

Blood-aqueous barrier can be circumvented by lowering intraocular pressure

(Schlemm canal/electron microscopy/aqueous humor)

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ABSTRACT Rhesus monkeys were injected intravenously with hypertonic urea (9 ml/kg body weight of 30% urea in 10% invert sugar) and the intraocular pressure was measured with an applanatic tonometer. When this pressure reached its minimum (20% of the normal value) horseradish peroxidase (molecular weight 40,000; radius of an equivalent hydrodynamic sphere about 2.5 nm; 0.5 g/kg body weight), was injected intravenously. Twenty minutes following peroxidase administration, either aqueous humor was sampled from the anterior chamber for biochemical determination of peroxidase activity, or one eyeball was enucleated and processed for light and electron microscopic localization of the enzymatic tracer. This experiment showed that: (1) therapeutic doses of hypertonic urea do not cause a breakdown of either the blood-retina or the blood-aqueous barriers; (2) as intraocular pressure decreases, peroxidase-containing blood flows back from the episcleral veins into the Schlemm canal; (3) macromolecules up to the dimensions of horseradish peroxidase leak through the intercellular clefts of the endothelium of the Schlemm canal, permeate the juxtacanalicular connective tissue and trabecular meshwork, and finally enter the anterior chamber. Thus, blood-borne substances can circumvent the blood-aqueous barrier when intraocular pressure is decreased, and administration of a hypertonic agent may represent a simple pharmacological device to cause penetration into the ocular chambers by drugs that are normally excluded from the interior of the eye.

In normal conditions, the aqueous humor has a composition different from plasma, and various substances, including many drugs, encounter difficulty in passing from blood into the interior of the eye. *Blood-aqueous barrier* is the term commonly used to define the entity which limits the exchange of materials, especially macromolecules, between the plasma and the ocular chambers. It is well known, however, that drugs which normally do not penetrate the blood-aqueous barrier gain access to the interior of the eye when administered immediately after penetrating wounds or after opening the cornea (paracentesis) and draining aqueous humor from the anterior chamber (1). Recently, ultrastructural tracer experiments in monkeys have demonstrated that the sudden fall of the intraocular pressure (IOP) to atmospheric values after paracentesis causes an inversion of the current of fluid in the aqueous pathways: from the episcleral veins, blood flows back into the Schlemm canal and macromolecular plasma constituents percolate into the anterior chamber through the permeable walls of the canal and the tissue of the trabecular meshwork (2). In the present paper, an experiment is described in which the blood-aqueous barrier is circumvented by pharmacological means, without anatomical damage to the eye structures. Monkeys were injected intravenously with hypertonic urea; as the intraocular pressure decreased, blood filled the Schlemm canal and a blood-borne macromolecular tracer (horseradish peroxidase,

HRPO) which is normally excluded from the interior of the eye was found in the trabecular meshwork and aqueous humor.

MATERIAL AND METHODS

Seven rhesus monkeys (*Macaca mulatta*) of both sexes and weighing 4.5-6.5 kg were used. The animals were anesthetized with pentobarbital (30 mg/kg body weight) and their IOPs were recorded with a Bausch and Lomb applanatic tonometer, whose floating tip was applied to the center of the cornea.

Experimental Animals. As a depressant of IOP, 9 ml/kg body weight of a commercial preparation of hypertonic urea (Urevert, Travenol Laboratories Inc., Deerfield, Ill.; 30% urea in 10% invert sugar) was slowly (8-10 min) injected into the small saphenous vein. This dose of urea causes a 80% decrease of IOP within 45-90 min from the end of the injection. Subsequently, the IOP remains stationary for 30 min to 3 hr and returns to normal in 5-6 hr.

In order to study the distribution of a blood-borne tracer in the ocular tissues following urea administration, HRPO (type II, Sigma; molecular weight 40,000; 0.5 g/kg body weight, dissolved in a total volume of 10 ml of phosphate-buffered saline) was slowly (3-5 min) injected intravenously in three monkeys after IOP reached its minimal value. In two animals, one eye was enucleated 20 min after the end of HRPO injection and processed for the ultrastructural demonstration of peroxidase activity (3). In the remaining animal, 20 min after HRPO injection aqueous humor was withdrawn from the anterior chamber with a 26 gauge needle inserted on a tuberculin syringe and its peroxidase activity was determined biochemically.

In order to establish that IOP depression was the only cause of tracer penetration into the eye cavity, HRPO was injected in three additional animals as soon as IOP had returned to normal. In two animals one eye was enucleated and processed for ultrastructural cytochemistry; the third animal was used for biochemical determination of peroxidase activity in the aqueous humor.

Injection of HRPO did not affect IOP, whereas enucleation of one eye sometimes delayed IOP recovery in the eye remaining *in situ*; for this reason, only one eye was studied in each animal. The eyeball was dissected and processed for light and electron microscopy as previously described (2). The following parts of the eye were examined with both the light and the electron microscopes: retina, ciliary body, iris, and sclero-corneal angle. Peroxidase activity in the aqueous humor was biochemically measured by determining the rate of decomposition of hydrogen peroxide with *o*-dianisidine as a hydrogen donor (4). The rate of color development was determined with a Beckman model 24 spectrophotometer at 460 nm and room temperature.

Abbreviations: IOP, intraocular pressure; HRPO, horseradish peroxidase.

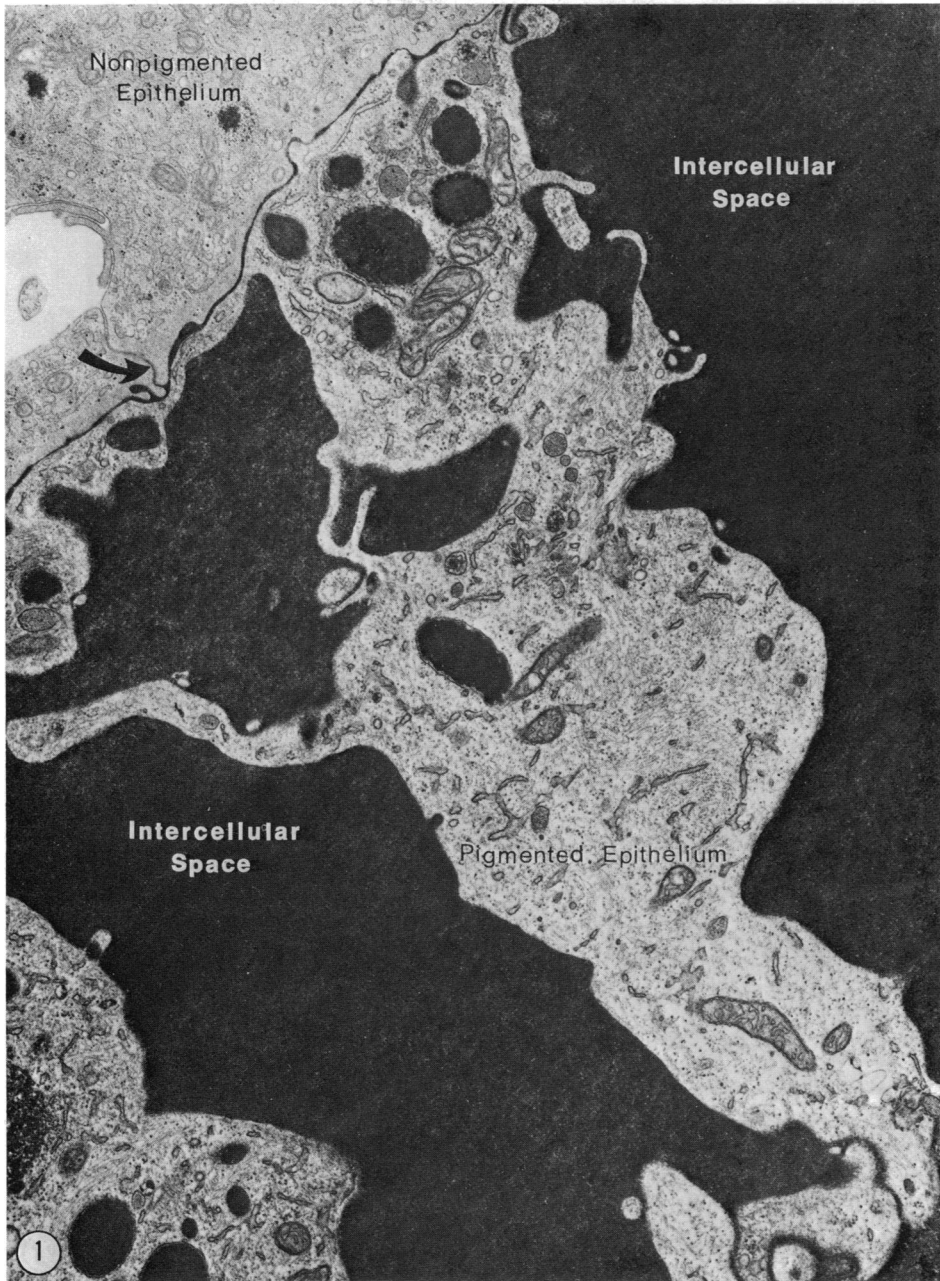


FIG. 1. All figures are taken from the eye of a monkey that was intravenously injected with hypertonic urea. When IOP reached its minimal value, HRPO was introduced into the blood stream. This figure is ciliary epithelium. In places, the intercellular spaces between pigmented cells are greatly distended and contain the dense product of the cytochemical reaction for peroxidase activity. HRPO also permeates the cleft between pigmented and nonpigmented cells, but its further progression toward the posterior chamber is blocked by the occluding junctions (arrow) which seal the intercellular spaces between nonpigmented cells. X11,550.

Control Animal. In one monkey 9 ml/kg body weight of phosphate-buffered saline was injected into the small saphenous vein. No significant change of IOP was observed following the injection. Ninety minutes later, HRPO was injected intravenously and, 20 min following tracer injection, aqueous humor was withdrawn for biochemical analysis.

RESULTS

Gross findings

As soon as IOP reaches 20% of its normal value following injection of hypertonic urea, inspection of the eye *in situ* re-

veals that in the proximity of the limbus numerous episcleral vessels are engorged with blood. Upon injection of HRPO and paracentesis, the primary aqueous humor has a pale pink-violet color, typical of HRPO in solution, whereas it is crystal clear when urea is omitted.

Biochemical findings

Normally, the aqueous humor of the monkey has no peroxidase activity. In the animal injected with HRPO, at the moment of maximal depression of the IOP, the aqueous humor contains a significant quantity of peroxidase (0.065 mg/ml). In both the animal injected with HRPO after IOP has re-

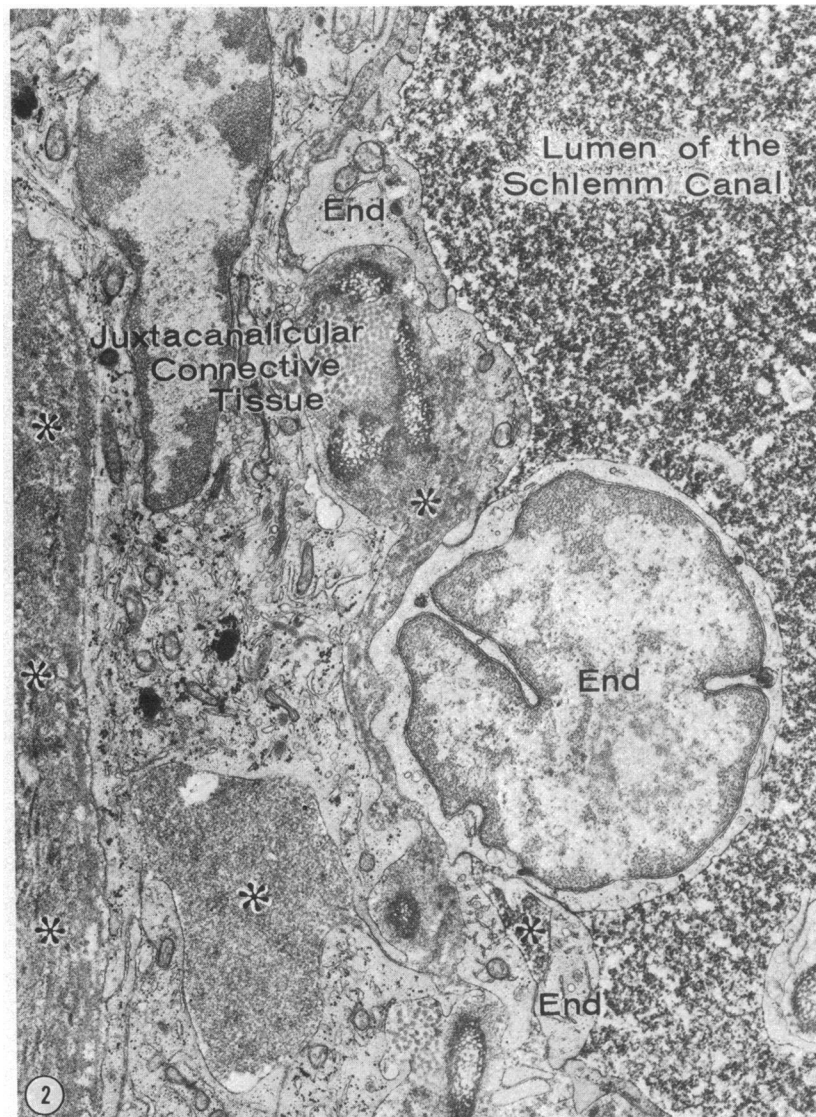


FIG. 2. Schlemm canal. The lumen of the canal contains HRPO. The tracer also fills in the juxtacanalicular connective tissue spaces (asterisks). End, endothelium of the Schlemm canal. $\times 13,000$.

turned to normal and the control animal injected with phosphate-buffered saline instead of urea and HRPO, there is no peroxidase activity in the aqueous humor.

Microscopical findings

It is well-known that following intravenous injection of HRPO in normal monkeys the blood-borne tracer diffuses through the walls of the vessels of the choroid and permeates the surrounding connective tissue spaces. However, HRPO does not enter the intercellular clefts of the retina, for the walls of the retinal capillaries (5) and the pigment epithelium (6) are impermeable to the tracer. Both the endothelium of the retina vessels and the pigment epithelium are commonly referred to as the anatomical sites of the blood-retina barrier. Tracer entry into the ocular chambers is prevented by the nonpigmented layer of the ciliary epithelium (7-11, 2) and the endothelium of the vessels of the iris (12, 2), and both of these are referred to as the anatomical sites of the blood-aqueous barrier. Normally, the Schlemm canal does not contain blood and tracer is lacking in its vicinity, i.e., in the juxtacanalicular connective tissue and trabecular mesh-

work (2). Microscopical examination of the eyes of the animal injected with HRPO at the moment of maximal depression of IOP reveals no change in the permeability properties of the structures which represent the site of the blood-retina and blood-aqueous barriers. In the retina, the interendothelial junctions of the capillaries and the occluding junctions of the pigmented epithelium are intact and prevent tracer diffusion into the nervous tissue. In places, the intercellular spaces between the pigmented cells of the ciliary epithelium are dilated and large vacuoles containing a moderate amount of HRPO are occasionally seen in the cytoplasm of the pigmented cells. However, the occluding junctions between nonpigmented cells are intact (Fig. 1) and the tracer content of the nonpigmented elements is negligible. In the iris, the endothelial cell junctions of the vessels are intact and impermeable to HRPO. The most remarkable effect of IOP depression is seen at the limbus. Blood containing tracer is found in the lumen of the Schlemm canal (Fig. 2). Furthermore, HRPO permeates the juxtacanalicular connective tissue spaces (Fig. 2) and the trabecular meshwork (Fig. 3). Clearly, blood from the episcleral veins, which normally

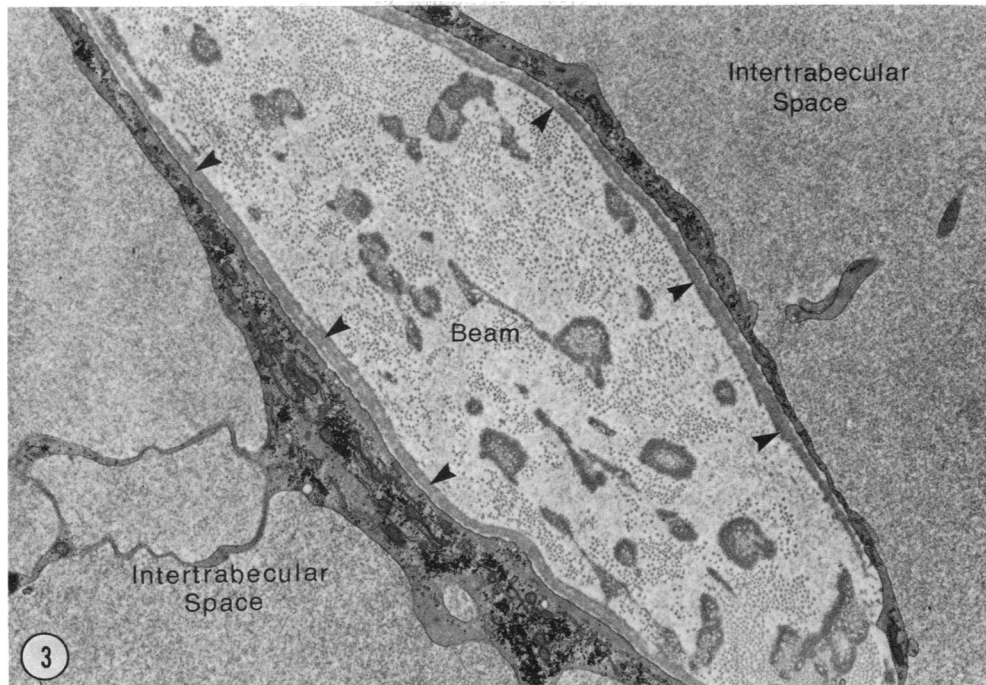


FIG. 3. Trabecular meshwork. Reaction product is seen in the intertrabecular spaces. The concentration of HRPO in the connective tissue core of the beams is low, but the enzymatic tracer clearly permeates the collagen fibrils and the basal lamina of the endothelium (arrowheads). $\times 13,200$.

drain the aqueous humor, invades the Schlemm canal, and blood-borne molecules, up to the size of HRPO, leak through the permeable walls of the canal into the surrounding tissue and the anterior chamber (Fig. 4). Tracer concentration, however, is higher in the lumen of the canal than in the juxtacanalicular connective tissue spaces and adjoining trabecular meshwork. The structure of the endothelium of

the Schlemm canal appears normal; giant vacuoles (13-15) and discontinuities such as those reported following paracentesis are absent (2). In the cytoplasm of the endothelial cells, the number of plasmalemmal vesicles loaded with tracer is very small. The interendothelial clefts have the usual 10 to 20-nm width except for one or two focal regions of closer membrane approximation. A few clefts are uniformly filled

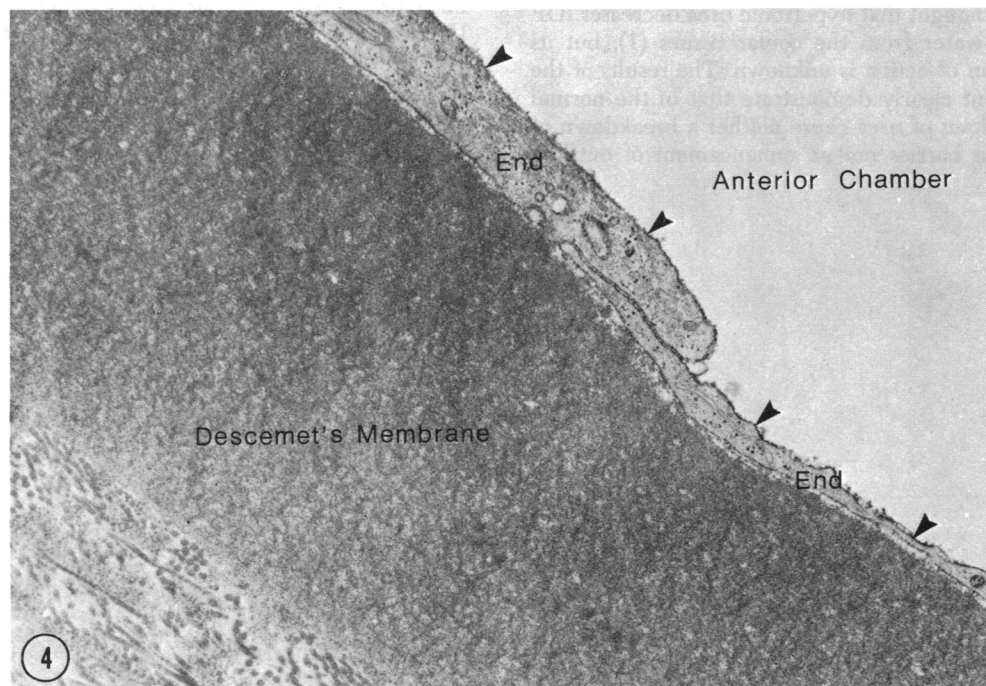


FIG. 4. Corneal periphery. HRPO is found in the Descemet membrane and it has penetrated the anterior chamber, as indicated by the thin layer of reaction product (arrowheads) on the surface of the corneal endothelial cells (End). $\times 19,200$.

with tracer throughout their length, whereas most of them display an abrupt gradient in tracer concentration at a narrow point or waist which may well correspond to a specialized intercellular junction. Thus, the most likely route for tracer movement across the endothelium of the Schlemm canal is via the intercellular clefts of the endothelium.

In the eyes which were processed for microscopy after IOP had returned to normal, there is no blood in the lumen of the Schlemm canal and tracer is absent in both the juxtacanalicular connective tissue and trabecular meshwork. Also, the structure of the ciliary epithelium is the same as in untreated animals.

DISCUSSION

This experiment demonstrates that hypertonic urea, when administered intravenously at the dose of 3 g/kg body weight, has no effect on the cell junctions which represent the structural counterpart of the blood-retina and blood-aqueous barriers. Nevertheless, blood-borne substances, in this instance HRPO (radius of an equivalent hydrodynamic sphere about 2.5 nm), do penetrate the interior of the eye. They do not cross, but rather circumvent the blood-aqueous barrier: as IOP decreases and aqueous humor reabsorption subsides, blood flows into the Schlemm canal from the episcleral veins and plasma constituents leak into the anterior chamber through the permeable walls of the canal and the spongy tissue of the trabecular meshwork. Thus, pharmacological depression of the IOP seems to represent a simple and harmless way to introduce into the anterior chamber drugs which do not normally cross the blood-aqueous barrier. These findings do not necessarily contradict the results by Okisaka *et al.* (16), who reported breakdown of the blood-aqueous barrier and selective destruction of the pigmented cells of the ciliary epithelium after administration of 2 M urea through the carotid artery. Obviously, the concentration of the hypertonic agent in the vascular bed of the eye is much lower following intravenous administration.

It is generally thought that hypertonic urea decreases IOP by withdrawing water from the ocular tissues (1), but its precise mechanism of action is unknown. The results of the present experiment clearly demonstrate that in the normal eye therapeutic doses of urea cause neither a breakdown of the blood-aqueous barrier nor an enhancement of outflow facility.

Tracer diffusion from the lumen of the Schlemm canal into the surrounding tissues seems to occur along the clefts between endothelial cells. One has the impression that only a proportion of the interendothelial clefts are patent, as is the case for muscle capillaries (17), and this might explain why tracer concentration around the canal is lower than in its lumen. Neither giant vacuoles (13-15), nor endothelial discontinuities (2) were observed; and consequently it is tempting to speculate that even in normal conditions the intercellular clefts of the endothelium represent the route for aqueous humor drainage, as previously suggested by Shabo *et al.* (18). However, the possibility cannot be ruled out that giant vacuoles were lacking because the IOP was very low (15), or that leakage of tracer through the walls of the Schlemm canal was caused by the hypertonicity of the blood contained in its lumen.

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