PROSTAGLANDINS, NONSTEROIDAL ANTI-INFLAMMATORY AGENTS AND EYE DISEASE*

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During the last decade an ever-increasing number of investigations have been carried out on the prostaglandins and their inhibitors. It has become clear that the prostaglandins are an important group of compounds. One function of these compounds is the mediation of certain aspects of the inflammatory response. Moreover, a host of nonsteroidal anti-inflammatory agents that inhibit the action of the prostaglandins have been elucidated.

It is the purpose of this report to review the interrelationships of the prostaglandins, their inhibitors, and their role in disorders of the ocular system and to present the results of studies designed to answer some of the questions about their actions and inhibition with respect to the eye.

PROSTAGLANDINS AND THE EYE

Mechanical irritation of the iris produces miosis, increased vascular permeability, and elevation of intraocular pressure in rabbits.\(^1\) Rabbit iris contains a substance which contracts smooth muscles and mimics the effects of stroking the iris. This substance is an unsaturated hydroxy fatty acid which Ambache named irin.\(^2\) After paracentesis, irin appears in secondary aqueous humor.\(^3\)

Acidic lipids extracted from human vesicular glands, which von Euler called prostaglandins, contract smooth muscle.\(^4\) The prostaglandins are 20 carbon, unsaturated fatty acids.\(^5\) Arachidonic acid (5, 8, 11, 14-eicosatetraenoic acid) and dihomo-\(\gamma\)-linolenic acid (8, 11, 14-eicosatrienoic acid) are the precursors of prostaglandin E\(_2\) (PGE\(_2\)) and prostaglandin E\(_1\) (PGE\(_1\)), respectively. Other prostaglandins are reported

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such as the A, F, and B series. The activity of the prostaglandin synthesis system varies among different tissues of the body.

The prostaglandins exist in virtually every organ system and their actions are diverse. They are involved in inflammation, pain, fever, smooth muscle contraction, gastric secretion, lipid and carbohydrate metabolism, cardiovascular responses, renal physiology, and blood coagulation, for example. Suggested therapeutic implications of this family of compounds include their use as bronchial dilators, gastric antisecretogogues, abortifacients, and antihypertensive agents. Different members of the family may have different actions on the same system.

The iris of rabbits and sheep contains PGE$_2$ and prostaglandin F$_2$ (PGF$_{2\alpha}$) which are felt to be the vasoactive agents of irin. Moreover, the iris of the pig eye, in vitro, is able to convert fatty acid precursor to prostaglandin. Thus, the eye contains these compounds and the machinery to manufacture them.

The effects of prostaglandins on the eye have been reviewed. Prostaglandins, administered topically, systemically, or intracameral, elevate intraocular pressure in the rabbit, cat, and monkey. Very small doses of prostaglandin produce this effect. With topical application of 0.5 $\mu$g of PGE$_1$, a significant rise in intraocular pressure occurs with a maximum elevation within the first 30 minutes. This is followed by a gradual decrease. Two hours after 50 $\mu$g of PGE$_1$ the intraocular pressure still remains above baseline values. The PGEs induce the greatest pressure response while PGF$_{2\alpha}$, PGA, and PGF$_1$ are respectively less effective. Differences between species of animals are noted.

The mechanism of the intraocular pressure response appears to be increased aqueous humor production. No decrease of outflow facility occurs after intracameral injections of PGE$_1$ in cannulated eyes of anesthetized rabbits. In awake, non-cannulated rabbits, tonography reveals an increase in total facility of outflow after topical PGE$_1$. The intraocular pressure response is less in the monkey after equivalent doses of PGE but a rise of outflow facility also occurs. PGE$_1$ increases trans-epithelial short circuit current in isolated ciliary body preparations, implying increased active transport. A breakdown of the blood aqueous barrier and increased pseudofacility caused by PGE may provide other possible explanations for its pressure effects. After topical application of PGE, extravasation of thorotrast particles occurs in the stroma of the ciliary and iris processes. After injection of PGE$_1$ into the vitreous humor there is alteration of the tight junctions of the non-pigmented epithelium allowing for leakage of protein into the anterior chamber in
rabbits. In addition, anterior segment fluorescein angiography shows increased vascular permeability in rabbit eyes. A marked increase of aqueous-humor protein is a concomitant feature in cat, monkey, and rabbit eyes after treatment with PGE₁ or PGE₂. The protein level in rabbit aqueous humor is ten to fifteen times as high one hour after the topical application of 5 µg of PGE₁ as compared to aqueous humor from untreated or control eyes. The reason for the increased aqueous humor production is not entirely resolved.

Other related features of the effect of prostaglandins on the eye are of interest. Most investigators report concomitant miosis. A consensual response in the fellow eye after treating one eye with intracameral prostaglandin is reported. The secondary messenger cyclic adenosine-3’,5’-monophosphate (cyclic AMP, c-AMP) is involved in the actions of prostaglandins. Adenyl cyclase, the enzyme which generates cyclic AMP, and c-AMP phosphodiesterase, which converts cyclic AMP to the metabolically inactive adenosine-5’-monophosphate, exist in ciliary processes and iris tissue. Cyclic AMP is present in aqueous humor, iris, ciliary body, and other ocular tissues. Adrenergic agents increase the amount of anterior but not posterior chamber aqueous humor c-AMP, and c-AMP increases outflow facility. Ciliary process adenyl cyclase is responsive to PGE₁ as is the cyclic AMP of rabbit iris and ciliary body in vitro.

The following studies were carried out to delineate the effect of topically applied PGE₂ on aqueous humor cyclic AMP in rabbits in vivo.

MATERIALS AND METHODS

³H-c-AMP (38.4 Ci/mMole) was obtained from New England Nuclear, c-AMP from Sigma, and scintillation fluid (3a40) from Research Products International Corporation. c-AMP-dependent protein kinase was purified from rabbit skeletal muscle by the method of Wastila and associates. The protein kinase inhibitor was purified through the trichloracetic acid precipitation step from rabbit skeletal muscle by the method of Walsh and co-workers. Charcoal (3 gm, Nutral A from Sigma) was suspended in 250 ml of 50 mM potassium phosphate buffer, pH 4.0, and stirred for 30 minutes at 4°C. Bovine serum albumin (0.5 gm, Sigma) and dextran (0.3 gm, mol. wt. 8200, Sigma) were dissolved in 250 ml of the same buffer and stirred for at least 30 minutes. The dextran and bovine serum albumin solution was then added to the charcoal suspension and was allowed to stir overnight at 4°C. This was made up fresh each week and was stirred for 30 minutes before each use.
Cyclic AMP was measured by a modification of the protein-binding method of Gilman. All procedures were carried out in an ice bath except where indicated. All components of the reaction mixture except the protein kinase were made up or diluted in 50 mM sodium acetate buffer, pH 4.0. Protein kinase was diluted in 10 mM potassium phosphate, pH 7.0.

The concentration of the components of the reaction mixture was adjusted so that there was enough $^3$H-c-AMP to give 25,000 counts per minute, enough protein kinase to bind between 12 and 20% of the c-AMP, and a maximal effective concentration of protein kinase inhibitor. Volumes normally used were 110 µl sodium acetate buffer, 20 µl $^3$H-c-AMP, 20 µl protein kinase inhibitor, and 50 µl binding protein. Test tubes containing the air dried aqueous humor samples were stirred vigorously with buffer on a vortex mixer to insure all c-AMP was in solution. The reaction was initiated by the addition of protein kinase. After two hours the reaction was stopped by the addition of 1 ml charcoal and the test tube was quickly stirred on a vortex mixer and centrifuged for five minutes at 1300 × g. Centrifugation was done at room temperature. The aqueous phase was decanted off the charcoal and counted in 10 ml scintillation fluid.

A standard curve of 0.25 to 16 p moles c-AMP was run with each experiment and duplicates were run at each concentration.

Each milligram of PGE$_2$ was dissolved in 0.1 ml of 95% ethanol and 0.9 ml of 0.02% sodium carbonate aqueous solution. Baseline intraocular pressure was measured in awake, wrapped animals under topical 0.5% proparacaine anesthesia with the Mackay-Marg tonometer. PGE$_2$, 5 µg in 5 µl, was applied to one eye and 5 µl of diluent to the other eye. Other rabbits received just the diluent. Thirty minutes later, intraocular pressure was remeasured and 0.1 ml of anterior chamber aqueous humor was removed with a tuberculin syringe. Duplicate samples of 100 µl aqueous humor were immediately pipetted into 1.5 ml absolute ethanol in 12 × 75 mm polystyrene test tubes. These were placed in boiling water until the ethanol boiled and were then cooled in an ice bath. They were then dried under a stream of air and the c-AMP assay was run in the same test tube.

RESULTS
At 30 minutes after instillation of PGE$_2$, the cyclic AMP in aqueous humor was significantly higher in the treated eyes than in the fellow eyes (Table I). This was associated with a significant elevation of intraocular pressure in the PGE$_2$-treated eyes. Ten eyes of rabbits treated just with diluent
TABLE I: EFFECT OF TOPICAL PGE₂ ON AQUEOUS HUMOR CYCLIC AMP IN 15 RABBITS

<table>
<thead>
<tr>
<th></th>
<th>Mean c-AMP ± SE (nM/L)</th>
<th>Mean ΔIOP ± SE (mm Hg)</th>
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</thead>
<tbody>
<tr>
<td>PGE₂ (5 μg)</td>
<td>53.3 ± 3.3*</td>
<td>+10.7 ± 1.4*</td>
</tr>
<tr>
<td>Fellow eye</td>
<td>34.1 ± 2.7</td>
<td>−0.3 ± 0.6</td>
</tr>
</tbody>
</table>

*Significant difference between eyes treated with PGE₂ and eyes treated with diluent at 30 minutes after treatment, paired t-test, p < 0.001.

had a mean c-AMP level of 25.7 ± 1.1 (SE) nM/L in their anterior chamber aqueous humor. The higher levels in the fellow eyes may reflect a consensual response.

The results of cyclic AMP in anterior chamber aqueous humor of untreated animals agree well with those previously reported.²⁷ It is not surprising but reassuring that PGE₂, *in vivo*, produces an increase of aqueous humor c-AMP. Further studies are needed to explain why the epinephrine-induced elevation of ocular cyclic AMP is associated with a fall in intraocular pressure while the PGE-induced aqueous humor cyclic AMP elevation is associated with a rise of intraocular pressure.

INHIBITION OF PROSTAGLANDINS

The direct actions of prostaglandins may be inhibited by a variety of compounds such as polyphloretin phosphate (PPP). The smooth muscle contraction in isolated preparations induced by PGE₂ and PGF₂α is inhibited by PPP. In the rabbit, close-arterial infusion or subconjunctival injection of PPP antagonizes the intraocular pressure elevation due to PGE.¹⁵ Topical instillation of PPP only limited the PGF₂α-induced pressure rise. A low molecular weight fraction of PPP contains the major prostaglandin-blocking activity. SubconjunctivalPPP also antagonizes the increased permeability of the iris due to topical PGE as documented by fluorescein iris angiography.²¹ Intraocular PPP fails to modify the effects of prostaglandin in the monkey eye.¹⁴ Some prostaglandin congeners selectively antagonize smooth muscle actions of the prostaglandins,³⁴ but no information is reported with respect to ocular studies along these lines. In our unpublished experiments, one of these compounds, 7-oxa-13 prostynoic acid administered topically in doses of 500 μg, does not ablate the intraocular pressure response of rabbit eyes to 5 μg of topical PGE₁. Another agent capable of blocking prostaglandin-induced intraocular pressure elevation in rabbits is imidazole.³⁵ If the elevation of intraocular pressure induced by prostaglandins is related to the generation of increased levels of intraocular cyclic
AMP, agents which increase the activity of phosphodiesterase, its inactivating enzyme, should antagonize the prostaglandin effect by lowering the cyclic AMP concentration. Although a variety of drugs, such as the methylxanthines, are known to inhibit phosphodiesterase, very few cyclic AMP phosphodiesterase stimulators are described. Imidazole is capable of increasing the activity of cyclic AMP phosphodiesterase \textit{in vitro}. The specificity of this effect is demonstrated by other studies.

Our \textit{in vivo} studies demonstrate the capability of imidazole to block the prostaglandin-induced intraocular pressure elevation in awake rabbits. However, imidazole also inhibits the elevation of intraocular pressure produced by topical nitrogen mustard, an action that is not prostaglandin-mediated. Thus imidazole is not a specific prostaglandin inhibitor but does have promising anti-inflammatory properties.

On the other hand, the endogenous synthesis and release of prostaglandins may be inhibited and thus their effects prevented. The aspirin-like compounds are such inhibitors. Aspirin and indomethacin inhibit the generation of PGE$_2$ and PGF$_{2\alpha}$ from arachidonic acid added to homogenates of guinea pig lung. The release of E prostaglandins from rabbit spleen is enhanced by the addition of arachidonic acid and abolished by aspirin and indomethacin. Aspirin, \textit{in vivo} and \textit{in vitro}, inhibits the production of prostaglandin in human platelets. Vane suggests that a mechanism of action of aspirin-like drugs, which are potent anti-inflammatory, analgesic and antipyretic agents, appears to be via inhibition of prostaglandin synthesis. Intermediary substances may be involved. Many families of compounds have such inhibitory action including the indomethacin analogs, arylacetic acids, fenamates, salicylates, pyrazolones, and others. Very similar rank order of potencies are described for these drugs in the \textit{in vitro} cell-free synthetase assay and the \textit{in vivo} standard carrageenin edema rat paw assay. The corticosteroids are very potent \textit{in vivo} anti-inflammatory drugs. Yet, conflicting results are reported with respect to their inhibition of prostaglandin biosynthesis. Some investigators find little effect, while others report inhibition of PGE$_2$ synthesis from arachidonic acid in skin homogenates. Possibly, corticosteroids inhibit the release rather than the synthesis of prostaglandins.

A differential sensitivity of the prostaglandin synthetase system exists in different tissues. For instance, iris enzyme \textit{in vitro} is 400 times less sensitive to indomethacin than spleen cell-free prostaglandin synthetase preparations. Prostaglandin synthetase in the conjunctiva appears to be somewhat more sensitive than that of iris. \textit{In vitro} studies of rabbit eyes show that aspirin and dexamethasone have little or no ef-
Prostaglandins

fect on uveal PG synthetase whereas indomethacin, phenylbutazone, and especially indoxole are quite active in this regard.51

In vivo, in the eye, one experimental model is capable of specifically delineating the effects of inhibitors of prostaglandin synthesis, although other prostaglandin-mediated ocular insults are responsive to such therapy. Arachidonic acid, applied topically to rabbit and monkey eyes, elevates intraocular pressure with a peak response at about 30 minutes.52-53 Arachidonic acid also increases anterior chamber protein and facility of outflow measured tonographically. These effects of arachidonic acid are similar to those of PGE2 applied topically. Pretreatment with indomethacin, 10 mg/kg or greater intraperitoneally, or aspirin as a 600 mg suppository, prevents the elevation of intraocular pressure, aqueous humor protein, and PGE induced by topical 2% arachidonic acid. Indomethacin, 2 mg/kg intraperitoneally, reduces the arachidonic acid effect on intraocular pressure by about 50% in rabbits. Topically applied indomethacin also is effective.53 The effect of indomethacin appears to be on the synthesis or release of prostaglandin from arachidonic acid in the eye as indomethacin has no effect on the rise of intraocular pressure seen after instilling 5 μg of PGE1 or PGE2. These data suggest that the presence, action, and inhibition of ocular prostaglandin synthesis can be demonstrated in vivo. Further studies show that indomethacin blocks the arachidonic acid-associated elevation of aqueous humor PGE itself.54 Either exogenous or endogenous precursor could be substrate. Conversion of arachidonic acid PGE2 may be occurring in the eye or its adnexa.50

A study was carried out of the effect of topical and intraperitoneal indomethacin on the elevation of intraocular pressure induced by arachidonic acid injected into the vitreous in order to clarify this point.

MATERIALS AND METHODS
Awake restrained rabbits were given indomethacin, 10 mg/kg, in aqueous suspension by intraperitoneal injection. Other un.injected rabbits were used as controls. Baseline intraocular pressure was measured one hour later by Mackay-Marg tonometry. Arachidonic acid, 1 mg in .02 ml of peanut oil, was injected into the vitreous humor of one eye and .02 ml diluent into the vitreous humor of the other eye of rabbits pretreated with indomethacin or no drug. Equal numbers of right and left eyes were treated with arachidonic acid. Intraocular pressure measurements were repeated at 30 minutes, 1 hour, 2 hours, and 4 hours after the injection of arachidonic acid. Similar experiments were carried out topically pretreating one eye of rabbits with 2 drops of 0.5% indo-
methacin suspension in water. Thirty minutes later intraocular pressure was determined, both eyes received an injection of arachidonic acid into the vitreous humor, and pressure was monitored.

RESULTS

Topical and systemic administration of indomethacin effectively reduced the intraocular pressure elevation induced by injection of arachidonic acid (Table II and III). This implies that there is intraocular production or release of prostaglandin from arachidonic acid and that the indomethacin had penetrated the eye.

The in vitro system using topical arachidonic acid is applicable to the screening of potential ocular anti-inflammatory agents. Subconjunctival injections of arachidonic acid or topical administration of aqueous solutions of sodium arachidonate may also be employed. The questions now arise as to which synthetase inhibitors are most effective in the eye and which clinical conditions are most amenable to such therapy.

PROSTAGLANDIN-MEDIATED OCULAR CONDITIONS

As the prostaglandins are physiologic constituents of uveal tissue that are capable of elevating intraocular pressure, a relationship to open-angle glaucoma can be envisioned. In one paper it is stated that none out of nine specimens of aqueous humor from patients with cataract contained greater than 2 ng/ml of PGE$_1$-like activity as compared to six out of eight aqueous humor samples from patients with open-angle glaucoma. The fact that only the patients with glaucoma were being treated with irritating drops casts doubt on the significance of this study. No significant difference is seen between aqueous humor from glaucomatous and non-glaucomatous eyes with regard to levels of PGE, as determined by radioimmunoassay, in another small series of patients in which the speci-

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**TABLE II: EFFECT OF INTRAPERITONEAL INDOMETHACIN ON THE INTRAOCULAR PRESSURE RESPONSE TO INTRAVITREAL ARACHIDONIC ACID IN RABBITS**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Animals</th>
<th>Mean Intraocular Pressure ± SD (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Indomethacin (10 mg/kg)</td>
<td>8</td>
<td>14.1 ± 2.0</td>
</tr>
<tr>
<td>Arachidonic acid (1 mg)</td>
<td></td>
<td>14.1 ± 2.0</td>
</tr>
<tr>
<td>Peanut oil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Indomethacin</td>
<td>8</td>
<td>16.1 ± 1.7</td>
</tr>
<tr>
<td>Arachidonic acid (1 mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peanut oil</td>
<td></td>
<td>16.8 ± 1.8</td>
</tr>
</tbody>
</table>

*Significant mean difference between eyes of individual rabbits receiving arachidonic acid into one vitreous and peanut oil into other vitreous, paired t-test, $p < 0.01$. 

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TABLE III: EFFECT OF TOPICAL INDOMETHACIN ON THE INTRAOCULAR PRESSURE RESPONSE TO INTRAVITREAL ARACHIDONIC ACID IN 8 RABBITS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Intraocular Pressure ± SD (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arachidonic acid (1 mg OU)</td>
<td></td>
</tr>
<tr>
<td>Diluent</td>
<td>13.9 ± 1.6</td>
</tr>
<tr>
<td>Indomethacin 0.5%</td>
<td>14.4 ± 1.4</td>
</tr>
</tbody>
</table>

*Significant mean difference between eyes of individual rabbits receiving indomethacin 0.5% to one eye and diluent to other followed 30 minutes later by arachidonic acid 1 mg into the vitreous of both eyes, paired t-test, p < 0.001.

mens were obtained both at surgery by outpatient paracentesis.\textsuperscript{58} It is most unlikely that prostaglandins relate to the etiology of primary open-angle glaucoma.

On the other hand paracentesis and uveitis are associated with elevated levels of aqueous humor prostaglandin. Following paracentesis, the site of disruption of the blood-aqueous barrier occurs within the ciliary processes.\textsuperscript{59} Aspirin (600 mg PR) prevents the disruption of the blood-aqueous barrier in the rabbit eye.\textsuperscript{60} Protein concentrations in secondary aqueous humor after paracentesis, in vivo, are much reduced in aspirin-pretreated rabbits. Paracentesis-produced PGE\textsubscript{2}-like activity is diminished by pretreatment of rabbits with aspirin (200 mg/kg).\textsuperscript{61} In rhesus monkeys, however, pretreatment with systemic aspirin or indomethacin does not inhibit the rise of anterior chamber protein after paracentesis or intraocular pressure after subconjunctival arachidonic acid in one study.\textsuperscript{62} Yet, pretreatment with aspirin does decrease the effusion of protein into the anterior chamber of humans after paracentesis.\textsuperscript{63} The inconsistency of results in two primate species is disturbing and needs further study.

It is attractive to examine the relationships between prostaglandins and uveitis. Many systemic inflammatory responses may be mediated by the prostaglandins.\textsuperscript{64} Bovine serum albumin injected into the vitreous of rabbit eyes creates an immunogenic uveitis. Eakins reports that PGE\textsubscript{1}-like activity equivalent to 45-150 ng/ml is found in the aqueous humor of such treated eyes as compared to less than 2 ng/ml in control aqueous.\textsuperscript{65} After paracentesis alone, the predominant PGE appears to be E\textsubscript{2}, 4-16 ng/ml.\textsuperscript{61} These differences are attributed to the migration of white blood cells into the eye in the former case and the prostaglandin release from these cells as opposed to from uveal tissue. Analysis of human aqueous humor for prostaglandin-like activity reveals that samples from untreated patients with uveitis contain substantial
amounts (20-56 ng/ml assayed as PGE₂) compared to specimens from eyes with cataracts or uveitis controlled with corticosteroids. Elevated levels of PGE also are reported to occur in the aqueous humor of human eyes with glaucomatocyclitic crisis and Behçet’s disease. In all of these studies the activity is measured on smooth muscle strips, sometimes specifically confirmed by chromatography. Quantitative analysis of the prostaglandins is a difficult process. Nevertheless, it can be concluded that prostaglandins are involved in inflammation in the eye as they are in inflammation elsewhere in the body.

A transport system exists for moving prostaglandins out of the eye, and experimentally induced uveitis in rabbits inhibits the in vitro active accumulation of prostaglandins by the isolated ciliary body. In addition, the ocular tissues have little prostaglandin dehydrogenase activity, the enzyme responsible for their degradation. These factors may be responsible, in part, for the PGE accumulation and contribution to the pathogenesis of chronic uveitis. Other organic anions are transported out of the eye. Probenecid blocks the secretion of some of these compounds, permitting anterior chamber accumulation. If this be the case, probenecid may enhance the in vivo ocular response to prostaglandins.

MATERIALS AND METHODS
Awake, restrained rabbits were pretreated with probenecid, 250 mg/kg intraperitoneally. Other rabbits were not so pretreated. Intraocular pressure was measured with a Mackay-Marg tonometer. One hour later, intraocular pressure was remeasured. Either PGE₂ (5 μg in 5 μl) or arachidonic acid (2% in peanut oil) was instilled in all eyes. Repeat measurements of intraocular pressure were carried out at 30 minutes, 1 hour, and 2 hours.

RESULTS
No differences in the pressure response to either PGE₂ or arachidonic acid occurred comparing the animals pretreated with probenecid to those animals not pretreated with probenecid (Table IV). Thus, probenecid may not inhibit the transport system for prostaglandins in vitro or the prostaglandin effect on pressure may be maximal. Probenecid is not a useful adjunctive agent for enhancing the in vivo ocular effect of prostaglandins in order to compare the effects of various prostaglandin inhibitors.

In other experiments, mechanical stimulation of the rabbit eye produces elevations of intraocular pressure and PG-activity that are significantly lower in indomethacin pretreated animals. Formaldehyde or
trigeminal nerve stimulation induce a rise in pressure that is not ameliorated by indomethacin and no significant PG activity. It is of interest that polyphloretin phosphate has a high molecular weight fraction that has almost no prostaglandin blocking activity but is a potent antagonist of hyaluronidase and formaldehyde.\textsuperscript{15,74} PPP also limits the rise of intraocular pressure and aqueous humor protein induced by topical nitrogen mustard.\textsuperscript{75} Yet, aspirin has little effect on the response of the eye to nitrogen mustard.\textsuperscript{60} It appears that this irritative stimulus is mediated neurologically and not by prostaglandin.\textsuperscript{76} Sensory denervation with retrobulbar alcohol prevents the protein effect of nitrogen mustard. The disruption of the blood-aqueous barrier after paracentesis, however, is not blocked by retrobulbar anesthesia. Argon laser radiation to rabbit irides also breaks down the blood-aqueous barrier and aspirin reduces this response.\textsuperscript{60} E prostaglandins found in the aqueous humor during peak laser response are reduced in indomethacin-pretreated animals.\textsuperscript{77}

Pretreatment of humans and rabbits with aspirin or indomethacin prevents the rebound rise of intraocular pressure after ocular compression.\textsuperscript{78} Preoperative compression may lead to an elevated ocular tension at the time of opening the globe and the therapeutic role of aspirin in this situation needs further study. Indomethacin also prevents the sustained elevation of pressure in rabbit eyes after the injection of alpha-chymotrypsin into the posterior chamber.\textsuperscript{79} The secondary rise of intraocular pressure after alkali burns in rabbits appears to be prostaglandin-mediated.\textsuperscript{80} Prostaglandin-like activity is elevated in aqueous humor after application of 20 \(\mu\l\) of 2N sodium hydroxide. Pretreatment with aspirin or indomethacin abolished this increase of intraocular pressure and aqueous prostaglandin.\textsuperscript{81} In addition, the hyperemia of the iris and conjunctiva produced after superior cervical ganglionectomy in the rabbit can be blocked by indomethacin.\textsuperscript{82} This interaction of the synthesis of prostaglandins and adrenergic responses suggests that epinephrine-
induced hyperemia also may be amenable to therapy with synthetase inhibitors.

Thus, a large number of ocular inflammations appear to be mediated by prostaglandins and many clinical conditions might be aided by treatment with the appropriate inhibitor of prostaglandin synthesis. Furthermore, the use of such drugs would obviate the toxic side effects of the corticosteroids. One is hampered by the paucity of knowledge about which clinical entities are so mediated and the experimental knowledge about which of the many PG synthesis inhibitors are most effective, especially when administered topically. The first step is to look at the nonophthalmologic literature in order to identify potential compounds of this nature. Next one should review the ophthalmologic literature in order to assess their relative potency on in vitro eye tissue synthetase, which may be very different from other tissues. However, most importantly, their relative in vivo ocular potency and toxicity must be discovered. This may differ from in vitro assessments for many reasons, including variations in the chemical state of the drug, ocular penetration, and the size of particle in suspension.

INHIBITORS OF PROSTAGLANDIN SYNTHESIS

What compounds are known presently to be inhibitors of prostaglandin synthesis in any system? In vitro, in nonocular tissue many agents show such activity. Great variability exists between various tissues. A partial listing by families of compounds would include indole acetic acids: indomethacin, fluoroindomethacin; arylacetic acids: ibuprofen, naproxen, metiazinic acid; anthranilic acids: meclofenamic acid, flufenamic acid, mfenamic acid, niflumic acid; pyrazolones: phenylbutazone, oxyphenbutazone, aminopyrine; and miscellaneous: indoxole, thiabendazole, aspirin, paracetamol, and clofibrate. In subcellular fractions, the ID$_{50}$ in order of decreasing potency generally is meclofenamic acid > niflumic acid or indomethacin > mfenamic acid > flufenamic acid > naproxen > phenylbutazone > aspirin or ibuprofen. The ID$_{50}$ doses of these compounds against prostaglandin synthetase correlate well with the ID$_{50}$ as anti-inflammatory agents, except for the fenamates. A general order of potency in vivo appears to be indomethacin > ibuprofen > metiazinic acid > fenamates > phenylbutazone > oxyphenbutazone > aspirin > aminopyrine in the rat paw test. In another in vitro study, the activities are indomethacin > naproxen > aspirin, and this rank correlated well with antipyretic, anti-inflammatory, and analgesic action. In addition, certain psychotropic drugs also inhibit prostaglandin synthesis.
The anti-inflammatory and analgesic properties of aspirin, indomethacin, and phenylbutazone are well documented. Further studies on non-ophthalmologic in vivo nonsteroidal anti-inflammatory activity are well reviewed for certain new compounds.94 Fenoprofen is effective in delaying the erythema of human skin after ultraviolet irradiation,87 reducing the pain and stiffness of rheumatoid arthritis,88 and suppressing fever.89 Flurbiprofen is a potent nonsteroidal anti-inflammatory agent. It is more potent than indomethacin and aspirin in certain types of rat inflammation.90 In a controlled study of 35 patients with ankylosing spondylitis, flurbiprofen is reported to be almost as effective as phenylbutazone when given orally.91 Ibuprofen is another agent in this group effective in the rheumatic disorders.92 The fenamates demonstrate antipyretic and anti-inflammatory activity,45 such as that of flufenamic acid in leprosy93 or rheumatoid arthritis.94 Clonixin is a new nonsteroidal anti-inflammatory drug95 with a nicotinic acid structure which reduces carrageenin pellet edema more potently than aspirin but less so than indomethacin.96 Naproxen inhibits rat paw edema as well as indomethacin and more so than aspirin or phenylbutazone.97 Another agent worth exploring is centchroman.98

Very few such new drugs have been investigated in the eye. In vitro, the effect of inhibitors on prostaglandin synthesis is different for the enzymes of spleen, uvea, and conjunctiva.51 Naproxen, phenylbutazone, and indomethacin are equally potent on anterior uvea, the latter being more potent on conjunctiva. Aspirin and dexamethasone have little activity. Indoxole is more active than any of the above drugs. In vivo, using the arachidonic acid model, our preliminary trials with topically administered drugs,53,55 indicate that aspirin and dexamethasone have little effect, whereas 10% oxyphenbutazone ointment, fenoprofen 2% solution and indomethacin 1% suspension are approximately equiactive. Parenthetically, Δ'-tetrahydrocannabinol, given intravenously, also inhibits the arachidonic acid-induced elevation of intraocular pressure and aqueous humor protein.99 In other studies, sodium arachidonate produces greater effects on intraocular pressure and aqueous humor protein than does arachidonic acid.56 Pretreatment with topically applied nonsteroidal anti-inflammatory agents reduces these effects. Indomethacin is more potent than either indoxole or pirprofen in vivo.

The following in vivo study was designed to compare the topical effects of a large number of inhibitors of prostaglandin synthesis on the intraocular pressure elevation produced by arachidonic acid in rabbits.

**MATERIALS AND METHODS**

Awake rabbits were restrained in canvas wraps. Intraocular pressure
was measured with a Mackay-Marg tonometer after topical 0.5% proparacaine anesthesia. Baseline measurements were made. One eye was treated with 2 drops of a solution or suspension of each drug made up just before use. The other eye of each animal received diluent. Equal numbers of right and left eyes were used. Thirty minutes later intraocular pressure was determined and 2 drops of 5% arachidonic acid made up freshly in peanut oil were applied to the cornea of both eyes. Intraocular pressure measurements were repeated at 30 minutes, 1, 2, and 4 hours thereafter. The drugs to be tested were made up in concentrations of 0.01%, 0.1%, 1.0% and 2.0%, and each concentration was tested topically in at least five eyes.

The following preparations were tested: solutions of naproxen sodium, fenoprofen sodium, sodium salt of flufenamic acid and sodium salt of meclofenamic acid in distilled water; suspensions of niflumic acid, indoxole, centchroman, clonixin, indomethacin, and oxyphenbutazone in distilled water; solution of flurbiprofen and suspensions of mefenamic acid, flufenamic acid and meclofenamic acid in distilled water and 1 N KOH buffered with saturated KH$_2$PO$_4$ to a pH of 7.4 to 7.8. In addition, the testing was repeated with mefenamic acid, flufenamic acid, meclofenamic acid, indoxole, and centchroman adding two drops of polysorbate 80 (Tween 80) to the preparations.

The data were analyzed for each drug, time, and concentration by tabulating the mean difference between control and treated eye ± SE and the mean change in the treated eye divided by the mean change in the control eye. The latter number subtracted from one, times one hundred, indicated percent of inhibition. This was plotted against log concentration and the approximate concentration of drug to produce 50% inhibition of the response to arachidonic acid was calculated.

RESULTS
As the maximum elevation of intraocular pressure produced by arachidonic acid occurred at 30 minutes, the comparison of the inhibiting effects of the various nonsteroidal anti-inflammatory agents was best demonstrated at this time (Table V). Dose-response relationships were found by both methods of calculation (see Methods). The optimum effective concentration was different for different agents.

Some of the drugs appeared to be more effective inhibitors than others. Indomethacin, flurbiprofen, clonixin, and meclofenamic acid at the 1% and 2% concentrations produced at least 65% inhibition of the arachidonic acid effect. At the 0.1% concentration only flurbiprofen was this active.
### TABLE V: COMPARISON OF INHIBITION OF INTRAOCULAR PRESSURE RESPONSE TO ARACHIDONIC ACID BY VARIOUS TOPICAL NONSTERoidal ANTI-INFLAMMATORY AGENTS

<table>
<thead>
<tr>
<th>Arachidonic Acid 5% OU</th>
<th>Form</th>
<th>No.</th>
<th>Mean ΔIOP ± SE, Control – Drug at 30 Min. (Mean ΔIOPdrug/Mean ΔIOPcontrol), 30-0 Min.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>2%</td>
</tr>
<tr>
<td><strong>Arylacet e Acids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naproxen sodium</td>
<td>soln</td>
<td>6</td>
<td>1.2 ± 0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(.89)</td>
</tr>
<tr>
<td>Flurbiprofen</td>
<td>soln</td>
<td>6</td>
<td>5.5 ± 0.9*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(.23)</td>
</tr>
<tr>
<td>Fenoprofen sodium</td>
<td>soln</td>
<td>8</td>
<td>4.8 ± 1.0*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(.40)</td>
</tr>
<tr>
<td><strong>Anthranilic Acids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mefenamic acid</td>
<td>susp</td>
<td>6</td>
<td>3.7 ± 0.6*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(.62)</td>
</tr>
<tr>
<td>Flufenamic acid, sodium salt</td>
<td>soln</td>
<td>6</td>
<td>2.2 ± 0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(.78)</td>
</tr>
<tr>
<td>Flufenamic acid</td>
<td>susp</td>
<td>6</td>
<td>4.5 ± 0.4*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(.46)</td>
</tr>
<tr>
<td>Meclomenamic acid</td>
<td>susp</td>
<td>6</td>
<td>6.5 ± 1.0*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(.34)</td>
</tr>
<tr>
<td>Meclomenamic acid, sodium salt</td>
<td>soln</td>
<td>6</td>
<td>5.3 ± 0.7*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(.49)</td>
</tr>
<tr>
<td>Niflumic acid</td>
<td>susp</td>
<td>6</td>
<td>4.5 ± 1.1*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(.47)</td>
</tr>
<tr>
<td><strong>Miscellaneous</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indoxole</td>
<td>susp</td>
<td>6</td>
<td>6.5 ± 0.6*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(.38)</td>
</tr>
<tr>
<td>Centchroman</td>
<td>susp</td>
<td>6</td>
<td>3.0 ± 1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(.58)</td>
</tr>
<tr>
<td>Clonixin</td>
<td>susp</td>
<td>5</td>
<td>7.6 ± 1.0*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(.21)</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>susp</td>
<td>6</td>
<td>8.5 ± 1.0*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(.13)</td>
</tr>
<tr>
<td>Oxyphenbutazone</td>
<td>susp</td>
<td>5</td>
<td>4.8 ± 0.7*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(.57)</td>
</tr>
</tbody>
</table>

*Significant difference between the eyes treated with drug and those treated with diluent, paired t-test, p < 0.01.
When polysorbate 80 was added to the drugs in suspension in order to provide better dispersion, an appreciably enhanced effect was seen only with indoxole. Indoxole treated in this manner almost completely blocked the arachidonic acid effect at the 1% concentration and was more effective than flurbiprofen at the 0.1% concentration (Table VI).

Although the dose-response curves were not linear, the approximate concentration of drug to half-inhibit the arachidonic acid-induced ocular hypertension could be calculated. This provided another means of ranking the drugs. Indoxole-polysorbate, flurbiprofen, meclofenamic acid, indomethacin, and clonixin were the most effective, in descending order of potency (Table VII).

In this comparative study of the topical administration of 14 nonsteroidal anti-inflammatory agents, an aqueous solution of flurbiprofen and aqueous suspensions of indoxole with polysorbate, indomethacin, meclofenamic acid, and clonixin demonstrate the greatest activity. Indoxole requires the addition of polysorbate 80, an emulsifying and dispersing compound, to be highly effective in blocking the arachidonic acid-induced elevation of intraocular pressure.

Many factors must be considered before extrapolating these results to the treatment of clinical disease. The arachidonic acid system is a good model of prostaglandin-mediated inflammation with a numerical endpoint of inhibition. It is obvious that the role of prostaglandins is not elucidated for most human clinical entities. Other mediators of inflammation exist, many of which appear susceptible to the effects of corticosteroids. The corticosteroids are weak inhibitors of prostaglandin synthesis. However, the agents tested may be less specific in their actions

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**TABLE VI: INHIBITION OF INTRAOCULAR PRESSURE RESPONSE TO ARACHIDONIC ACID BY VARIOUS TOPICAL NONSTEROIDAL ANTI-INFLAMMATORY SUSPENSIONS WITH POLYSORBATE-80**

<table>
<thead>
<tr>
<th>Arachidonic Acid 5% OU</th>
<th>No.</th>
<th>Mean ΔIOP ± SE, Control - Drug at 30 Min. (Mean ΔIOP&lt;sub&gt;drug&lt;/sub&gt;/Mean ΔIOP&lt;sub&gt;control&lt;/sub&gt;), 30-0 Min.</th>
<th>2%</th>
<th>1%</th>
<th>0.1%</th>
<th>0.01%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mefenamic acid</td>
<td>6</td>
<td>3.7 ± 1.0 (.54) 4.0 ± 1.3 (.55) 2.2 ± 1.5 (.78) 1.0 ± 1.4 (.89)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flufenamic acid</td>
<td>3</td>
<td>3.0 ± 1.7 (.72) 2.3 ± 2.1 (.74) 0.3 ± 2.1 (.96) 0.3 ± 1.5 (.97)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meclofenamic acid</td>
<td>6</td>
<td>5.0 ± 1.0* (.42) 4.0 ± 1.0* (.29) 3.7 ± 1.5 (.45) 0.7 ± 1.5 (.91)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indoxole</td>
<td>6</td>
<td>8.0 ± 1.4* (.03) 6.5 ± 1.3* (.26) 3.0 ± 1.6 (.67)</td>
<td>3.3 ± 2.9 (.60) 2.7 ± 1.5 (.77) 2.0 ± 1.0 (.80) -0.7 ± 1.5 (.09)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Centchroman</td>
<td>3</td>
<td>3.3 ± 2.9 (.60) 2.7 ± 1.5 (.77) 2.0 ± 1.0 (.80) -0.7 ± 1.5 (.09)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant difference between the eyes treated with drug and those treated with diluent, paired t-test, p < 0.01.
and should be tested in other ocular inflammations. In addition, the effect of arachidonic acid on intraocular pressure shows some variability. Comparing the effect of potential inhibitors by calculating the ratio of pressure rise in the treated and control eyes takes this variability into account.

The preparation of drug certainly plays a role in its effect. Permeability is an important issue. The comparative penetrability through the cornea of the agents tested is unknown. Some are solutions and others are suspensions. Flurbiprofen is the only highly effective soluble drug in the present study. Dispersion may affect permeability and the dose-response characteristics. At high concentrations the differences between 1% and 2% may be small in drugs that disperse poorly. Niflumic acid, oxyphenbutazone, and indoxole do not disperse well. Indoxole is much more effective in a dispersed suspension. Additives that alter pH, dispersion, and solubility may be important in finding the best topical nonsteroidal anti-inflammatory compounds.

Noncorticosteroid anti-inflammatory agents are effective in a variety of human and animal ocular conditions. Hydrocortisone 2½% and indomethacin 1%, or these drugs given systemically, reduce intraocular inflammation created in rabbits by intracameral bovine serum. Indomethacin 0.1% is the minimally active dose in this model. Indomethacin 1% does not alter wound healing or the course of corneal herpes infection. In other studies, indomethacin 0.5% is as effective as dexamethasone 0.1% in rabbit uveitis, and indomethacin 0.25% inhibits xenograft reactions. Water suspensions of indomethacin 1% enter the rabbit eye. Inflammation enhances its penetration. Oral

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**Table VII: Drugs Ranked by Approximate Concentration to Produce 50% Inhibition of Response to Arachidonic Acid**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indoxole + Polysorbate 80</td>
<td>0.03</td>
</tr>
<tr>
<td>Flurbiprofen</td>
<td>0.06</td>
</tr>
<tr>
<td>Meclofenamic acid</td>
<td>0.09</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.2</td>
</tr>
<tr>
<td>Clonixin</td>
<td>0.4</td>
</tr>
<tr>
<td>Fenoprofen</td>
<td>1.0</td>
</tr>
<tr>
<td>Mefenamic acid</td>
<td>1.1</td>
</tr>
<tr>
<td>Flufenamic acid</td>
<td>1.8</td>
</tr>
<tr>
<td>Niflumic acid</td>
<td>1.8</td>
</tr>
<tr>
<td>Indoxole</td>
<td>1.8</td>
</tr>
<tr>
<td>Meclofenamic acid, sodium salt</td>
<td>2.0</td>
</tr>
<tr>
<td>Centchroman</td>
<td>&gt;2.0</td>
</tr>
<tr>
<td>Oxyphenbutazone</td>
<td>&gt;2.0</td>
</tr>
<tr>
<td>Flufenamic acid, sodium salt</td>
<td>&gt;2.0</td>
</tr>
<tr>
<td>Naproxen</td>
<td>&gt;2.0</td>
</tr>
</tbody>
</table>
oxyphenbutazone is effective in human ocular inflammatory disease.\textsuperscript{107,108} Aqueous suspensions of this drug are anti-inflammatory in rabbit uveitis in concentrations down to 0.5%.\textsuperscript{109} Oxyphenbutazone, as a 10% ointment, enters the human eye, does not elevate human intraocular pressure, and is as effective as prednisolone 2.5% in postoperative inflammation.\textsuperscript{110} It also is effective in the treatment of superficial eye injuries\textsuperscript{111} and episcleritis.\textsuperscript{112} It does not hasten galactose-induced cataracts in rats.\textsuperscript{113} Fenoprofen 1% is effective in rabbit uveitis.\textsuperscript{114}

The obvious next steps will involve therapeutic trials of the best agents found in the arachidonic acid studies in models of animal disease and ultimately human disease states.

**SUMMARY AND CONCLUSIONS**

The prostaglandins produce elevation of intraocular pressure and breakdown of the blood-aqueous barrier. They act via the secondary messenger system, cyclic AMP. Although the pathogenesis of many forms of ocular inflammation, both external and internal, is unclear, it is evident that some forms of ocular inflammation are prostaglandin-mediated, at least in part. Others may be totally mediated by prostaglandin synthesis. At present the corticosteroids are the mainstay of therapy of these conditions. However, the corticosteroids are poor inhibitors of prostaglandin synthesis and have many deleterious side effects such as induction of ocular hypertension, cataract, and infection. The search for new agents that will obviate these side effects and be more specific for the disease process is crucial. The discovery that the mode of action of many nonsteroidal anti-inflammatory agents is via inhibition of prostaglandin synthesis places a premium on elucidating which of these agents is most effective and least toxic in the eye and by which route of administration. The arachidonic acid screening model is ideal for initially choosing which agent has the greatest potential clinically. Arachidonic acid, a PGE\textsubscript{2} precursor, when given topically also elevates intraocular pressure and aqueous humor protein, and these effects are blocked by the nonsteroidal anti-inflammatory drugs. This occurs if the arachidonic acid is injected into the vitreous humor, too, providing evidence that this in vivo model involves intraocular mechanisms. Utilizing the arachidonic acid system, a comparative study of nonsteroidal inhibitors of prostaglandin synthesis shows that the most effective of 14 agents were flurbiprofen solution and suspensions of polysorbate-dispersed indoxole, meclofenamic acid, indomethacin, and clonixin. Animal uveitis is not an ideal model for the human condition. Nevertheless, proving the superior efficacy of a
Prostaglandins

screened drug in this system will identify those drugs to be tested in the human disease states. Only after the very few best drugs of this nature are identified should the ultimate steps of human testing be initiated.

ACKNOWLEDGMENTS

Centchroman was provided by the Centra Drug Research Institute, Lucknow, India; oxyphenbutazone by Geigy Pharmaceuticals, Summit, New Jersey; fenoprofen sodium by Lilly Laboratories for Clinical Research, Indianapolis, Indiana; indomethacin by Merck, Sharp and Dohme, West Point, Pennsylvania; mefenamic acid, flufenamic acid and sodium salt, and meclofenamic acid and sodium salt by Parke-Davis and Company, Ann Arbor, Michigan; clonixin by Schering Corporation, Bloomfield, New Jersey; niflumic acid by The Squibb Institute for Medical Research, Princeton, New Jersey; naproxen sodium by Syntex Research, Palo Alto, California, and indoxole and flurbiprofen by the Upjohn Company, Kalamazoo, Michigan. Segments of the older literature review for this paper were freely adapted from a paper “Prostaglandins and the Eye” by Steven M. Podos and Bernard Becker presented at the 1973 American Academy of Ophthalmology and Otolaryngology meeting and published in Symposium on Ocular Therapy, volume 7, edited by I. H. Leopold, C. V. Mosby Co., 1974. Dr Kenneth Eakins suggested the use of polysorbate 80.

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