A NEW APPROACH TO THE MEDICAL MANAGEMENT OF GLAUCOMA, FROM THE BENCH TO THE CLINIC, AND BEYOND

The Proctor Lecture

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lthough prostaglandins (PGs) were said to have detrimental ocular effects, they and their derivatives were found later to represent a new approach to the medical management of glaucoma, being local hormones rather than the heretofore used agonists of the autonomic system or inhibitors that are totally foreign to the body. An esterified PG prodruk, PGF2α-isopropyl ester (PGF2α-IE), was found to be a particularly effective and potent ocular hypotensive agent, but PGs, in general, offer great therapeutic advantage in terms of their local and systemic pharmacokinetics and mechanism of effect. By increasing uveoscleral outflow, the hypotensive effect of PGs is not limited by episcleral venous pressure that may be particularly elevated in sleep. Furthermore, this mechanism, in contrast to the aqueous humor production-reducing effect of β-blockers, provides around-the-clock pressure reduction. This was actually shown to be the case with latanoprost, the active ingredient of Xalatan (Pharmacia, Uppsala, Sweden), the first PG-based glaucoma drug released in the United States and Europe.

It is argued that, in the future, not only the extent but also the mechanism of pressure reduction should be considered in the selection of therapy for a given patient. The major goal of this article is, however, to provide an account of the many hurdles and frustrating obstacles that had to be overcome in the development of Xalatan, from the original misconceptions, through the development of new concepts, to its therapeutic realization.

During much of the 20th century, glaucoma therapy was dominated by drugs relating to the mediators or inhibitors of the sympathetic and parasympathetic systems. In the absence of prior evidence of an autonomic role in the control of intraocular pressure (IOP), this was apparently because these mediators were discovered in the 19th century and their study dominated pharmacology during the first decades of the 20th century. But then, a new class of autacoids was discovered in the 1930s, named prostaglandins (PGs) by von Euler1 in 1934, and found in the 1960s and 1970s to be produced by, and to affect virtually all, tissues of the body.2 Consequently, much effort was devoted to the synthesis of large numbers of PG derivatives aimed at the development of PG-based therapeutic agents for cardiovascular, gastrointestinal, and other major areas of pharmacology, but these efforts eventually faded out. In my judgment, both the initiation and cessation of these attempts were premature, because both preceded the understanding of the pharmacodynamics of PGs.

The plethora of studies on the ocular effects of PGs, including the numerous clinical studies with PGF2α-IE, which became the first PG derivative used to demonstrate the feasibility of this new approach to glaucoma management as well as the mechanism of PG-mediated lowering of the IOP, have been repeatedly reviewed.3–7 An account of the development of latanoprost, with its superior selectivity for the F type of PG receptor, making it therefore essentially free of the conjunctival hyperemic and corneal sensory side effects of naturally occurring PGs, is described in this issue of IOVS by Johan Stjernschantz in his Proctor Medal award lecture.8

It is for this reason that I write here primarily about my own involvement and the work of my own laboratory, although I do not mean to imply that all the relevant ideas were generated by my group.

The First Hurdle: The Concept that PGs Increase IOP and Have Other Deleterious Ocular Effects

My exposure to, and interest in, PGs dates back to 1964, when I started a postdoctoral fellowship with Hugh Davson. I learned then that he had given up research on aqueous humor dynamics, because the required cannulation of the eye invariably led to some breakdown of the blood–aqueous barrier (BAB), preventing evaluation of normal physiologic or pharmacologic influences. A search for the mediator of this annoying effect led Ambache et al.,9–11 in the 1950s, to discover, in crude iridal extracts, lipidic mediators that reproduced some of the signs of ocular irritation and that were subsequently shown to include PGs. This discovery, however, did not help to protect the BAB, because there was no known inhibitor of the PG system. Thus, we focused in the 1960s on experiments that did not require cannulation of the eye, including the elucidation of transport mechanisms that control the unique and remarkably stable microenvironment of the retina and the brain.12–14 Interestingly, these studies led me back to PGs a few years later, after I established my own laboratory at Columbia University.

Meanwhile, in the late 1960s, Upjohn (Kalamazoo, MI) had begun to provide free PG samples, and several researchers began to administer PGs to the eyes of experimental animals (primarily rabbits), mostly through cannulas inserted into the anterior chamber also for measuring IOP, in amounts sufficient to produce signs of ocular irritation and inflammation. These included BAB breakdown and large increases in IOP, which these researchers must have assumed to be the primary ocular effects of PGs, based on the findings of Ambache and Brummer.10 Being aware of the unphysiological state of the cann-
lated eye, I viewed these results with much skepticism, which I did not hesitate to air at scientific meetings. Yet, the resultant concept that PGs are the mediators of ocular inflammation became more and more generally accepted during the 1970s.\(^\text{15}\) This preoccupation with the role of PGs in ocular inflammation did, however, lead to the demonstration that PGs are synthesized by ocular tissues, most notably by the anterior uvea, but are not effectively metabolized within the eye.\(^\text{16,17}\) This led me to the hypothesis that there must be an active transport mechanism for the removal of PGs from intraocular fluids.

### The PG Transport System and Its Implications

My already-mentioned interest in the homeostatic transport processes of the blood–ocular and blood–brain barriers extended over the years to several classes of substances of known biological importance.\(^\text{14}\) Only the so-called organic acid transport system presented an enigma, because its known substrates, such as iodipamide, were not produced within the body and had no known physiological roles. It was one of the great challenges of this field to find the natural substrate of this transport system. However, our first reports\(^\text{18,19}\) demonstrating that PGs are transported by this system were received with great skepticism, because PGs, being fatty acid derivatives, were assumed to freely cross cellular membranes. Starting in 1970, we conducted in my laboratory—with the able cooperation of Erica Salvador, Roger Baroody, and Martin Wallenstein, among others—a series of experiments demonstrating that not only the normal functioning of the eye and the brain, but also the pulmonary metabolism and renal excretion of PGs and PG derivatives depend on this active PG transport system.\(^\text{18–21}\)

Our studies of these transport processes gave us a unique insight into the ocular and systemic pharmacokinetics of PGs and also led me to the conclusion that PGs, functioning in the eye like local hormones, are uniquely well suited to achieve localized effects, after topical application, without systemic side effects.\(^\text{9}\) This became a particularly important consideration after the recognition of the systemic and central side effects of the eye drop formulation of a cardiac drug, timolol, because it called attention to the fact that topical ocular instillation may result in a more effective systemic delivery of a drug than oral administration, by avoiding the “first-pass” effect of hepatic metabolism.\(^\text{22}\)

Our studies of the kinetics of the ocular PG transport system also indicated that PG concentrations in the intraocular fluids (IOFs) are normally maintained at very low levels—orders of magnitude lower than those resulting from the doses of PGs that were introduced in the early 1970s into cannulated eyes, yielding very large IOP increases and other ill effects. The resultant concept that PGs are the mediators of the ocular irritative response and inflammation was also supported at that time by several other observations, most notably in the studies of Kenneth Eakins et al.\(^\text{16}\) showing very large increases in IOP.

The First Breakthrough: A Tonometer Probe that Allowed Us to Study the Whole Time Course of IOP Changes in Uveitis

By that time we had already shown in our PG transport studies that experimental uveitis eliminates the ability of the anterior uvea to accumulate PGs.\(^\text{23}\) This suggested that, during uveitis, the PG accumulation in the aqueous humor may be due not only to increased PG synthesis, but also to impaired PG removal across the BAB. This realization gave me the impetus to undertake a long-term study to define the development and resolution of the various signs of uveitis, including the whole time course of its effect on IOP over several weeks. This was made possible by a breakthrough in 1972, when, with the help of Maurice Langham, we were able to obtain a floating-tip IOP sensor that allowed us to repeatedly measure the IOP of unanesthetized rabbits, without any sign of BAB breakdown.

Intravitreal injection of bovine serum albumin caused a biphasic inflammatory response, with respect to iritis, BAB breakdown, and cellular invasion of the anterior chamber, and a profound decrease in IOP.\(^\text{24}\) The first phase peaked within the first 2 days, followed by a temporary recovery to normal values. The lowest IOP was observed on day 2, and it was preceded by, or paralleled with, a loss of anterior uveal PG transport capacity. This capacity did not show substantial recovery, before a second phase of inflammation, associated with a second cellular invasion of the anterior chamber (peaking at around 12 days), became evident. Only the first phase of this uveitic response was observed when bacterial endotoxin was injected. This led us to the finding that all commercial protein preparations we tested contained such endotoxins.\(^\text{25}\)

The Ocular Effects of Exogenous PGs on Eyes Not Traumatized by Anterior Chamber Cannulation

We found no ill effects when, in our studies on the ocular PG transport system,\(^\text{15,26}\) we injected intravitreally amounts of PGs in excess of those that had been shown previously to cause BAB breakdown when introduced into the anterior chamber. These and many other observations eventually confirmed my conviction that the IOP increases observed in earlier experiments were due to the combined effects of excessively high PG doses and of endogenous mediators released by the trauma of anterior chamber cannulation. However, the implications of these conclusions were generally ignored throughout the 1970s.

It was a very refreshing experience, therefore, when in the fall of 1975, while I was instructing in the histology laboratory, a first-year medical student introduced himself to me and demonstrated not only great familiarity with the PG field, including my own prior publications, but also expressed skepticism regarding the prevailing views on the ocular effects of PGs. This is how I met Carl Camras, who had worked in eye research at Yale as a college student. From some experiments he had done there, Carl had independently concluded that the role of PGs in ocular inflammation is primarily a hypotensive one. Carl demonstrated a keen ability to analyze and critically evaluate relevant publications, as well as to defend his viewpoints vigorously and effectively—virtues he never compromised over the years. Carl joined my laboratory in the summer of 1976, and, using the aforementioned tonometer probe, produced a seminal paper,\(^\text{27}\) demonstrating that low doses of PGs can reduce IOP in unanesthetized rabbit eyes.

Unfortunately, further studies in rabbits revealed that the demonstrated IOP reduction, lasting for a few hours with a single topically applied PG dose, could not be maintained with repeated twice-daily topical applications, because of rapid development of tachyphylaxis, which could not be overcome by increasing the PG dose. In fact, doubling the PG dose from 5 to 10 μg on the fifth day of treatment increased, rather than decreased, the IOP. Tachyphylaxis to the IOP effects of PGs had also been reported earlier, in short-term studies on cannulated eyes,\(^\text{28,29}\) but there was no guidance in the literature as to the possibility of overcoming it.

We postponed the publication of this most disappointing finding for several years,\(^\text{30}\) hoping to first establish its mechanism. Assuming that it had to do with BAB breakdown, we redoubled our ongoing efforts to elucidate the true role of PGs in the mediation of the ocular irritative and inflammatory response and the role of our PG transport system in its prevention. Carl had also worked on one of these projects showing that the large initial IOP increase after nitrogen mustard (NM)
application could be blocked by prior alcohol denervation or by capsaicin-induced neuropeptide depletion, but not by indomethacin. This indicated that the initial IOP response to ocular irritation is mediated by neuropeptides rather than PGs, whereas a second phase of IOP increase, occurring at 3 to 7 hours after NM application, was not similarly blocked but could be reduced by indomethacin. These experiments clearly demonstrated that PGs are just one of the mediators of the complex phenomenon of the ocular irritative response, even in rabbits.

Because one of my coworkers, Susan Merritt, had already trained six owl monkeys to accept handling and tonometry for our IOP studies on the autoregulation of cholinergic mechanisms, Carl also tested the effects of PGs on their eyes. The IOP reduction obtained was very impressive, yet the findings were rather disappointing: even a 40-fold higher dose of PGE2 than was observed to effectively reduce IOP in rabbits was found ineffective in this species. Significant IOP reduction was obtained with 1000 μg per eye, but this dose also produced some initial pressure increase and miosis, and in some animals even a few cells were detected in the aqueous humor of the treated eyes. There was some contralateral IOP effect that appeared to be increased when alternate eyes were treated with the same single dose of PG at several-week intervals. These and other findings suggested that, in these very small-bodied animals (only 0.8–1.0 kg body weight), topically applied drugs can have a systemic effect, thus complicating the interpretation of results. These findings also indicated that there are astonishing species differences with respect to both the nature of ocular response to PGs and the doses required to obtain an effect. The differences in these two divergent species made it impossible to predict the possible responses of the human eye to similar treatment. The eyes of these nocturnal monkeys, in particular, could not be assumed to be an adequate model for the diurnal type of eyes of humans.

By the beginning of the 1980s, our studies, combined with available information on morphologic and behavioral differences in mammals, convinced me that the data obtained from the rabbit, which has a monitoring type of visual system, are irrelevant with respect to the searching-type human eye, which, as we later concluded, has a very different protective mechanism, in which PGs also play an important role, but a role very different from their observed effects on rabbit eyes.

The Unique Sensitivity of the Rabbit Eye to BAB Breakdown and Its Apparent Evolutionary Significance

Using many different experimental models, ranging from paracentesis to x-ray or NM-induced ocular irritation and intravitreal endotoxin or antigen-induced uveitis, I became more and more convinced that the great vulnerability of the rabbit eye to BAB breakdown is not a freakish evolutionary oversight. Rather, it represents a uniquely well-developed prophylactic mechanism to allow the entry of the blood-borne components of the clotting mechanism, required for the sealing of corneal wounds, into the anterior chamber. The importance of this mechanism becomes evident if we consider that the avascular cornea overlying the essentially protein-free aqueous humor is the only part of the body surface that has no ready access to such a clotting mechanism. Because the lateral placed, protruding, unprotected eyes of a rabbit, which allows the virtually 360° field of vision required to monitor its environment for predators, are very vulnerable to injury, there must have been a particularly high selective pressure in this species for a mechanism to allow plasma proteins to enter the anterior chamber after penetrating ocular injury. This, in fact, is a truly prophylactic mechanism in the rabbit, because its aqueous humor can become plasmoid even before corneal penetration occurs, when the corneal surface or the surrounding skin is irritated.

In contrast, for species with great dependence on high visual acuity (such as primates, particularly arboreal primates), such prophylactic breakdown of the BAB would be detrimental because of the interference—caused by the resultant light-scattering of the increased protein content in the anterior chamber—with the very high visual acuity required by such species. However, the frontally oriented, deeply seated eyes in these species are much better protected, thus minimizing corneal injuries, although greatly limiting the visual field. Although the BAB breakdown mechanism in rabbits is mediated by axon reflexes releasing substance P and other neuropeptides, it became clear that PGs play at least a modulatory role in BAB breakdown in this species. We argued, therefore, that tachyphylaxis to PGs may also be unique to the rabbit eye, rendering it particularly unsuitable for predicting the effects of PGs on the human eye.

Selecting More Appropriate Animal Models

For these reasons, we decided to use rhesus monkeys (Macaca mulatta) instead, because this diurnal species has much more humanlike eyes, and macaques have been shown, particularly by the elegant studies of Ernst Bárany, Anders Bill, and Paul Kaufman, to exhibit many physiological and pharmacological responses very similar to those exhibited by the human eye.

However, to develop optimal glaucoma drugs, we expected to assay many dozens of PGs in different concentrations and formulations. Because of their high cost of maintenance and difficulty of handling, we judged monkeys alone insufficient for our purposes. Because of their ease of handling and relatively well protected, searching-type eyes, we chose cats as a second model. And, indeed, we found cats to be much less sensitive to BAB breakdown than rabbits.

Maintained IOP Reduction in Cats and Rhesus Monkeys, with Daily Topical PG Application

Because any drug advocated for the treatment of chronic simple glaucoma must maintain effective IOP reduction indefinitely, we initiated a long-term study in cats that continued for 9 months. Effective IOP reduction was maintained so long as a dose of 100 μg of PGE2 was applied topically to the eye once or twice daily. These studies, together with shorter term studies in rhesus monkeys, clearly demonstrated that in contrast to rabbits, continuous PG treatment in cats maintains IOP reduction without tachyphylaxis or breakdown of the BAB. Virtually all ocular parameters that could be assessed noninvasively were evaluated in at least some of the six cats studied. All these parameters, which included ERG, the integrity of intracellular muscle function as determined with dynamic pupillography, maintenance of endothelial cellularity as assessed by specular microscopy, and complete biomicroscopic evaluations, were found to remain normal.

These were the key experiments that finally allowed me to sufficiently convince myself, and thereby to be able to begin convincing others, that PGs could be used for long-term reduction of IOP for the medical management of glaucoma—i.e., that our ideas, dating back to the early 1970s, could indeed be reduced to practice.

Coincidentally, it was at this time, in 1980, when I first heard about the Bayh-Dole Act, which encouraged, and in essence required, the patent protection of findings arising from National Institutes of Health (NIH)—sponsored research that have therapeutic potential, as well as their licensing for commercial development. Accordingly, with the help of university
attorneys, I applied for a patent for the use of PGs to reduce IOP in glaucoma.38

The Side Effects of PGs on the Ocular Surface and the Need to Reduce the Topically Applied Dose

The anticipation of using PGs to reduce IOP in patients led me to put the same dose of 100 µg PGE₂, that we used on the cats into my own eye, but I quickly washed it out, because I experienced a very irritating foreign-body sensation. Actually, I should have been prepared for this effect, because we had noted that after topical PG application, cats would hold their treated eyes closed for varying periods, indicating some irritation of the ocular surface. Luckily, we soon realized that the duration of eye closure in these cats was dependent on the dose and type of PG applied. I found that these animals’ reactions correlated with the extent of the foreign-body sensation that I experienced, after putting various PGs in different doses into my own eyes.

Such comparison also made me realize that some of these side effects on the ocular surface must be due to the very high topical PG concentrations required to deliver effective amounts to intraocular target sites, because of the low permeability of the cornea to PGs. This concept was also contrary to the accepted view on PGs before our work, which was that these lipid mediators could readily dissolve in and pass through membrane lipids. This, of course, implied that they could be expected to freely penetrate even tight-junctional membranes, such as the corneal epithelium. Contrary to this a priori view, our studies demonstrated that the basic cell membrane, thus also the tight-junctional corneal epithelium, represents an effective permeability barrier to PGs.39

The Search for a Commercial Partner

By this time, officers of the newly established Office of Science and Technology Development at Columbia University had approached the relevant pharmaceutical companies in the United States, but none was inclined to license my patent, presumably because of the still prevalent prejudices against the ocular use of PGs. It was then that, through the mediation of my colleague and friend, Endre Balazs of Healon fame (Pharmacia & Upjohn), we established contact with Pharmacia in Uppsala, Sweden. Much credit is due to Bengt Agerup of Pharmacia, who could see the significance of our findings, and who became a great supporter of this project within the company.

The Pharmacokinetic and Pharmacologic Advantages of Local Hormones Such as PGs

Our studies on the control of cholinergic sensitivity showed, for example, that from a physiological point of view, pilocarpine is particularly ill-suited for use as a topical ocular hypotensive agent because of its antagonistic effects within the eye, decreasing uveoscleral outflow while facilitating conventional outflow. We could even demonstrate that under some conditions, it increases rather than decreases IOP.40,41 These and other considerations led me to develop the concept that the use of local hormones has great therapeutic advantages over the use of neurotransmitters and their analogues. In contrast to these, local hormones must have evolved to have a consonant effect in the diffusional domain of their site of release.42,43 They also offer great advantage when applied topically, because of the very specific distinguishing characteristic of local hormones—i.e., their rapid inactivation in, and removal from, the circulation, virtually eliminating the possibility of systemic side effects.44 These apparent advantages of this new approach convinced me to stay with this project and to fight for its success, overcoming all obstacles.

Esterified PGs, as the First Approach for Overcoming Local Side Effects

In the ensuing collaboration with Pharmacia, we initially hoped to minimize the known effects of sensory irritation and conjunctival hyperemia by finding a PG ester (or diester) that was stable enough not to be rapidly de-esterified on the ocular surface, hence remaining inactive, yet hydrolyzed by tissue esterases rapidly enough after it enters the cornea to have a full intraocular hypotensive effect. By that time, we were collaborating with Bahram Resul, a medicinal chemist who was the first person hired by Pharmacia for this project, in 1983. Bahram synthesized many different esters and sent them to us to test in our cat and monkey IOP models. In addition, by measuring the isotonic contraction of bovine iris sphincter preparations in vitro, we could estimate the rate of hydrolysis of various PG esters within this tissue, from the time elapsed between their addition to the bathing fluid and the onset (or half-time) of contraction. We could also estimate the efficacy and potency of the free PG released, by determining the maximum sphincter muscle contraction achieved. As we could create beautiful structure–activity relationships that could also be confirmed in vivo by measuring miosis in cats,45 Bahram and I were very optimistic that we would find a PG derivative with reduced local side effects, but support for this project within Pharmacia started to dwindle.

The Savior Arrives and Sets His Sights Very High

The arrival of Johan Stjernschantz, who joined the project in 1986, was a godsend. Johan immediately saw the great value of this new approach to glaucoma management and gave up the higher management position he was originally hired for by Pharmacia to become the project leader. Johan set his sights very high: to go beyond esterification alone and to develop a PGF₂α derivative that would retain the potency and efficacy of PGF₂α esters but would not have the known local side effects of foreign-body sensation and conjunctival hyperemia, which would have limited its use to cases of glaucoma that could not be controlled by milder drugs.

At first, I was disappointed by the obvious delay that ensued as a result of this high standard, because in my case the foreign-body sensation caused by PGF₂α-IE and some of the higher esters was quite tolerable, at least compared with some of the PG-free acids I had tried. I thought that we should at least make PGF₂α-IE available to patients with glaucoma that was not controlled by other drugs. But finally, I had to accept as an unfortunate fact of life that a company cannot go to the expense of long-term toxicology and phase 3 studies unless the drug is acceptable for a large enough segment of patients, making it commercially viable.

Carl Camras, who had returned to New York by then and was working at Mt. Sinai with Steve Podos, continued to advocate making PGF₂α-IE available for clinical use. When in the summer of 1988, Carl, Johan, and I went to Umeå, Sweden, to visit Albert Alm (who by then had conducted very important phase 2 studies with PGF₂α-IE in human volunteers), Carl kept insisting that patients are used to such side effects, but then admitted that he had never put any PGs into his own eye. That was easily remedied. We did put a drop of the PGF₂α-IE into one of Carl’s eyes, and he quickly admitted that even though the foreign-body sensation was tolerable, many patients would not comply with its daily use.

Johan Stjernschantz and Bahram Resul, with their teams, worked many long days producing new PG derivatives that they tested in Uppsala and we in New York. They came up with latanoprost, the isopropyl ester form of a 17-phenyl PGF₂α derivative (the active ingredient of Xalatan) in record time. For this, they deserve great credit. The series of steps that led to
this derivative, as well as its pharmacology, are described in the presentation of Johan Stjernschantz, the coreipient of the 2000 Proctor Medal.

The Next Crisis
I thought my job would end with the completion of the laboratory research, but biology is not a science of the readily predictable. I was ready to settle back, primarily to our fascinating studies on accommodation and presbyopia with Paul Kaufman and others, knowing that the PG project was in good hands, when I received a crisis call from Johan. In the toxicology study in France, they noted that the irides of the latanoprost-treated eyes in some monkeys became noticeably darker. I was stunned. “And in the rabbits?” I asked after I recovered my wits. “None of the rabbits,” was his response. Species differences had struck again! This time, however, closer to home, because the unwanted effect occurred in a primate. On the other hand, I had convinced myself so thoroughly by then that local hormones can do no wrong that I was able to regain my composure. My first hope was that the increased pigmentation might reflect some peculiarity related to the characteristic coloration of the rhesus monkeys’ eyes.

At that point, however, the initiation of the phase 3 studies was at stake, and Johan therefore asked me to try to figure out what was going on. My job was easy: I simply had to work day and night to educate myself in melanin chemistry and the fascinating world of melanosomes which, as I expected, had many aficionados in dermatology. It did not take me long to learn about the fundamental difference between the melanin system of the skin and the eye—namely, that dermal melanocytes continually produce melanin and transfer it to keratocytes, whereas iridial melanocytes are continent and presumably have no mechanism to lose melanin, which itself has no intracellular turnover. Thus, if PGs stimulate melanogenesis even to a minimal extent, it would obviously have to result eventually in an observable darkening of a light-colored iris. There was no need to assume that the iridial pigmentation we observed was due to proliferation of melanocytes.

After many long transatlantic calls and emergency meetings in Uppsala, we were able to persuade management to continue with the project, thus gaining some time to learn more about the melanin system and to initiate some studies.

Luckily, there were some very good electron microscopic studies available in rhesus monkeys showing that their iridal melanocytes are morphologically different from those of the human eye and that sulfur-containing phaeomelanin is a major form of pigment in rhesus monkeys, rather than eumelanin, which is the major form in most humans (or in pigmented rabbits, for that matter). In short, there was reason to believe that the increased iridial pigmentation that occurred in monkeys but not in pigmented rabbits, might not necessarily occur in humans. Thus, Johan’s decision to perform one of the toxicology studies in a primate, resulting in the early discovery of this side effect, turned out to be a godsend. It led to the inclusion of photographic documentation of the eye color of all patients when they were enrolled in the phase 3 studies and periodically during the whole study. This allowed the documentation of the extent, incidence, and time course of this fascinating effect.

The Third (Not Entirely Unexpected) Crisis
In the meantime, our efforts to gain a better understanding of the melanin system, the peculiarities of the iridal melanocytes, and the possible effects of PGs thereon, continued full steam. Thus, by the time the first human case of increased pigmentation was reported in the United Kingdom, we had reason to assume what later would be experimentally supported—that PGs produced within the globe may be involved with the normal maintenance of iridial pigmentation and that this phenomenon may simply represent just one more normal physiological mechanism mediated or modulated by endogenous PGs.

The future of the whole project depended on the answer to the question of what happens to the pigmentation after treatment is stopped, which the protocol of the phase 3 study was designed to answer. If the pigmentation continues to increase after treatment is stopped, as soon as increased pigmentation is noted in a given patient, the project must be terminated, at least temporarily, because this would imply that the exogenous PGs induce a self-propagating, and thus possibly malignant, process. If the effect is rapidly reversed, it would imply that PGs stimulate very excessive melanin production that is partially compensated for by loss of melanosomes, which is likely to occur also during treatment. In this case, further studies would be required to study the fate of these melanosomes, including the possibility that they accumulate in the trabecular meshwork.

Anxious Waiting
The ensuing months were nerve wracking, until the third possibility was shown to be the case. After the pigmentation increase was noted and the treatment stopped, the pigmentation remained constant in all cases, showing neither a continuing increased nor a rapid decrease and implying that this side effect on eye color is primarily of cosmetic concern. This, however, does not mean that I did not consider the effect to be a potentially very important finding, particularly because my extensive reading and the studies we initiated revealed how little is known about iridial melanocytes and their physiological functions. My great interest in this phenomenon took me back to my long-time interest in the homeostasis of IOP composition and the microenvironment of intraocular tissues. Given the capacity of the melanin system to scavenge free radicals, I began to consider the possible role of iridial melanocytes in free radical and hydrogen peroxide management of the aqueous humor.

Beneficial Potentials and Missed Opportunities
The possible beneficial effects of PG-induced stimulation of the melanin system of the iris began to intrigue me, particularly when I found two articles in journals not usually monitored by researchers in our field. These provided evidence that iridial pigmentation is not stable in all individuals throughout their lifetimes. One study provided cross-sectional data strongly suggesting that iris color can change in adults. Moreover, I noted that if PGs stimulated melanogenesis even to a minimal extent, it would obviously have to result eventually in an observable darkening of a light-colored iris. There was no need to assume that the iridial pigmentation we observed was due to proliferation of melanocytes.

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The possible beneficial effects of PG-induced stimulation of the melanin system of the iris began to intrigue me, particularly when I found two articles in journals not usually monitored by researchers in our field. These provided evidence that iridial pigmentation is not stable in all individuals throughout their lifetimes. One study provided cross-sectional data strongly suggesting that iris color can change in adults. Moreover, I noted that if PGs stimulated melanogenesis even to a minimal extent, it would obviously have to result eventually in an observable darkening of a light-colored iris. There was no need to assume that the iridial pigmentation we observed was due to proliferation of melanocytes.

In the meantime, our efforts to gain a better understanding of the melanin system, the peculiarities of the iridal melanocytes, and the possible effects of PGs thereon, continued full steam. Thus, by the time the first human case of increased pigmentation was reported in the United Kingdom, we had reason to assume what later would be experimentally supported—that PGs produced within the globe may be involved with the normal maintenance of iridial pigmentation and that this phenomenon may simply represent just one more normal physiological mechanism mediated or modulated by endogenous PGs.

The future of the whole project depended on the answer to the question of what happens to the pigmentation after treatment is stopped, which the protocol of the phase 3 study was designed to answer. If the pigmentation continues to increase after treatment is stopped, as soon as increased pigmentation is noted in a given patient, the project must be terminated, at least temporarily, because this would imply that the exogenous PGs induce a self-propagating, and thus possibly malignant, process. If the effect is rapidly reversed, it would imply that PGs stimulate very excessive melanin production that is partially compensated for by loss of melanosomes, which is likely to occur also during treatment. In this case, further studies would be required to study the fate of these melanosomes, including the possibility that they accumulate in the trabecular meshwork.
showed that eye color changes occurred both in the twins up to 24 years of age and in the parents during adulthood. Interestingly, approximately 15% of both the young-adult and the adult population exhibited such changes in the sample of white twins.\textsuperscript{49} This was about the same percentage as the overall incidence of PG-induced eye color change in the phase 3 Xalatan study.\textsuperscript{8,5} This led us to the working hypothesis that approximately 15% of white persons retain a mechanism that influences the melanin synthesis of iridial melanocytes past infancy. Considering the typically uneven initial coloration in the eyes that are most likely to show this PG-response,\textsuperscript{8,50} our working hypothesis further assumed that this type of iridial coloration is due to age-dependent focal or regional loss of pigmentation that can be reversed by PGs.

If you will excuse me for a nonscientific observation: Ever since we observed this phenomenon and I read the literature on eye color changes, I was looking in a conscious way at the irides of just about everybody I met. And I became convinced that the multicolored irides with irregular areas of hypo- or de-pigmentation and that exhibit the greatest tendency toward PG-induced increased pigmentation do not occur in children or young adults. Thus, it must represent an age-dependent focal loss of pigmentation. It would also be of interest to compare the appearance of the iris in persons with ocular hypertension and in normal individuals and also in those with ocular hypertension and normal persons who do and do not have a tendency toward development of glaucomatous changes. I must emphasize that I do not believe in “iridology,” but it is indeed likely that age-dependent loss of melanin from the iridial melanocytes reflects some local insufficiency, possibly insufficient endogenous PG production that may also contribute to the pathophysiology of the outflow routes and may reflect an underlying condition that affects the whole eye, contributing even to age-related macular degeneration.\textsuperscript{51}

The Possible Role of the Iridial Melanin System in Intraocular Homeostasis

Compromised melanin systems, as reflected by age-dependent loss of iridial pigmentation, may also contribute to other age-dependent ocular diseases by compromising, for example, the normal homeostasis of aqueous humor composition, thus compromising the maintenance of the avascular tissues of the globe. There is considerable evidence, for example, that hydrogen peroxide levels in the aqueous humor may cause or contribute to cataractogenesis.\textsuperscript{52} The maintenance of the avascular portion of the trabecular meshwork can be expected to be just as vulnerable as the lens to the deterioration of aqueous humor composition. Given that most of the iridial melanocytes lie on, or their processes reach, the anterior surface of the iris and because of the rough involuted surface of the iris, these cells are ideally positioned to allow their rapid metabolic exchanges with aqueous humor. Because the contemporary view regards the melanocyte–melanin system not just as a sunscreen (as it used to be regarded) but assigns to it much more complex, multifaceted, protective functions,\textsuperscript{53–55} it is high time to study the role of uveal melanocytes in the maintenance of normal intraocular processes and functions.

This melanogenic side effect, particularly if it stimulates further studies, may lead not only to a better understanding of the function of these long-ignored cells, but, as has been the case with many side effects, may also lead to important new therapeutic approaches.

The Role of the Iris in Protecting Other Intraocular Tissues from Light Damage

It has always surprised me—and surprises me even more now—that many people in eye research who are interested in the adverse effects of light study only the lens: this, in spite of the fact that the most sensitive part of the lens, its germinative zone, receives virtually no light, whereas even the center of the lens, where all the light passes through, absorbs very little of it—and, in the case of a clear young lens, virtually none. By contrast, the iris absorbs much, if not all, of the light impinging on it, and the more light that reaches the eye, the more iris surface is exposed to it. Thus, the iris is likely to be the organ possessing the most highly developed mechanism to cope with the damaging effect of light. Furthermore, compared with the skin, which has a continuous turnover of cells and melanin, the light-absorbing cells of the iris do not. Thus, these cells must cope with light irradiation for a lifetime, and, judging from the very low incidence of iridial disease, manage to do so remarkably well. Thus, the study of iridial melanocytes, which has begun to gain more momentum in the past few years,\textsuperscript{56} may hold the key to the understanding of how biological systems can most effectively cope with the damaging effects of light.

Nocturnal Versus Diurnal Aqueous Humor Dynamics and My Hopes for Combined Formulations of a PG and a β-Blocker

Such formulations had much theoretical advantage, and my laboratory had shown such unexpectedly good additivity that jointly with Johan we obtained a separate patent to assure that Pharmacia would use the advantages of this approach. I advocated the marketing of formulations with different concentrations of latanoprost and a β-blocker. Most important, I also advocated effective studies evaluating the advantages of this approach in terms of improved compliance and specific efficacy—primarily, the efficacy of the tempering of the IOP spikes associated with awakening.\textsuperscript{57,58}

I had become convinced over the years that these IOP spikes are due to the switch from a nocturnal to a diurnal type of aqueous humor dynamics—that is, the switch from a lower rate of aqueous humor secretion\textsuperscript{59} and an outflow presumably dominated by the uveoscleral route (partly in view of increased episcleral venous pressure) during sleep to the diurnal type of increased aqueous humor production and increased conventional outflow facility (due in part to the pilocarpine-like effect of accommodative efforts).

I had already reviewed some experimental findings and had presented theoretical considerations strongly suggesting that even such short episodes of IOP increases may be sufficient to cause the accumulation of permanent damage to the optic nerve head.\textsuperscript{58,59} Considering that glaucomatous damage develops over years or decades, one can appreciate how even the smallest chance of minimal damage caused by some pressure spikes—the not always reversible collapse of a few capillaries, for example—could eventually cause the optic nerve damage in glaucoma.

We have evolved to be able to switch over safely from sleep to wakefulness, with the slowly increasing light and the gentle stirrings of the dawn, over a considerable period, allowing the associated physiological processes to follow each other in a safe sequence. Evolution could not, however, prepare us to cope with the rapid switch from sleep to the awake state that began to occur routinely only in our alarm-clock–driven lifestyle. It seems possible and even likely that the more rapid the awakening, the more likely that various phases of the changeover will overlap. For example, aqueous humor secretion may increase while episcleral venous pressure is still at its nighttime high, causing a transient IOP increase.

Thus, I would have liked to include the study of at least one combined formulation with the lowest effective β-blocker concentration, because we already know that β-blockers are incapable of reducing IOP during sleep.\textsuperscript{59} On the other hand,
latanoprost was actually shown—as would be expected based on its known effect of increasing uveoscleral outflow—to provide 24-hour IOP reduction. For these reasons, at least some studies on the efficacy of combined Xalatan and \( \beta \)-blocker therapy should emphasize the evaluation of the reduction of IOP spikes during the period of awakening. Clearly, combined formulations also should include preparations with lower latanoprost concentrations for those patients who show a greater than average sensitivity to the ocular hypotensive and/or the side effects of PGs.

**Other Hopes and Plans that Were Frustrated**

My hope was not only that this new approach to glaucoma management would yield new drugs, but also that it would open up new avenues of study toward the understanding of the role of IOP and different types of IOP abnormalities in the glaucomatous process. I am convinced that in the new century the question is not just going to be how much to reduce IOP in a given patient (i.e., target pressure), but also how to reduce it—i.e., what aspect(s) of transient or maintained (nocturnal or diurnal) IOP elevations have to be protected against in any given patient (i.e., target mechanism).\(^{59,60}\)

**My Concern over Increasing Commercial Pressures on Academic Research**

The pressure to patent and license technologies derived from academic research will unquestionably continue under the Bayh-Dole Act for decades to come. This will increasingly alter the orientation of academic research and its reward structure and will continue to alter the contributions society expects from scientists and universities. As was pointed out in a major article in the New York Times\(^ {63}\) that was based largely on a lengthy interview with me and appeared just before my Proc- tor Lecture, so far the public has not benefited from by the Bayh-Dole Act in terms of more affordable drug prices. Unfortunately, the authors failed to address my concern that although the Bayh-Dole Act encourages the patenting of inventions, it fails to address the problems of a completely outdated patent system that was never designed for, and is totally unsuited to, the field of therapeutics as it exists today. New types of licensing agreements also must be developed to reflect the collaboration of two different types of institutions. Even more important, investors should not be required to assign their patents to their universities unless their roles and responsibilities are clearly defined in a mutually acceptable manner.

Furthermore, the inventor should have the option of assigning a patent, with or without further personal involvement in the developmental phases. If the inventor elects to return to academic research without further demands on his or her time and effort, the university must assume all further responsibilities for the management of any resultant licenses when it becomes the owner of the patent under current rules. On the other hand, if the inventor elects to continue with the project, he or she must be made part of the university’s team that the company reports to and negotiates with. Furthermore, the licensing agreement in this case must reflect an agreement of joint development between two entities of equal status.

Most of all, the best-effort clauses must have real teeth, and the licensing agreement should have provisions for the periodic insertion of new best-effort deadlines, particularly as the collaborative research reveals new line extension products under the existing patent or as additional patents are awarded.

My preference would be for the government to establish an institute providing an alternative, where needed, for the development of drugs that emerge from government-sponsored research. This entity should invest in the development of a drug according to the anticipated benefit to the public. After completion of phase 2 and in some cases even Phase 3 studies, this institute would negotiate, jointly with the inventor and his or her institution, with pharmaceutical companies for the manufacturing and distribution of the drug.

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**References**


