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The aqueous flare (AF) of an intact rabbit eye was measured by a photoelectric instrument. Local application of prostaglandin E1 (PGE1) and its precursor arachidonic acid (AA) gave an almost identical increase of the AF. The response to AA but not to PGE1 was inhibited by pretreating the eye locally with a solution of indomethacin. The ability of indomethacin to inhibit the aqueous flare response (AFR) to an agent is assumed to indicate that a kind of prostaglandin is the effector of the AF. Indomethacin blocked the AFR to infrared irradiation of the iris and to intravenous administration of endotoxin but not to subcutaneous administration of α-melanocyte-stimulating hormone (α-MSH).

The rabbit eye responds to both mechanical and chemical traumata with miosis, local ocular vasodilation, a sustained rise in intraocular pressure, and an increased capillary permeability, seen as a breakdown of the blood-aqueous barrier resulting in a marked rise in the protein content of the aqueous humor. This was first demonstrated by Wessely1 in 1908, and has since been repeatedly confirmed. This inflammatory reaction can be mimicked by locally applied prostaglandins (PGs),2 which can be formed by the tissues of the iris and the ciliary body. Some systematically administered substances can also cause a breakdown of the blood-aqueous barrier, resulting in an aqueous flare response (AFR). Thus, certain peptides of pituitary origin are known to yield an AFR when given subcutaneously to rabbits. The AF-producing ability of the different amino acid chains closely follows their melanocyte stimulating activity, whereas, it seems to be independent of their corticotropic activity. Finally, endotoxins of different bacteria give an AFR when administered intravenously.3

Since traumata of different kinds as well as topically and systemically administered drugs are able to provoke very similar effects, it does not seem unlikely that there is a common factor responsible for the final step. In fact, Beitch and Eakins4 in 1969 attributed this role to prostaglandins. It is known5-10 that indomethacin and aspirin-like drugs inhibit the conversion of PGE1 and PGE2 from their precursor, arachidonic acid (AA). It has also been shown that aspirin reduces the increase of protein in aqueous humor after paracentesis and argon laser radiation of the iris11 indicating that these effects are mediated by

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Fig. 1. Aqueous flare increase (AF-increase) after stimulation with 5 μg prostaglandin E₂ (PGE₂) given locally to both eyes; 2 drops of 2 per cent arachidonic acid (AA) in peanut oil given locally to both eyes; infrared irradiation (IR) of the iris of both eyes; 5 μg endotoxin of *P. mirabilis* (E) given intravenously and 20 μg per kilogram of α-melanocyte-stimulating hormone (MSH) given subcutaneously. On Day 1, the right eye was pretreated with a solution of 10 mg of indomethacin (I) in 1 ml of distilled water. On Day 2, no pretreatment with indomethacin was given to either eye. Ordinate: AF-increase given in 10 log of Qₐₚₙₐₜₜ-values (see text). Identical numbers of the values of Day 1 and Day 2 refer to the same rabbit.

PG's. However, there was no blocking activity of aspirin to 1 per cent nitrogen mustard- and MSH-stimulation. In the present study the response to a series of agents has been recorded by measuring the aqueous flare photoelectrically in situ. Any damage to the eye is thereby avoided and the course of the protein leakage can be followed during the interesting period. The aims of this study have been: (1) to follow the course of the AFR to topical administration of PGE₂ and AA, and the blocking activity of indomethacin to these agents; and (2) to test the ability of indomethacin to block the AFR to infrared irradiation, endotoxins given intravenously and α-MSH given subcutaneously.

**Materials and methods.**

**Animals.** Adult pigmented rabbits of mixed strains, weighing between 2.0 and 4.0 kilograms were used. They were given pellets and water ad libitum. The rabbits were used again for new experiments after the aqueous flare and all other inflammatory criteria had been absent for at least seven days.

**Chemical and mechanical stimulation.** Topical administration to the cornea of 2 drops of 2 per cent arachidonic acid (Sigma) in peanut oil (w/w). The solution was freshly made at the beginning of each experiment.

**Endotoxin of Proteus mirabilis** was given intravenously in a dose of 5 μg in 1 ml. distilled water. The preparation was freshly made at the beginning of each experiment.

**α-Melanocyte-stimulating hormone (CIBA)** given subcutaneously in the back of the neck in a dose of 20 μg per kilogram.

**Indomethacin treatment.** It is known that both oil solution and water suspension of indomethacin is readily absorbed by the rabbit eye following topical administration to the cornea. Thus we have chosen this mode of administration of indomethacin to one eye of a rabbit in order to use the other eye as a control. The indomethacin used is a water-soluble preparation (Dønen, Copenhagen). Ten milligrams of this is dissolved in 1 ml. of distilled water at the beginning of each experiment. The pH of this solution is about 7.7 to 7.8.

**Experiments and results.** One eye of a rabbit was pretreated with 0.1 ml. indomethacin locally...
applied to the cornea four times every 15 minutes. One hour after the first treatment we tried to provoke a breakdown of the blood-aqueous barrier in both eyes using the different stimuli mentioned above. The AF was measured for about five seconds every half an hour until the maximum flare was covered or, if no increase occurred, for five to six hours after stimulation. In those cases where there was a reduction of the AFR after indomethacin treatment, an AFR was again elicited the next day in both eyes, without pretreatment of one eye with indomethacin, in order to prove that both eyes are about equally sensitive to the stimuli. The measurements were made with a photo-electric instrument, described in the technical note at the end of this paper. The AF was measured in arbitrary units and the results are given in

\[ \frac{Q_{\text{max}}}{Q_{\text{max}} - Q_{\text{before}}} \]

values = \frac{\text{flare density after treatment}}{\text{flare density before treatment}}

**Indomethacin.** When indomethacin was given four times every 15 minutes in a concentration of 10 mg. per milliliter there was no change in the aqueous flare for five to six hours after administration.

**Prostaglandins.** With a dose of 5 μg PGE₂ there was a 35- to 40-fold increase in six eyes, a 15- to 20-fold increase in four eyes, and a 7- to 10-fold increase in four eyes. Mean value: 22.5 (Fig. 1). The maximum occurred 1 to 1.5 hours after stimulation. At about 30 minutes after application there was an intense conjunctival injection, an epiphora, a chemosis, and a miosis. There was no blocking activity of indomethacin to the AFR (seven rabbits) (Fig. 1). The eye pretreated with indomethacin was irritated to the same degree as the other eye.

**Arachidonic Acid.** Two drops of 2 per cent arachidonic acid gave a 4- to 10-fold increase of the aqueous flare in two eyes, a 10.5- to 20-fold increase in four eyes, and a 20.5- to 40-fold increase in four eyes. Mean value: 18.5 (Fig. 1; Day 2). The maximum occurred 1 to 1.5 hours after stimulation (Fig. 2). At about 30 minutes after application there was an external irritation of the eye identical with that seen after stimulation with PGE₂. With indomethacin the aqueous flare increase was totally abolished in all cases (five rabbits) (Fig. 1, Day 1). The eye pretreated with indomethacin was less irritated than the other eye. On Day 2, when no pretreatment with
Indomethacin was given, we obtained "equal" (within the errors of the method) responses in both eyes to stimulation with AA.

Infrared irradiation. After infrared irradiation of the iris we obtained a miosis and a 6- to 15-fold increase in the flare intensity (14 eyes). Mean value: 9.0 (Fig. 1, Day 2). The maximum occurred 45 to 60 minutes after irradiation (Fig. 3). The AF-increase of the eye pretreated with indomethacin was in three rabbits 40 to 50 per cent, in two rabbits 20 to 30 per cent, and in two rabbits 0 per cent of the AF-increase of the other non-pretreated eye (Fig. 1, Day 1). The miosis usually seen during infrared irradiation seemed to be inhibited to the same degree as the AFR by treatment with indomethacin. On Day 2, when either eye was pretreated with indomethacin, we obtained "equal" (within the errors of the method) responses to irradiation in both eyes.

Endotoxin. Five micrograms of endotoxin of *Proteus mirabilis* gave a 3- to 12.0-fold increase of the AFR in four rabbits. Mean value: 6.4 (Fig. 1, Day 2). The maximum occurred between three and five hours after stimulation. No external signs of inflammatory reaction or measurable miosis could be seen. In three rabbits, the AFR was totally abolished by indomethacin. In one rabbit the AFR was in the pretreated eye; 18 per cent of the AF-increase in the other eye (Fig. 1, Day 1). On Day 2 when no pretreatment with indomethacin was given, we obtained "equal" (within the errors of the method) responses in both eyes to stimulation with endotoxin.

α-MSH. After 20 µg per kilogram of α-MSH given subcutaneously we obtained a 1.5- to 3.3-fold increase of the AFR in at least one eye of 12 rabbits (Fig. 1, Day 2). The maximum occurred about two hours after the α-MSH injection. There were no external criteria of inflammatory reaction or measurable miosis. Pretreatment with indomethacin did not inhibit the AFR but rather seemed to facilitate it (Fig. 1, Day 1). The probability for indomethacin to facilitate instead of inhibit the AFR to α-MSH was tested with
Wilcoxon’s test for paired differences and was found to be $p > 0.98$.

**Discussion.** Our data confirm that the AFR to AA and PGE$_3$ are almost identical and that prior administration of indomethacin prevents this response to AA—but not to PGE$_2$—stimulation. Thus it is possible that the AA-induced AFR is mediated by a release of PG's of E$_2$- or F$_2$-type as indomethacin is known to inhibit the conversion of AA to PGE$_2$ and PGF$_2$α. Still it has not been proved whether it is the AA administered that is converted to PG or if the AA applied merely stimulates the eye to endogenous synthesis of PG.

The infrared irradiation applied gives just a minimal trauma to the iris and is not perceived as painful when caught on the back of the hand and, therefore, local anesthetics are unnecessary. Compared with the argon laser radiation used by Neufeld, Jampol, and Sears, and Unger, Perkins, and Bass, where a burn could be seen on the iris, this trauma is very faint and no histologic changes are seen in the iris at the site of the irradiation. However, in both cases, the AFR is significantly inhibited by aspirin and indomethacin, respectively. Thus it is reasonable to assume that this AFR is mediated by PG's synthesized somewhere in the eye, as the AFR can be totally inhibited by indomethacin in some cases. It is most likely that the partial inhibition seen in some cases is merely due to too low a concentration of indomethacin in the eye tissues for complete inhibition. As the AFR elicited by endotoxin from *P. mirabilis* given systemically is reduced by indomethacin, we suggest that this AFR, too, is mediated by PG's in the same way as the AFR after infrared irradiation.

The disruption of the blood-aqueous barrier induced by a α-MSH is, however, not prevented by indomethacin, suggesting that PG's are not the mediator of the α-MSH response. As a matter of fact the AFR seemed rather to be facilitated by pretreatment with indomethacin, in the sense that the $Q_{max}$ values were generally higher in the eye given pretreatment with indomethacin than in the other. Neufeld, Jampol, and Sears also noted the highest protein concentration after stimulation with MSH in a rabbit that had been pretreated with aspirin systemically. Our percentage of positive AFR's was, however, the same as that found by Dyster-Aas and Krakau, that is 40 per cent in an unselected material. The reason for the considerable variation in AFR from animal to animal is unknown, but to some extent it is dependent on the age of the animals.

Our results confirm the suggestion made by Dyster-Aas and Krakau that the AFB to trauma and MSH are mediated in different ways although there are identical histologic changes of the ciliary processes in both cases. It was earlier known that the MSH-induced effect is not markedly influenced by either local anesthetics or antihistamines as is the trauma effect, and now we have found that they seem to have different modes of reaction to indomethacin, suggesting that PG's synthesized in the eye mediate the effect to trauma but not to MSH. Neither is the refractoriness for trauma the same as that for MSH.
A technical note on the instrument for flare measurements. In a previous paper, a photoelectric instrument for measurements of the aqueous flare in the anterior chamber has been described. An inconvenience was the mode of registration of the density and for this reason the present modification has been constructed, in which the flare density is recorded as an ink written record. An optical system built like a slit lamp, furnishes a narrow beam of light. When this beam (B) passes the anterior chamber an aqueous flare is produced due to the presence of small amounts of protein in the aqueous. The slit lamp is rigidly connected with a system for observing and measuring the aqueous flare. An objective lens forms an image of the flare and its surroundings in front of a photomultiplier tube. By means of a beam splitter part of the light also reaches the observer. The vibration frequency of the flare to be measured is adjusted to coincidence with a mark, seen by the observer. In front of the photomultiplier tube one fixed and one vibrating aperture are placed. These correspond to the areas A1 and A2 in the anterior chamber (Fig. 4). Only the light from the common (dotted) area will hit the multiplier. As the vibrating aperture moves with a frequency of 20 cps., this area will scan the flare. The signal from the multiplier tube will consist of an alternating current with a frequency twice the vibration frequency generated by the light from the flare, plus a component from the background, which may also be somewhat illuminated from diffused light. The latter component can be considered a constant if the background is evenly illuminated. The signal from the multiplier is fed to an alternating current (AC) amplifier and then to a band-pass filter. Only the light intensity difference between the flare and at the sides of the flare will be important.

A sphere of glass, sufficiently impure to let a fine flare appear, is used as a standard. The mean value of the flare in this standard sphere was by the light from the flare, plus a component in infrared irradiation, endotoxin, a-MSH. Both test ball and the normal rabbit’s eye have a rabbit, well accustomed to the method, gave values around five units; standard deviation 0.61.

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Lysosomes and melanin granules of the retinal pigment epithelium in a mouse model of the Chediak-Higashi syndrome.

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The origin of giant granules in the retinal pigment epithelium of the beige mouse was investigated with electron microscopy and ultrastructural histochemistry. These granules were found to contain melanin and acid phosphatase. Apparently they arise from fusions of primary lysosomes with melanin granules which are already enlarged from multiple fusions among melanosomes. Therefore, the giant granules are not primary lysosomes, nor are they simply enlarged melanin granules as suspected from light microscopic studies. A deficiency of primary lysosomes in the pigment epithelium results, suggesting a defect in intracellular digestion similar to that found in the leukocytes of Chediak-Higashi patients and several animal models. Affected humans probably have defective digestion in their retinal pigment epithelium also; which could impair the renewal process for rod outer segments. Thus, Chediak-Higashi patients may show an increased susceptibility to light damage due not only to hypopigmentation, but to defective intracellular digestion, as well.

The Chediak-Higashi syndrome is a rare human disorder of recessive inheritance which results in decreased ocular cutaneous pigmentation, photosensitivity, nystagmus, and recurrent pyogenic infections. The increased susceptibility to infection is attributed mainly to a block in intracellular digestion, though neutropenia and lowered chemotaxis of leukocytes are contributing factors. Phagocytic leukocytes contain abnormally large granules which represent multiply fused lysosomes. Cells with such enlarged lysosomes fail to degranulate properly and are unable to carry out normal digestion. The pigment epithelium also contains enlarged granules, but these were reported to be abnormal melanin deposits, and their possible relation to lysosomes has not been investigated.

The beige mutant of the C57 black mouse is a good animal model for the Chediak-Higashi syndrome. We have studied its retinal pigment epithelium using electron microscopy and acid phosphatase histochemistry to determine the relations of the abnormally large granules to melanin granules and to lysosomes.

Materials and methods. Eyes were obtained from three black mice (C57BL/6 N1H- +/+ ) and three beige mutants (C57BL/6 N1H- bg/bg) aged 25 to 33 days. The central retina and choroid were excised in fixative and carefully sliced with a sharp razor blade into pieces that were 200 to 400 μm wide. These tissue sections were fixed for 180 minutes at 4° C. in 2.5 per cent glutaraldehyde and 1 per cent sucrose buffered to pH 7.2 with 50 mM sodium cacodylate. Some of the tissue sections were processed directly for ultrastructural studies. Others were washed overnight in 50 mM sodium cacodylate (pH 7.2) with 8 per cent sucrose at 4° C., rinsed in 200 mM Tris maleate at pH 5 for 60 minutes, and then were incubated at 37° C. for 60 minutes in one of the following media for acid phosphatase histochemistry.

The incubation medium for the demonstration of acid phosphatase contained 5 ml. H2O, 5 ml. 50 mM MnCl2, 10 ml. 200 mM Tris maleate (pH 5.0), 10 ml. 25 mM cytidine 5'-monophosphate, 3 Cm. sucrose, and 20 μl. 0.2 per cent lead nitrate.

Control tissues were treated by one of the following methods: (1) heated at 60° C. for 30 minutes prior to incubation; (2) incubated in medium with 10 mM sodium fluoride added; (3) incubated in medium without cytidine 5'-monophosphate or other substrate; and (4) left unincubated and not postfixed.

Following incubation the tissues were rinsed briefly in 200 mM Tris maleate (pH 5.0) at 37° C. and then in 120 mM cacodylate buffer (pH 7.2) at room temperature. They were postfixed and embedded for electron microscopy. Only the first 100 μm of each piece was sectioned in order to avoid artifacts related to limited penetration by the histochemical media. Sections were examined and photographed with a JEM 100B electron microscope, unstained or after uranyl acetate and lead citrate staining.

Results. Comparative morphology. The retinal pigment epithelium of the beige mouse contains giant granules of irregular shapes which may approximate the sizes of nuclei (Fig. 1). These granules may be as large as 3 μm wide and 11 μm long. They are especially conspicuous for their high density (light and electron opacity). They contain material which is structurally identifiable as melanin, but they differ from typical melanin granules in that their content is not homogeneous, and their position in the cell is usually central rather than apical. The smaller (melanin) granules which are present abundantly in the apical portions of normal retinal pigment epithelium are absent.