no positive labelling for TNF-α. In patients with glaucoma, TNF-α has been shown to be upregulated in both the optic nerve head and the retina. It has been proposed that TNF-α may contribute to GON by exerting a direct effect on the axons of the retinal ganglion cells and indirectly by inducing nitric oxide synthase 2 (NOS2) in astrocytes.

Glutamate is a major excitatory neurotransmitter in the retina and may also play a role in GON (Figure 7.5). It is released by the presynaptic cells and acts through various postsynaptic receptors, including the *N*-methyl-D-aspartate (NMDA) receptor. Oxidative stress interferes with the reuptake of glutamate by Müller cells, thereby increasing the extracellular concentration. If excessive

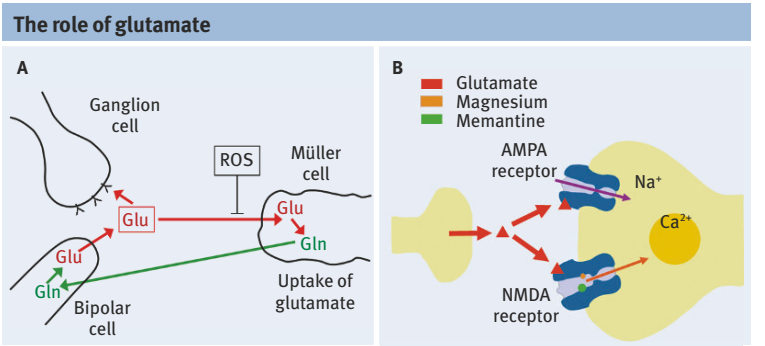

Figure 7.5 The role of glutamate. AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; Ca^{2+}, calcium; Gln, glutamine; Glu, glutamate; Na^{+}, sodium; NMDA, *N*-methyl-D-aspartate; ROS, reactive oxygen species. **A**, glutamate is released by bipolar cells, stimulates the ganglion cells and is taken up by the Müller cells. ROS block the glutamate uptake and thereby lead to excitotoxicity. **B**, the effect of increased glutamate levels can partially be blocked by memantine or magnesium.

amounts of glutamate are released or if glutamate clearance is insufficient, neuronal death can occur by a process known as excitotoxicity.

Excitotoxicity is mediated by over-stimulation of the NMDA receptor and non-NMDA receptor, a mechanism that has been demonstrated in the retina. Over stimulation of these receptors leads to excessive levels of intracellular calcium which, in turn, leads to activation of nitric oxide synthase and an excess accumulation of superoxides ($O_2^{\cdot-}$) and NO. This damages the macromolecules causing lipid peroxidation, DNA damage, leading to mitochondrial dysfunction and, in some cases, cell death.

Early clinical studies have found there to be elevated glutamate levels in the vitreous of patients with glaucoma. Animal studies also support this. More recent investigations, however, have failed to confirm these initial findings of elevated glutamate levels in patients with glaucoma. The role of glutamate excitotoxicity in glaucoma therefore remains unclear.

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Chapter 8

Pathogenesis of glaucomatous optic neuropathy

The previous chapters have discussed many different elements that contribute to glaucoma and glaucomatous optic neuropathy (GON) in particular. This chapter will summarize and explain these different elements into one concept. GON is characterized by two major elements:

- the loss of retinal ganglion cells and their axons (mainly by apoptosis)
- tissue remodelling leading to the characteristic cupping of the optic nerve head (ONH).

One of the major molecules to cause apoptosis is peroxynitrate ($ONOO^-$). Peroxynitrate is produced if nitric oxide (NO) diffuses into axons in which high concentrations of peroxide, due to reperfusion, are present (Figure 8.1). As with peroxynitrate, but unlike NO, peroxide cannot penetrate intact cell membranes. Peroxynitrate, although very reactive with a short half-life, can diffuse to some extent within the axons both in the direction of the lateral ganglion geniculate nucleus, and towards the retina causing damage on both sides (Figure 8.2).

The upregulation of NO in the astrocytes is part of the activation of these cells either by mechanical or reperfusion cell stress (Figure 8.3). This demonstrates nicely how different parameters can have the same effect, and is typical for a multifactorial disease.

The major cause of oxidative stress, occurring predominantly in the axons of the ONH, is reperfusion caused by unstable ocular perfusion. This perfusion is unstable if intraocular pressure (IOP) fluctuates to a level which exceeds the capacity of autoregulation or if, even at a normal IOP level, autoregulation is disturbed. Disturbed autoregulation occurs mainly in patients with primary vascular dysregulation (PVD), which also causes low blood pressure (BP) and simultaneously, by interfering with autoregulation, increases sensitivity to any reduction in BP (Figure 8.4).

The production of peroxynitrate

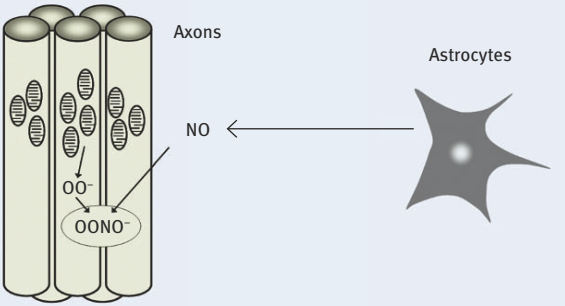

Figure 8.1 The production of peroxynitrate. NO, nitric oxide; $ONOO^-$, peroxynitrite; OO^-, superoxide anion. The reaction between the superoxide anion (produced by many mitochondria located in the axons of the optic nerve head) and NO produces the highly damaging peroxynitrite. Reproduced with permission from Neufeld AH. Surv Ophthalmol 1999; 43:S129–35.

Glaucomatous damage caused by peroxynitrite

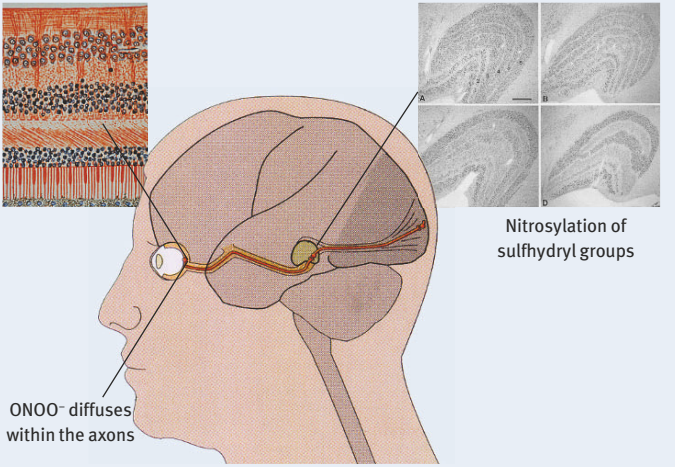

Figure 8.2 Glaucomatous damage caused by peroxynitrite. $ONOO^-$, peroxynitrite. Peroxynitrite can be produced anywhere but may also diffuse within the axons from the optic nerve head, both in the direction of the lateral ganglion geniculate nucleus and towards the retina, causing damage on both sides. Top right photographs reproduced with permission from Weber et al. Invest Ophthalmol Vis Sci 2000; 41:1370–9.

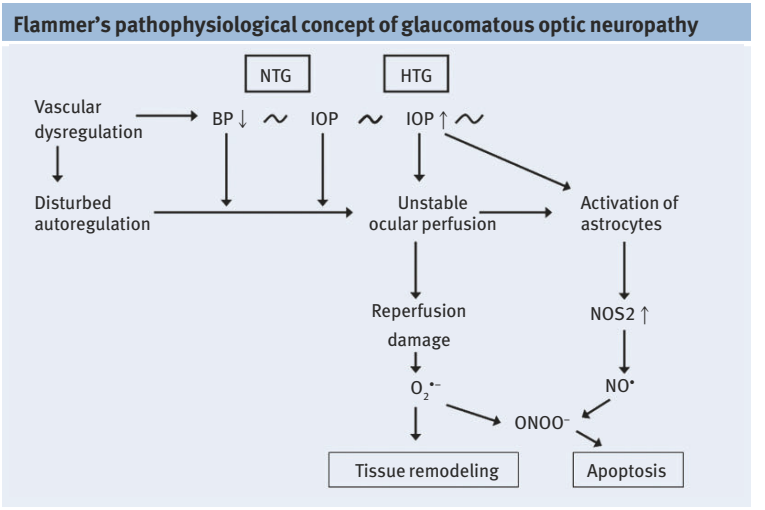

Figure 8.3 Flammer's pathophysiological concept of glaucomatous optic neuropathy. BP, blood pressure; HTG, high tension glaucoma; IOP, intraocular pressure; NO, nitric oxide; NOS2, nitric oxide synthase 2; $O_2^{\bullet-}$, superoxide anion; $ONOO^-$, peroxynitrate. This concept has two major pathways: the activation of astrocytes leading to increased NO production and an unstable blood flow leading to oxidative stress. Blood flow is unstable if IOP fluctuates on a high level or if autoregulation is disturbed.

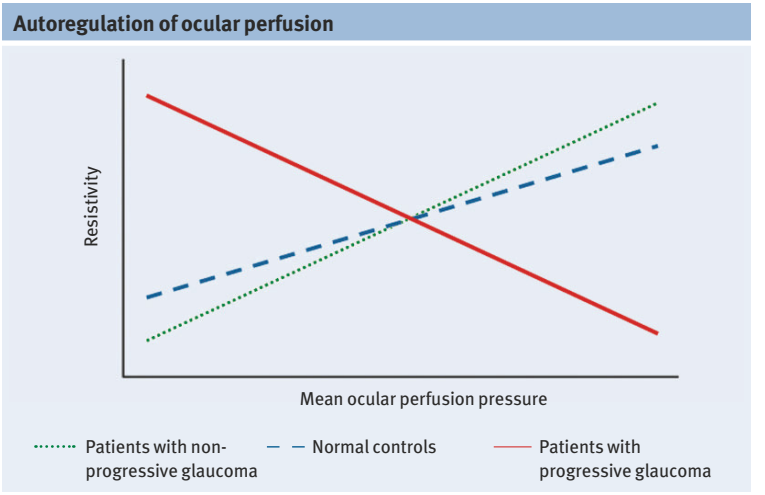

Figure 8.4 Autoregulation of ocular perfusion. As mean ocular perfusion drops the resistivity to flow is reduced in healthy subjects to keep flow constant (autoregulation). Whereas patients with non-progressive glaucoma behave like healthy controls, patients with progressive glaucoma show a disturbed autoregulation despite a normal intraocular pressure.

The fact that PVD occurs more often in women than in men, in Japanese populations than in American populations, and in academics than in blue collar workers, explains why these factors have a statistically significant relationship with GON; this is particularly the case in patients with normal tension glaucoma (NTG). PVD also contributes to disc hemorrhages explaining why the latter are associated with the progression of GON, and why they occur more often in women and patients with NTG (Figure 8.5). The involvement of sleep apnea in GON can also be explained by reperfusion injury. Sleep apnea causes a transient reduction of oxygen supply. Reperfusion leads to upregulation of matrix metalloproteinase-9 (MMP-9), which is also elevated in the blood of people with sleep apnea, explaining the association between NTG and floppy eyelid syndrome. Reperfusion also explains the mechanism by which episodes of a marked drop in blood pressure (so-called 'shock-like states') and orthostatic blood pressure dips, are risk factors for GON.

Reperfusion, together with glial cell activation, explains how several different risk factors eventually contribute to the same type of damage. It also highlights that high tension glaucoma (HTG) and NTG are not two separate diseases but rather two ends of a continuum. Furthermore reperfusion also gives rise to new therapies which will be discussed in the next chapter.

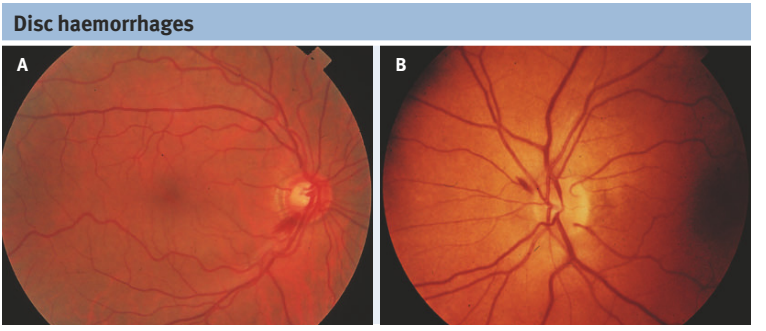

Figure 8.5 Disc haemorrhages. A, in a patient with glaucoma. **B**, in a person with primary vascular dysregulation.

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Chapter 9

Treatment strategies

Importance of reducing intraocular pressure

Many controlled studies have shown the beneficial effect of a reduction in intraocular pressure (IOP). The mechanical stress on the optic nerve head (ONH) is lowered, the probability of glial cell activation is reduced, and ocular blood flow (OBF) is improved, especially in patients with a disturbed autoregulation. While there is agreement that a reduction in IOP is helpful, the question arises as to whether alternative or additional treatments are feasible. We need to deal with this question for the following reasons:

- It is not always possible to reduce IOP to a safe level – often a reduction to this extent is achieved only by surgery which has its own side-effects (eg, cataract formation).
- Reducing IOP by drugs can result in drug related side-effects, this also includes vasoconstriction by certain classes of drugs or drug combinations.
- It is the authors' daily experience that even when reaching the target IOP, some cases will progress as shown in Figure 9.1.

It should also be possible in the future to bring patients to a state where IOP levels within a normal physiological range will no longer be harmful.

Methods to reduce intraocular pressure

It is often stated that the IOP level alone is important and not the method by which a reduction in IOP is achieved. However, this is unlikely to be true. There is still debate as to whether a reduction of IOP by surgery is better than a reduction achieved through taking medication. There is strong evidence that differences exist even within the same classes of drugs (eg, among the different β-blockers). The differences among the many classes of drugs may be even larger. Unfortunately, however, little research has been done in this area but there seems to be some agreement that car-

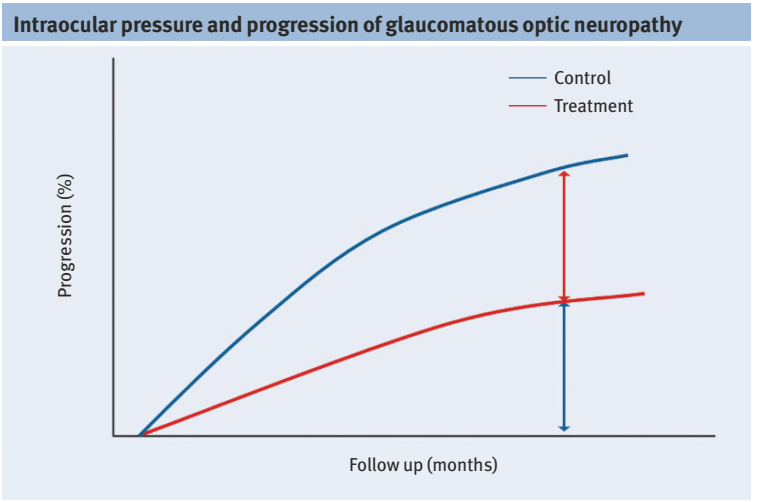

Figure 9.1 Intraocular pressure and progression of glaucomatous optic neuropathy. Reduction of intraocular pressure reduces the probability for progression but does not stop progression in all patients.

boanhydrase inhibitors improve OBF, particularly the regulation of OBF. Figure 9.2 shows a list of drugs that lower IOP and Figure 9.3 summarizes the mechanism of action of these drugs. There are, however, potential treatments for glaucoma which may prevent the development of any damage without reducing IOP.

Non-intraocular pressure lowering treatments

With the emergence of new risk factors, such as disturbed OBF or vascular dysregulation, investigations have aimed to target these risk factors using new treatment strategies. As these treatments are targeted toward factors other than IOP, they are often referred to as non-IOP lowering treatments (Figure 9.4).

While most drugs belonging to this new treatment strategy are still in the experimental phase, others such as magnesium, ginkgo, salt, and fludrocortisone are already used by some physicians. In order to visualize the individual mechanisms that may be targeted by treatment Figure 9.5 illustrates the pathophysiological concept of glaucomatous optic neuropathy (GON) introduced by Josef Flammer. Using this concept we would like to explain the different modes of action of these treatment strategies. The section numbers that follow correspond to the numbers in Figure 9.5.

Drugs that lower intraocular pressure

Active agent	Generic examples	Method of administration: Eye drops	Method of administration: Tablets
Adrenergic agonists	Dipivefrin	•	
	Brimonidine	•	
	Apraclonidine	•	
	Epinephrine	•	
β blockers	Timolol	•	
	Levobunolol	•	
	Betaxolol	•	
	Carteolol	•	
	Levobunolol	•	
Carbonic anhydrase inhibitors†	Acetazolamide		•
	Methazolamide		•
	Dorzolamide	•	
	Brinzolamide	•	
Cholinergic agonists	Pilocarpine	•	
	Carbachol	•	
Prostaglandin analogues	Travaprost	•	
	Latanaprost	•	
	Brimatoprost	•	
Fixed combinations			
β blocker and adrenergic agonist	Timolol/Brimonidine	•	
β blocker and carbonic anhydrase inhibitors	Timolol/Dorzolamide	•	
	Timolol/Brinzolamide	•	
β blocker and prostaglandin analogue	Timolol/Travaprost	•	
	Timolol/Latanoprost	•	
Pilocarpin and β blockers	Pilocarpin/Timolol	•	
	Pilocarpin/Metipranolol	•	
	Pilocarpin/Carteolol	•	

Figure 9.2 Drugs that lower intraocular pressure. †In addition to lowering IOP, carbonic anhydrase inhibitors also improve ocular blood flow as well as the regulation of blood flow.

1A Inhibition of the epidermal growth factor receptor (EGFR)

Mechanical stress leads, via stimulation of the EGFR, to activation of astrocytes and, in turn, the upregulation of several gene products including nitric oxide sythase 2 (NOS2). Therefore blockage of the EGFR, by a tyrosine kinase

Mechanisms by which drugs reduce intraocular pressure

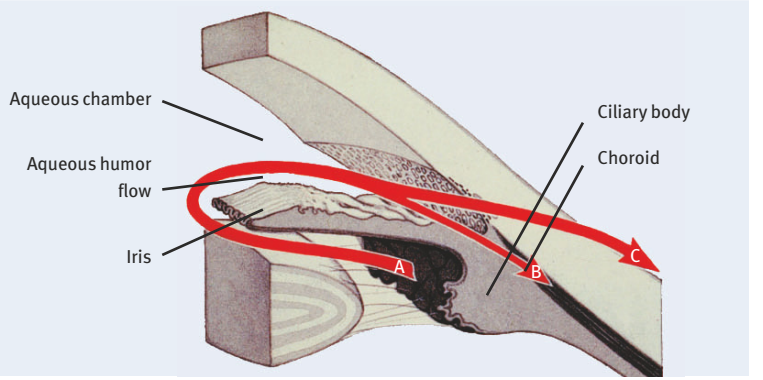

Figure 9.3 Mechanisms by which drugs reduce intraocular pressure. A, reduction of aqueous humor production (β-blockers and carbonic anhydrase inhibitors). **B,** improvement of uveoscleral outflow (prostaglandin analogues). **C,** improvement of trabecular outflow (miotics and α-agonists).

Non-intraocular pressure lowering substances

Substance	Active agent	Mechanism of protection
Bilberry	Anthocyanin	Free radical scavenging properties
Calcium channel blocker	Nifedipine, amlodipine, diltiazem	Improvement of blood flow and regulation of blood flow
Coffee	Polyphenolic flavonoids Monocyte chemotactic protein	Free radical scavenging properties selective scavenger of peroxynitrite
Fish/fish oil	Omega-3 fatty acids	Increase the production of uncoupling proteins and thereby improve ATP-independent heat production
Ginkgo biloba	Polyphenolic flavonoids Terpenoids	
Magnesium (tablet, effervescent, sachet)	Mg^{2+}	Physiological calcium channel blocker which improves vascular regulation
Melatonin	*N*-acetyl-5-methoxyryptamine	Free radical scavenging properties
Mineralocorticoid	Fludrocortisone	Increase in blood pressure
Red wine	Polyphenolic flavonoids Anthocyanins	Free radical scavenging properties, enhances the production of vasodilating factors, inhibits the production of vascular endothelial growth factor
Salt tablets	Sodium chloride	Increase in blood pressure
Tea	Polyphenolic flavonoids	Free radical scavenging properties, iron chelating properties

Figure 9.4 Non-intraocular pressure lowering substances. ATP, adenosine triphosphate.

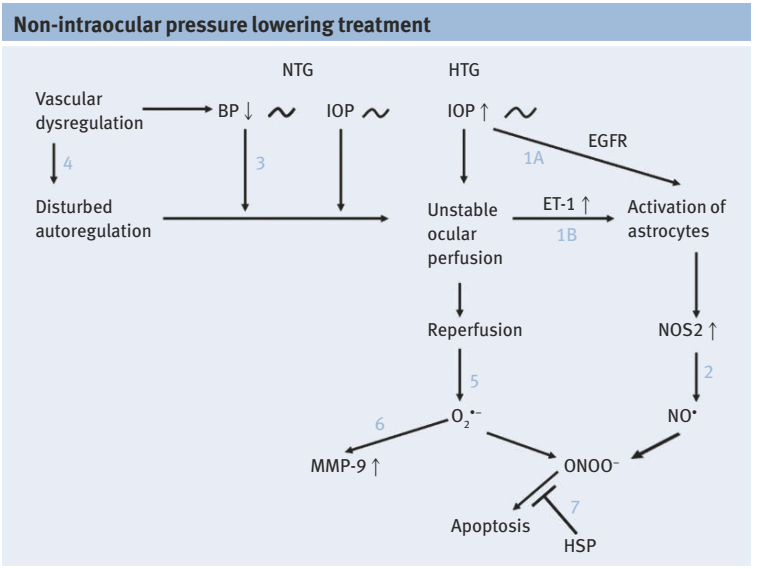

Figure 9.5 Non-intraocular pressure lowering treatment. BP, blood pressure; EGFR, epidermal growth factor receptor; ET-1, endothelin-1; HSP, heat shock protein; IOP, intraocular pressure; MMP-9, matrix metalloproteinase 9; NO, nitric oxide; NOS2; nitric oxide synthase 2; $O_2^{\bullet-}$, superoxide anion; $ONOO^-$, peroxynitrite. The different modes of action of non-IOP lowering treatments using Flammer's pathophysiological concept of glaucomatous optic neuropathy. The numbers in this figure correspond to the section numbers in the text.

inhibitor, prevents activation of the astrocytes. Interestingly, in animal studies such treatment also leads to a reduction in the loss of retinal ganglion cells. This indicates that the activation of astrocytes is, indirectly, relevant in GON. Whether such an approach will be used in the treatment of human glaucoma cannot be predicted at the moment.

1B Inhibition of the effect of endothelin-1

Endothelin is involved in the regulation of blood flow, the activation of glial cells and in axoplasmatic transport. The effects of endothelin can be partially blocked by a number of different drugs such as calcium channel blockers (CCBs) including magnesium (a physiological CCB), and dipyrimadole. Endothelin blockers are particularly promising but as yet not registered for glaucoma treatment.

2 Inhibition of nitric oxide synthase

The induced expression of nitric oxide synthase (NOS2) causes a marked increase in nitric oxide (NO). NOS2 can be inhibited by the drug aminogua-

nidine, a nucleophilic hydrazine compound. Aminoguanidine is an oral insulin stimulant for type 2 diabetes mellitus, however it also seems to prevent the formation of advanced glycation end products. It is also a relatively specific inhibitor of NOS2 which is why it has been studied in experimental glaucoma (Figure 9.6). Results showed that aminoguanidine was capable of preventing the development of GON. Such treatment appears to be very promising but clinical studies are not yet available.

3 Increase of systemic blood pressure

Generally, low blood pressure, orthostatic hypotension, nocturnal over-dipping and shock-like states increase the probability of GON deterioration. We can therefore assume that an increase in blood pressure in patients with hypotension may improve prognosis, although there are only a few interventional studies which support this. Treating systemic hypotension with vasoconstrictive drugs will increase blood pressure, yet may reduce OBF further and consequently is not recommended. Blood pressure, however, can be safely raised by simply increasing salt intake (by 1–5 g daily, taken in the evening). In severe cases a low dose of fludrocortisone (eg, 0.1 mg twice/week) is helpful. Fludrocortisone is a mineralocorticoid which has fewer side effects than glaucocorticoids especially when given in low doses. Fludrocortisone treatment not only slightly increases blood pressure and reduces nocturnal dips, but also indirectly improves the regulation of blood flow.

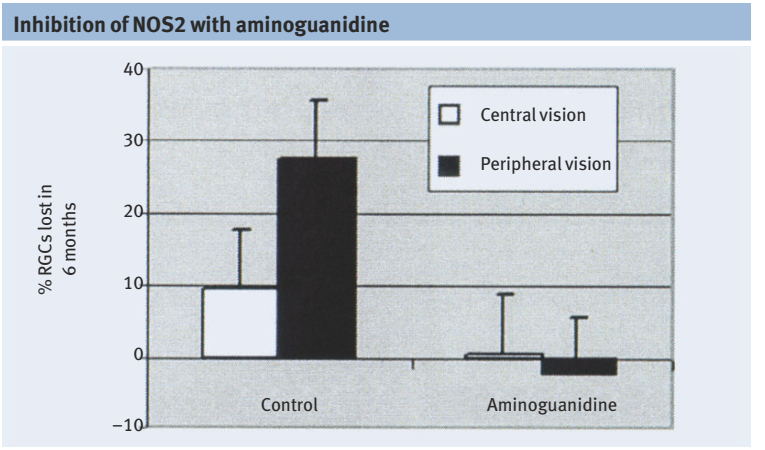

Figure 9.6 Inhibition of NOS2 with aminoguanidine. RGCs, retinal ganglion cells; NOS2, nitric oxide synthase 2. NOS2 inhibition with aminoguanidine prevents loss of retinal ganglion cells in rat eyes with moderately increased introcular pressure. Reproduced with permission from Neufeld AH. Brain Res Bull 2004; 62:455–459.

4 Improvement of vascular regulation and autoregulation

Vascular dysregulation is a major risk factor for GON. It increases sensitivity to a rise in IOP or a decrease in blood pressure. The consequences are manyfold and have already been discussed. One approach is to avoid the major challenges of regulation by avoiding cold, emotional, or mechanical stress as far as possible, and also by decreasing and stabilizing IOP and increasing blood pressure. In addition, one can try to improve vascular regulation in itself. Among the IOP lowering drugs, only carbonic anhydrase inhibitors (particularly dorzolamide) have been proven to not only increase OBF but also to improve the regulation of OBF, and thereby decrease the likelihood of reperfusion injury (Figure 9.7).

CCBs given at a low dose also improve OBF and its regulation. A regular dose is not necessary to achieve an effect on dysregulation and would cause side-effects particularly in patients with primary vascular dysregulation (PVD). It is often claimed that CCBs might reduce blood pressure even further, however this rarely

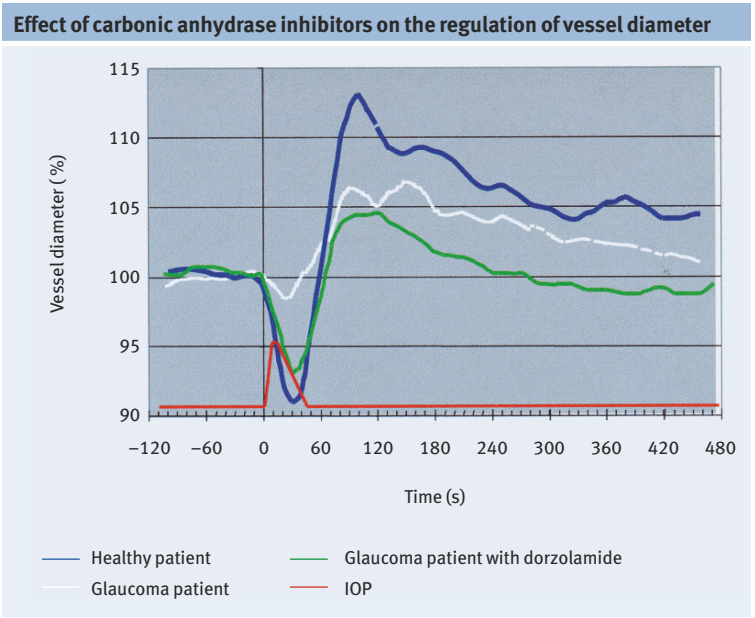

Figure 9.7 Effect of carbonic anhydrase inhibitors on the regulation of blood vessel diameter. IOP, intraocular pressure. A short rise in IOP leads to a short constriction of the retinal arteries, followed by a prolonged vasodilation. In glaucoma patients this vascular response to IOP is blunted. Pre-treatment with dorzolamide improved vascular regulation. Reproduced with permission from Nagel et al. VASA 2002; 31:230–4.

occurs. CCBs can even cause a slight increase in blood pressure at night, and this is known as the paradoxical effect. There is a debate as to which CCB may be best for patients with glaucoma. Some clinicians prefer to use liposoluble CCBs (eg, nimodipine) as they cross the blood–brain barrier more readily and therefore act mainly in the CNS (these are known as centrally acting CCBs). We know, however, that water soluble molecules (eg, nifedipine) will reach the optic nerve head by diffusing from the choroids (Figure 9.8). Water-soluble CCBs act mainly in the periphery, at the corneal and ocular vessels including the optic nerve. They are, therefore, the authors' first choice. CCBs partly inhibit the effects of endothelin, a molecule known to be involved.

Magnesium is a physiological CCB and given at relatively high doses (eg, 10–20 mmol) it has a mild effect without any major side-effects. Consequently magnesium is very often used in our treatment.

Dipyridamol, a drug used in the past as a platelet inhibitor, also inhibits the effect of endothelin, and improves OBF (Figure 9.9). Unfortunately the long-term role of dipyrimadole on patients with glaucoma has not been studied.

It is often asked whether an improvement in OBF is helpful or whether it might even be harmful in certain conditions. Both carbonic anhydrase inhibitors (CAIs) and CCBs do not cause an increase in OBF in patients with a normal OBF. They improve or normalize a functionally disturbed OBF. Furthermore, such an improvement in OBF is paralleled by an improvement in visual field, indicating that normalization of OBF is beneficial for the eye.

Visual fields of a 25 year old women with marked vasospasm and borderline excavated optic nerve head

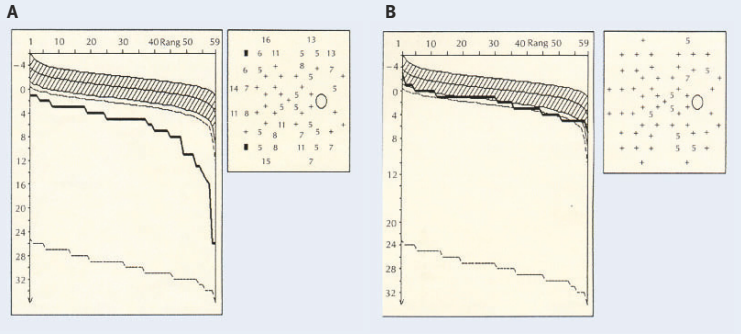

Figure 9.8 Visual fields of a 25 year old women with marked vasospasm and borderline excavated optic nerve head. The visual field was assessed using the Octopus program G1. The results are presented with the Bebie curve and comparison display printout. **A,** the visual field before treatment. **B,** visual field after 1 week of treatment with nifedipine.

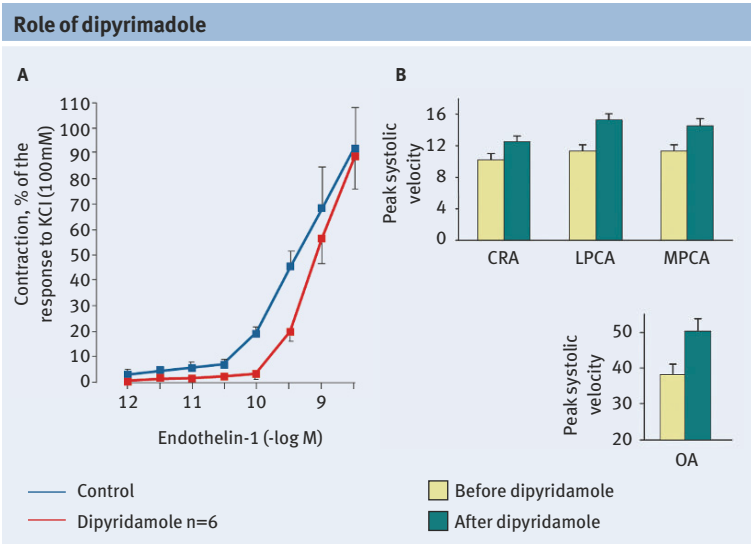

Figure 9.9 Role of dipyrimadole. CRA, central retinal artery; KCl, potassium chloride; LPCA, lateral posterior ciliary artery; MPCA, medial posterior ciliary artery; OA, ophthalmic artery. **A**, dipyrimadole shifts the dose response curve to the right. **B**, this inhibition of endothelin leads to improvement of ocular blood flow.

Omega-3-fatty acids (omega 3-FAs) have a number of different effects including the modulation of intracellular calcium ion release, and in turn the stabilization of circulation. Omega 3-FAs (Figure 9.10) also increase the production of uncoupling proteins and thereby improve adenosine triphosphate-independent heat production, which is most probably impaired in patients with vascular dysregulation.

Cacao beans (Figure 9.11) from the seed of *Theobroma cacao* contain a subclass of flavonoids, called flavan-3-ols, which are reported to increase endothelial nitric oxide synthase (eNOS), and thereby NO, which improves endothelium-dependent vasorelaxation. The use of cacao beans has been studied in cardiology but has not yet been studied in the context of glaucoma.

5 Combat oxidative stress

Free radicals are involved in a number of inflammatory and degenerative diseases. Oxidative stress occurs under a condition of high energy consumption, light exposure, or (age-related) decline in coping capacity to deal with free radicals. In glaucoma, an additional major factor is most likely to be a repeated mild reperfusion injury. Oxidative stress is involved in the pathogenesis of GON

Omega-3 fatty acids

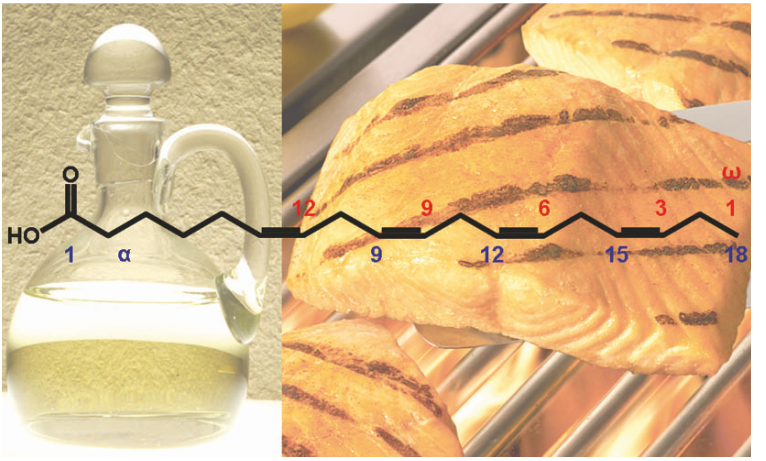

Figure 9.10 Omega 3 fatty acids. The term ω-3 signifies that the first double bond is the third carbon-carbon bond from the terminal methyl end (ω). Omega-3 fatty acids are commonly found in fish and fish oil.

Dark chocolate

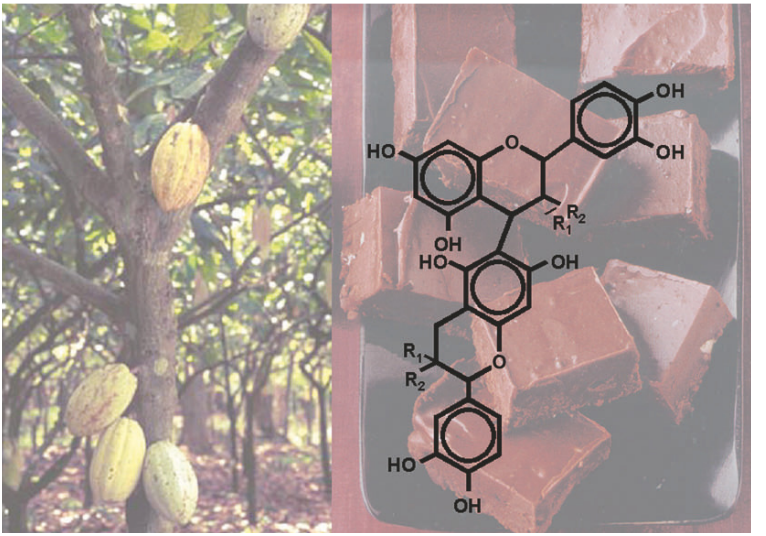

Figure 9.11 Dark chocolate. Dark chocolate (right) is made from the seed of *Theobroma cacao* (left). Cacao is particulary rich in a subclass of flavonoids, namely, flavan-3-ols and their oligomers (procyanidins).

where free radicals cause damage to retinal ganglion cells and their axons. In addition, oxidative stress leads to degeneration of the trabecular meshwork and alterations in the aqueous outflow pathway, leading to an increase in IOP which, in turn, also damages retinal ganglion cells. From a therapeutical point of view, therefore, the situation may be improved by stabilizing OBF (ie, reducing and stabilizing IOP and/or increasing and stabilizing blood pressure or improving autoregulation) or by treating with antioxidants.

As previously mentioned, the main characteristic of an antioxidant is its ability to neutralize free radicals. Highly reactive free radicals and reactive oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. There are a number of substances with antioxidant activity that could theoretically be beneficial, but only a few have been studied in relation to glaucoma. What follows is a discussion of various classes of compounds with antioxidant activity and the natural sources in which they can be found.

Flavonoids

Flavonoids, are a group of naturally occurring compounds which are widely available in nature and are ubiquitous in food sources such as vegetables, berries, fruits, chocolate and plant-derived beverages such as tea or red wine. More than 5,000 different flavonoids have been identified.

Flavonoids are an important class of phenolic compounds. One thing that all of the different phenolic compounds have in common is that their molecular structure includes a phenol ring, a ring of six carbon atoms with at least one hydroxyl (OH) group attached (Figure 9.12). When a compound has more than one phenolic group it is referred to as a polyphenol. Polyphenols provide much of the flavour and color to fruits and vegetables. This is due to the fact that the electron cloud around the aromatic ring of a phenol, has the capacity to absorb light at a visible wavelength. Moreover, a phenol is very easily oxidized. It is this ease of oxidation (polyphenols act as reducing agents) that gives polyphenols their antioxidant properties. The hydroxyl group of a phenol enables these compounds to readily donate an electron to free radicals in order to neutralize them.

Ginkgo

Polyphenol antioxidants are found in a wide array of phytonutrients. *Ginkgo biloba* leaf extract is an example of the most widely sold phytomedicine in Europe, where it is used to treat the symptoms of early-stage Alzheimer's disease, vascular dementia, peripheral claudication, and tinnitus of vascular origin. It is also one of the ten best-selling herbal medications in the United States. The

General chemical structure of polyphenols

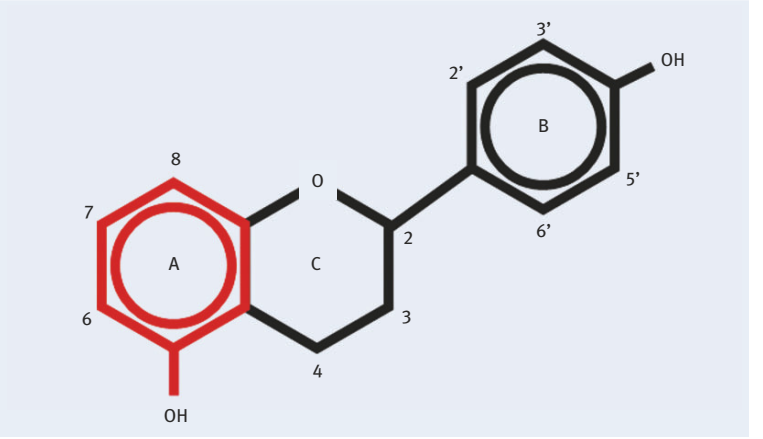

Figure 9.12 General chemical structure of polyphenols. Polyphenol flavonoids are compounds containing more than one phenolic ring. Phenols are a class of chemical compounds consisting of a hydroxyl group (-OH) attached to an aromatic hydrocarbon group.

ginkgo tree is one of the oldest living tree species, having been growing on earth for 150–200 million years. Ginkgo is indigenous to China, Japan and Korea where it exists in remote mountainous areas. The active components of ginkgo are extracted from the leaves and the seeds of the ginkgo fruit which contain flavonoids and terpenoids (Figure 9.13). Recently, the effect of ginkgo biloba extract as a potential antiglaucoma therapy has raised much interest.

The major targets of oxidative stress, relevant in the development of GON, are the mitochondria. It is therefore preferrable to have a drug which protects the mitochondria, particularly those of the optic nerve head. Unfortunately, this cannot simply be achieved by an increased intake of vitamins such as vitamin C or E. Only molecules which reach the inner membrane of the mitochondria are of potential use. Ginkgo contains a number of substances, including polyphenolic flavonoids, that have been proven to protect the mitochondria from oxidative stress and thereby also protect the retinal ganglion cells. Moreover, ginkgo has been shown to improve visual fields. A daily dose of 120 mg is sufficient and acceptable.

Tea and coffee

Polyphenols are found in a variety of other naturally occurring substances. Both green and black tea are rich sources of flavonoids catechin (C), epicatechin (EC), and epigallocatechin (EGC) (Figure 9.14). Green tea has one of the highest levels of phenolic compounds among food, about 35% by dry weight.

Ginkgo biloba

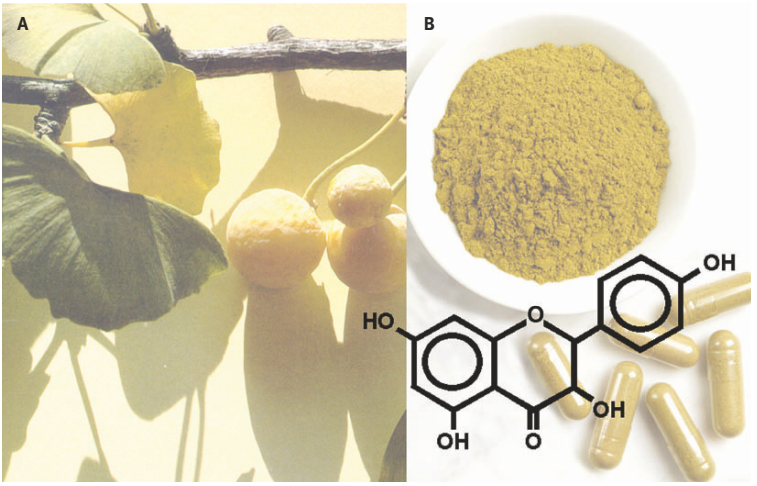

Figure 9.13 ***Ginkgo biloba*****. A**, the ginkgo tree is indigenous to Asia. **B**, one of the abundant polyphenols in ginkgo is kaempferol. The medicinal components of ginkgo are extracted from the leaves and the seeds of the ginkgo fruit which contain flavonoids (a subgroup of polyphenols) and terpenoids.

Tea

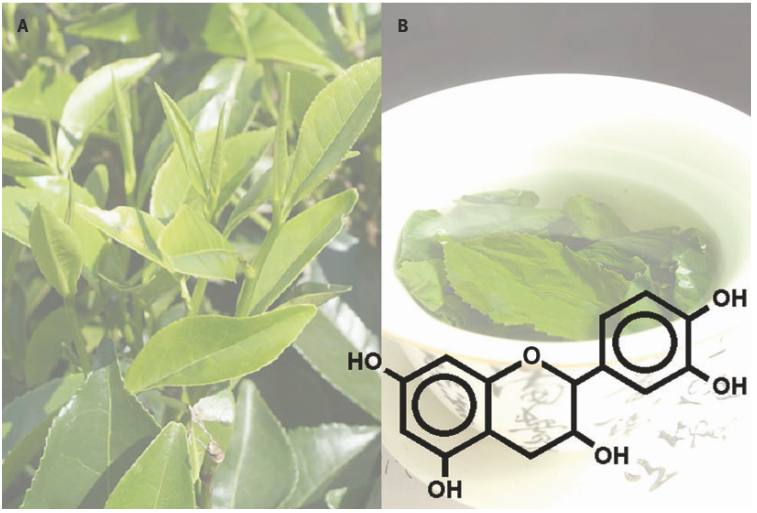

Figure 9.14 Tea. A, epicatechin. **B**, green tea. Both green and black tea are a rich source of flavonoids such as catechin and epicatechin. The concentration of these flavonoids is higher in green tea than in black tea.

The difference between black and green tea is how the leaves are processed after picking. In black tea, catechins are converted to complex fermentation products, namely theaflavins (TFs) and thearubigins (TGs), which give black tea its characteristic color.

Coffee also has good antioxidant properties due to polyphenolic compounds. In addition, coffee contains the molecule 3-methyl-1,2-cyclopentanedione (MCP) which is recognized as a selective 'scavenger' of peroxynitrite ($ONOO^-$) The chemistry of MCP enables it to easily donate a proton to peroxynitrite in order to neutralize it and this is brought about by the chemical conversion of one of its hydroxyl groups (OH) which becomes oxidized to a carbonyl group (C=O) as shown in Figure 9.15.

Other naturally occurring compounds containing polyphenols include dark chocolate and red wine.

Red wine

In 1819 the Irish physician Samuel Black first noted that the French suffer from a relatively low incidence of coronary heart disease, despite having a diet rich in saturated fats. The drinking of red wine, to which is attributed the low incidence of coronary heart disease in France, is known as the French paradox.

Coffee

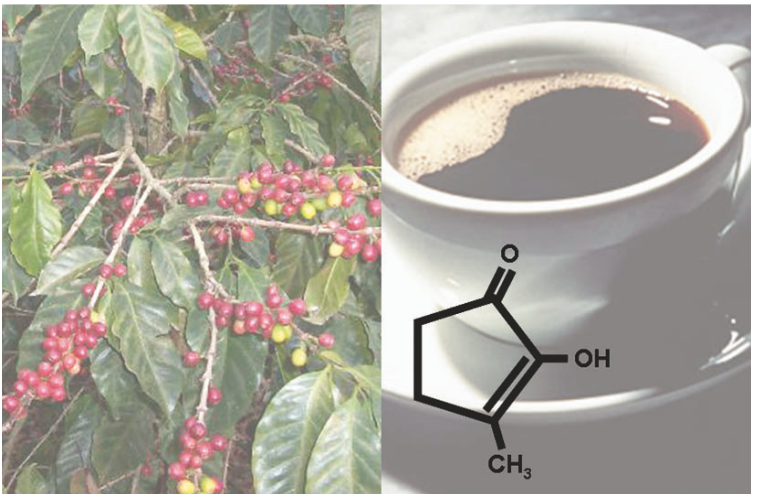

Figure 9.15 Coffee. A, coffee berries, which contain the coffee bean, are produced by several species of small evergreen bush. **B**, coffee contains polyphenols (not shown) as well as the compound 3-methyl-1,2-cyclopentanedione (MCP).

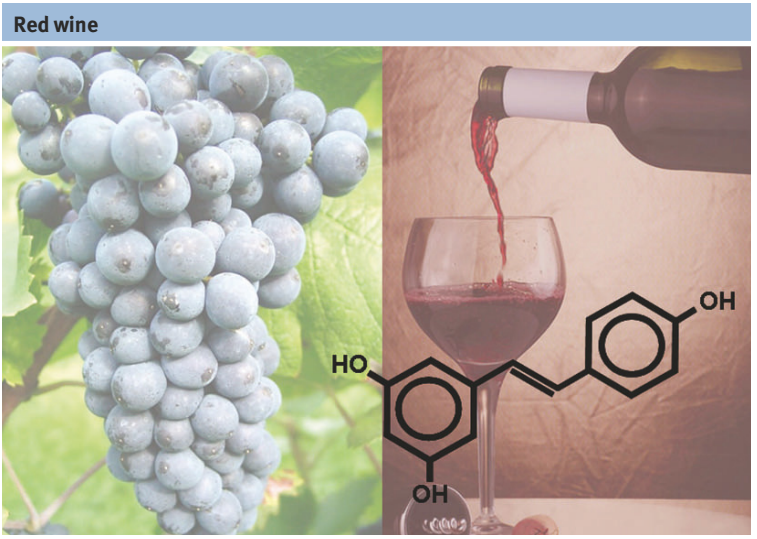

Figure 9.16 Red wine. The color pigment in red wine originates from the skin of the blue grapes. Red wines exhibit a stronger antioxidant capacity than white wines due to their phenolic content. This figure depicts the chemical structure of resveratrol, a polyphenol found in red wine.

When news of this paradox became known in the United States in 1991, the consumption of red wine increased by 44% and some wineries began lobbying for the right to label their products as health food.

Red wines exhibit a stronger antioxidant capacity than white wines due to their phenolic content (Figure 9.16). In addition, red wines strongly inhibit the synthesis of endothelin-1 (ET-1). Resveratrol, a polyphenol of the skin of grapes, is found in red wine. Resveratrol reduces the extracellular levels of vascular derived endothelial growth factor. One of the mechanisms by which polyphenols in red wine improve endothelial function is their ability to stimulate the production of eNOS and promote the production of NO, which induces vasodilation.

Tomatoes

Lycopene ($C_{40}H_{56}$) is another class of antioxidant phytochemicals and is a bright red carotenoid pigment mainly found in tomatoes (Figure 9.17).

The color of lycopene is due to its many conjugated carbon double bonds. Each double bond reduces the energy required for electrons for transition to higher energy states, allowing the molecule to absorb visible light of progres-

Tomatoes

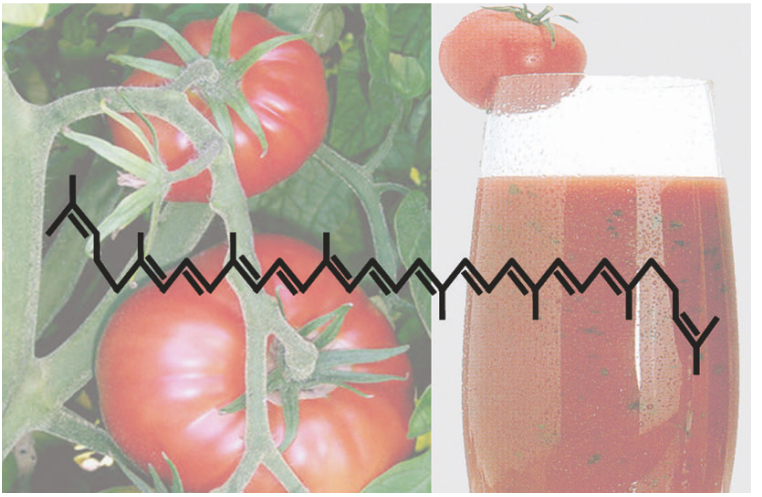

Figure 9.17 Tomatoes. Tomatoes contain a bright red carotenoid pigment called lycopene ($C_{40}H_{56}$). The color of lycopene is due to its many conjugated carbon double bonds.

sively longer wavelengths. Lycopene absorbs most of the visible light (except red light), which is why it appears red. It acts as an antioxidant by donating its electrons to free radicals. When lycopene is oxidized (loss of an electron), the double bonds between carbon atoms are broken, cleaving the molecule into smaller molecules.

Berries

Anthocyanins, are found in different types of fruits and berries such as blueberries and bilberries. Bilberries (*Vaccinium myrtillus*), are another class of substance which have antioxidant properties. The use of bilberry for the treatment of conditions affecting eyesight dates back to World War II when British Royal Air Force pilots first reported an increase in night-time vision after ingesting bilberries. In addition to polyphenolic rings, anthocyanins possess a positively charged oxygen atom in their central ring which enables them to readily scavenge electrons (Figure 9.18). While the compounds mentioned so far neutralize free radicals by donating an electron, anthocyanosides neutralize free radicals by accepting an electron.

Quinones

We will briefly discuss two more classes of compounds with antioxidant activity. The first, belongs to a class of organic compounds called quinones, namely,

Blueberries

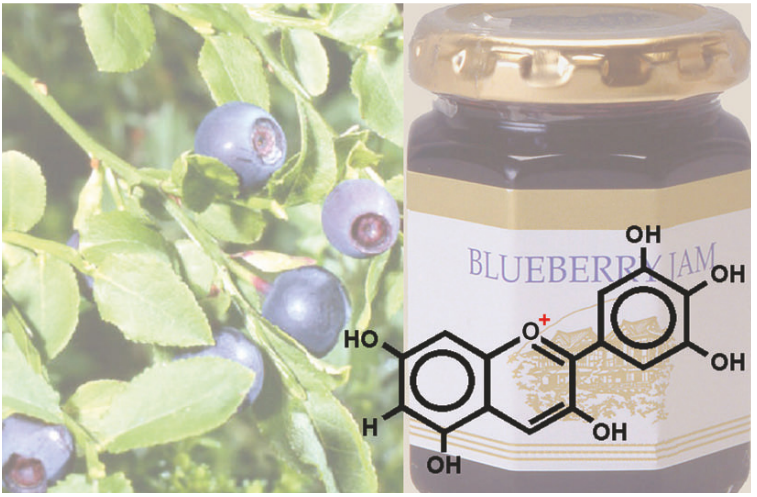

Figure 9.18 Blueberries. Blueberries contain anthocyanosides. The chemical structure of anthocyanin (example depicted is delphinidin) not only contains phenolic rings but also consists of a central ring with a positively charged oxygen atom.

ubiquinone (Coenzyme Q10 [CoQ10]). Quinones are compounds which have a fully conjugated cyclic dione structure, such as that of benzoquinones. CoQ10 is a benzoquinone, where Q refers to the quinone chemical group, and 10 refers to the isoprenyl chemical subunit. This compound is present in all human cells acting as a coenzmye for the inner mitochondrial enzyme complexes involved in energy production. In other words, in each human cell, food energy is converted into energy in the mitochondria with the aid of CoQ10. It is often used in the form of supplements as treatment for some of the very rare and serious mitochondrial disorders. Ubiquinone also has strong antioxidant properties. CoQ10 has been shown to prevent lipid peroxidation and DNA damage induced by oxidative stress. Figure 9.19 shows the reduced and oxidised forms of ubiquinone. Ubiquinone has been studied well in dermatology but unfortunately studies of its use in glaucoma are lacking.

Melatonin

Melatonin (N-acetyl-5-methoxytryptamine) is an indoleamine, secreted by the pineal gland (Figure 9.20) as well as by the retina, lens and the gastrointestinal tract. Indoleamines, are substituted indole compounds (aromatic heterocyclic organic compounds) that contain an amino group.

Coenzyme Q10

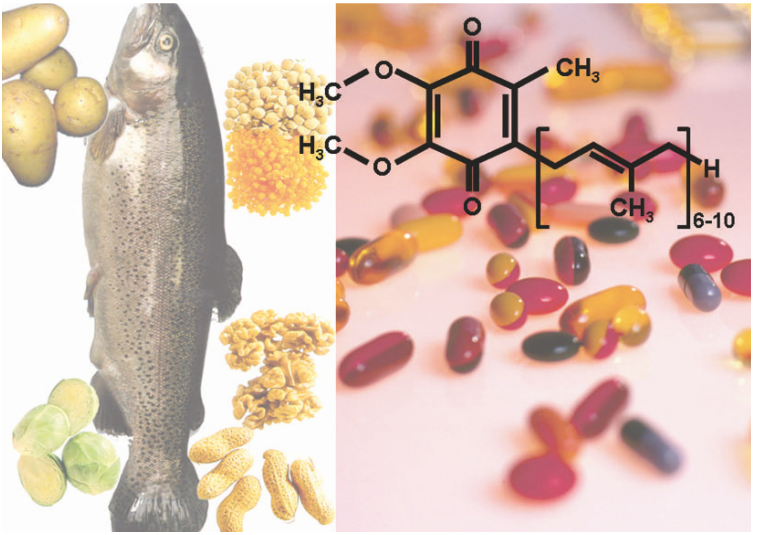

Figure 9.19 Coenzyme Q10. Coenzyme Q10 also known as ubiquinone is a benzoquinone. Examples of dietary sources of ubiquinone are fish (eg, mackerel, herring).

Melatonin

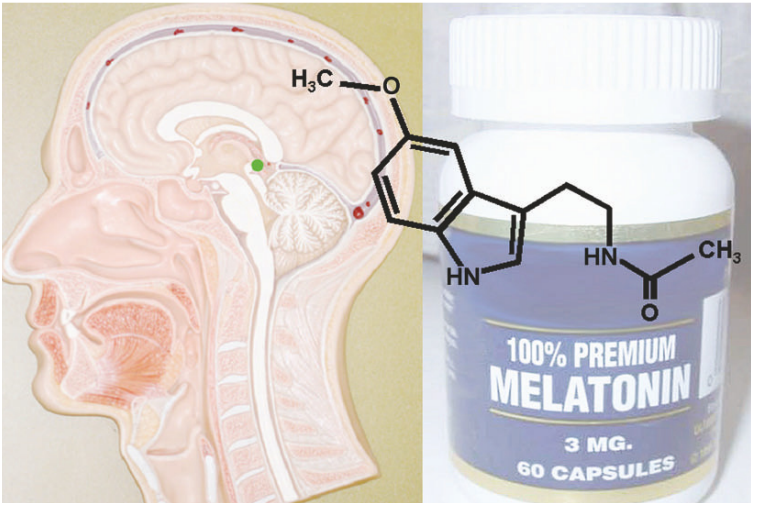

Figure 9.20 Melatonin. Melatonin (N-acetyl-5-methoxyryptamine) is an indoleamine, secreted by the pineal gland. Melatonin is a free-radical scavenger, and because melatonin production decreases with age, it may also be an important factor in the aging process.

Melatonin is naturally synthesized from the amino acid tryptophan (via synthesis of serotonin) by the enzyme 5-hydroxyindole-*O*-methyltransferase. Melatonin is found in all living creatures from algae to humans, at levels that vary in a diurnal cycle. A basic biological function of melatonin is as an antioxidant. In fact, in many lower life forms it serves this purpose already. Melatonin has been shown not only to neutralize free radicals but also to stimulate a number of antioxidative enzymes.

6 Inhibition of matrix metalloproteinase-9 (MMP-9)

MMP-2 and MMP-9 are upregulated in the astrocytes of patients with glaucoma, and MMP-9 is also upregulated in the circulating lymphocytes of these patients (Figure 9.21). These MMPs, in particular MMP-9, are involved in both retinal ganglion cell loss and in tissue remodelling. MMP-9 can be inhibited pharmacologically by GM6001, which is also known as Ilomastat (*N*-[(2R)-2-(hydroxamidocarbonylmethyl)-4-methylpentanoyl]-L-tryptophan methylamide). Studies reveal that inhibition of MMP-9 with GM6001 prevents retinal ganglion cell loss in animal models. Moreover, MMP-9 knockout mice do not show apoptosis of retinal ganglion cells even when the optic nerve is ligated. It is most likely that MMP-9 does play a role in the pathogenesis of GON.

7 Stimulation of heat shock protein production

Heat shock proteins (HSPs) are produced by cells when they are subjected to stress (eg, elevated temperatures, oxidative stress). The upregulation of these

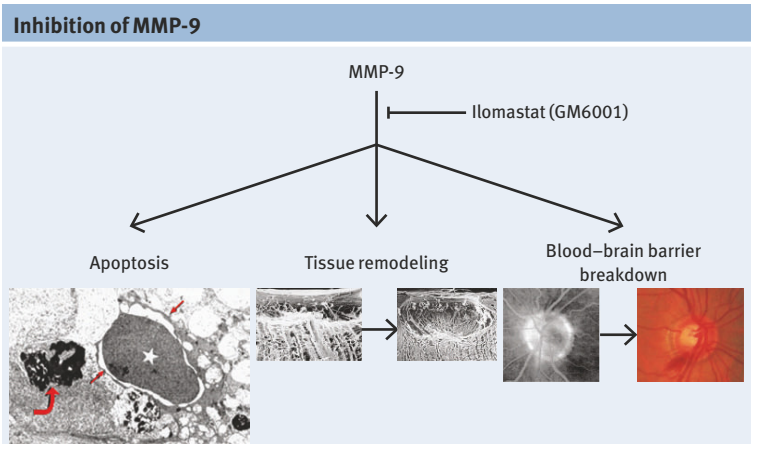

Figure 9.21 Inhibition of MMP-9. MMP-9, matrix metalloproteinase-9. Ilomastat blocks the activity of MMP-9 and thereby prevents the development of glaucomatous optic neuropathy in experimental models.

proteins is a protective mechanism as they act as molecular chaperones protecting the three-dimentional structure of other proteins. HSPs protect neurons from nitrosative stress and excitotoxicity. In a rat model, pharmacologically induced upregulation of HSPs, by the systemic administration of the compound geranylgeranylacetone, protected retinal ganglion cells from glaucomatous damage. Whether treatment with this drug or the natural stimulation of HSPs (eg, by sauna baths) is beneficial in the case of human glaucoma needs to be studied.

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9.9B: Modified from: Kaiser HJ, Stümpfig D, Flammer J (1996) Int Ophthalmology 19:355-358
9.21: Modified from: Mozaffarieh M, Grieshaber MC, Orgül S, Flammer J (2008) Surv Ophthalmol 53(5):479-505

Further reading

The text in this book is based on numerous publications and in order to keep the text concise and readable, individual publications have not been referred to or cited in the text. Instead, the authors direct the reader to their internet homepage: **www.glaucomaresearch.ch.**

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