Osmotically induced retinal detachment in the rabbit and primate
Electron microscopy of the pigment epithelium

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Previous work has shown that the injection of a hyperosmotic solution into the rabbit vitreous will cause rapid detachment of the retina. The present experiments show that osmotic detachment also occurs in the monkey eye. No anatomic changes were found to account for the early separation of the retina, but within a few minutes of detachment in the rabbit or several hours in the monkey, the retinal pigment epithelium microvilli lose their normal organization. This may be a nonspecific response to separation from the outer segments. After longer periods of osmotic detachment in the rabbit, the RPE cells became edematous and the microvilli shrunk and disappeared. Although some cells ruptured at the apical membrane and large cysts were often observed above the cell junctions, the intercellular tight junctions always appeared intact. Osmolarity should be accounted for in the evaluation of intravitreal injections for man, and care should be used to avoid injection of concentrated solutes near the retina.

Key words: intravitreal injection, hyperosmotic solution, osmotic pressure, retina, retinal pigment epithelium (RPE), photoreceptor outer segments, retinal detachment, electron microscopy, tight junctions, blood-retinal barrier

Intravitreal injection is an accepted mode of delivery for a variety of drugs. However, recent experiments on the rabbit eye have shown that the intravitreal injection of only 0.05 ml of a 500 mOsm solution can cause retinal detachment, apparently initiated by water flow across the retinal pigment epithelium (RPE) and/or cellular damage to the RPE and photoreceptors. Information is still needed on whether the same phenomena occur in the primate eye, which has more extensive retinal vasculature, and on whether anatomic changes accompany or can account for osmotic detachment.

Methods
As reported previously, hyperosmotic intravitreal injection experiments were performed on a large number of Dutch rabbits weighing approximately 1.5 kg and anesthetized with pentobarbital, 12 mg/kg. Samples for electron microscopy were retained from roughly half of the eyes studied, including normal eyes, control eyes that received injections of hypo- or iso-osmotic solutions, and eyes that received hyperosmotic injections and suffered retinal detachment. For the present study, experiments were also performed on one cynomolgus and two rhesus monkeys (Table I) under ketamine anesthesia (50 to 100 mg, intramuscularly, repeated as necessary).

Injections were made by passing a small-bore
needle through an opening in the pars plana and depositing 0.05 ml of solution into the center of the vitreous gel. Osmolarity of the injected solutions was measured on a Fiske Model 130 osmometer. All experiments were monitored by contact lens biomicroscopy and indirect ophthalmoscopy to note any effects on the retina. Intracocular pressure was estimated in selected experiments with a Schiitz tonometer (4/5.5 = 4 scale readings with a 5.5 gm weight). The pressure readings in the rabbit have been described elsewhere; we report here only the readings for the monkey experiments.

Rabbit eyes were enucleated for electron microscopy at selected intervals (ranging from 15 sec to more than 24 hr) after the midvitreal injection of various solutions at different osmolarities (see Results). The solutions used in the primate experiments and the length of observation before enucleation are shown in Table I. Within seconds of enucleation, all eyes were immersed in cold fixative (1.5% to 2% glutaraldehyde with 0.1M cacodylate buffer at pH 7.4 and 300 to 350 mOsm) and sectioned at the pars plana. Photographs of the posterior segment within 1 min of enucleation faithfully preserved the clinical appearance of the retina immediately before enucleation.

Tissues were fixed for a minimum of 24 hr, and then samples for microscopy were taken from the parafoveal region of the primate eyes and the posterior pole of the rabbit eyes. In most eyes, the retina was already detached, so that the RPE surface was exposed for scanning electron microscopy (SEM). In control eyes or eyes injected with solutions that did not cause detachment, a small fragment of retina was gently chipped from the RPE surface. Primate tissues were postfixed in 1% OsO₄. Both rabbit and primate tissues were dehydrated in ethanol for transmission electron microscopy (TEM) and in acetone (except for a few rabbit samples treated with ethanol) for SEM. Samples for SEM were critical-point dried, coated with gold, and examined with a Coates and Welter field emission microscope. Samples for TEM were embedded in Epon 812, stained with uranyl acetate and lead citrate, and examined in thin section with a Siemens Elmiskop 1A.

Results

Osmotic detachment in the primate. Midvitreal hyperosmotic injections had basically the same effects regardless of whether NaCl, mannitol, or penicillin was used (Table I).
Fig. 2, a and b. a, 1 min (posterior retina opacified but still attached). The RPE appears similar to that of controls; fragments of outer segments are attached because adhesion was still firm. b, 2 min (retina glistening and beginning to separate). Outer segment fragments are no longer attached; small microvilli cover most of the RPE surface, and aggregates of tall microvilli are spaced roughly 10 μm apart. (Magnification bar (b) = 10 μm and applies to all of Fig. 2.)

From 1 to 3 min. Retinal edema developed, primarily in the posterior pole (the animals were supine), and the fovea appeared as a cherry red spot; however, the retinal vessels remained normal in appearance.

From 5 to 15 min. The edema faded somewhat; irregular elevations of the retina appeared and coalesced into low blebs.

From 20 min to several hours. Small blebs enlarged into bullous detachments that covered much of the posterior fundus (Fig. 1).

From 12 hr to 3 days. Detachment persisted, and the retina appeared to degenerate; these eyes were functionally blind, judging by minimal pupillary responses and the behavior of the animals.

The initial appearance of retinal edema mimicked the cherry-red spot of central retinal artery occlusion, but the vessels showed no constriction or alterations in flow by ophthalmoscopy and contact lens biomicroscopy. Schiötz tonometry readings were normally

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4.5 to 6/5.5 but changed to 1/5.5 (or 7/15) immediately after the midvitreal injection of 0.05 ml of fluid. Anterior chamber paracentesis in one eye (Table I) lowered the reading to 3/5.5 but had no effect on the retinal edema. In two eyes, paracentesis was performed prior to vitreous injection, and the pressure remained low (7 to 11/5.5) for 15 to 30 min after hyperosmotic injection; here, retinal changes were indistinguishable from those in eyes without paracentesis.

**Microscopy of the RPE surface.** The injection of any strong (>1000 mOsm) hyperosmolar solution into the rabbit vitreous causes immediate opacification of the retina, followed by the formation of a bullous detachment. Fig. 2, a to f, shows the typical sequence of changes at the RPE surface which was observed regardless of whether NaCl, Na aspartate, sucrose, mannitol, or EDTA was used to induce the detachment. The speed and severity of these changes were dependent upon the osmolarity of the injected solution. Solutions of less than 500 mOsm (including isotonic saline and distilled water) had no effect. Injections of 500 to 1000
mOsm produced inconsistent and often only partial damage. Injections of more than 2000 to 5600 mOsm invariably and promptly produced the changes in Fig. 2. Large cysts at the cell boundary were more prominent in some preparations than others (Fig. 3, a) but were observed with all of the solutions used for injection. The anatomic changes after EDTA were most severe relative to the osmolarities used; only 0.05 ml of a 533 mOsm EDTA solution caused destruction of the RPE surface (similar to Fig. 2, f). Within the first 10 to 15 min after penicillin injection, the anatomic changes were identical to the sequence shown in Fig. 2. However, after longer exposure to penicillin the RPE cells developed a distinctive matting of fine microvilli (Fig. 3, b) instead of the tuftlike aggregates of swollen microvilli noted after other solutions (cf. Fig. 2, e).

Changes at the surface of the monkey RPE after osmotic detachment were less severe. The normal monkey RPE (Fig. 4, a) has two types of vertically oriented microvilli: short cylindrical ones which cover most of the surface and tall thick ones which appear in

Fig. 2, e and f. e, 35 min (extensive retinal detachment). Most of the cells are denuded except for small central tufts of distended villi; huge cysts, many of which have burst open, balloon up between the cells. f, 50 min (and longer). The microvilli are almost totally lost, and cystic changes are prominent.
clumps spaced 5 to 10 μm apart. After the intravitreal injection of hyperosmolar mannitol, penicillin, or NaCl (Fig. 4, b), the posterior retina was detached, and the underlying RPE surface showed a loss of the longer villi and disorganization and matting of the smaller villi.

In both rabbit and primate, SEM of the photoreceptor surface showed swelling and curling at the tips of the outer segments after exposure to hyperosmotic solutions (Fig. 5, a and b). By TEM (Fig. 5, c), the damaged outer segments showed abnormal separation of the lamellae and curling of the terminal discs within the outer membrane.

Transmission microscopy. The remaining
Fig. 4. Surface of the rhesus RPE. a, Normal eye; note the periodic clumps of tall microvilli (arrow). b, At 3¾ hr after detachment induced by midvitreal injection of 1600 mOsm mannitol; the tall microvilli are gone and the short ones are disarrayed. (Magnification bar = 10 μm.)

figures compare the TEM appearance of normal RPE with the appearance at different stages of osmotic detachment. When separation of the rabbit retina first became evident at 1 to 3 min after a strong hyperosmotic injection (see Fig. 2), the ultrastructure of the RPE (Fig. 6, a and b) was still relatively normal, with the microvilli retaining their vertical organization. However, after 15 min or longer of osmotic stress there was severe damage to the RPE (Figs. 6c and 7) which varied in appearance, depending on the speed and extent of the detachment (compare Figs. 2 and 3). Note that the intercellular tight junctions did not appear (at this level of magnification) to be ruptured, despite the obvious stress upon other parts of the cell membrane. In some of the rabbit preparations, cellular swelling and apical rupture were less prominent, but large apical cysts were present (Fig. 8). These cysts were generally located at the cell boundary (see Fig. 3) above the tight junctions (Fig. 8, a), although they may appear to one side (Fig. 8, b) if the section is oblique to the cell boundary.

The changes in the monkey eye were simi-
Fig. 5. Rabbit outer segments before and after osmotic detachment. 

a. Normal photoreceptor surface; some fragments of RPE pigment are adherent. 
b. Photoreceptor surface 35 min after intravitreal injection of 5600 mOsm NaCl (same eye as in Fig. 2, e). 
c. Outer segments 25 min after injection of 1600 mOsm mannitol (same eye as Fig. 6c). (Magnification as in Fig. 2b for SEM and Fig. 6b for TEM.)
lar to those in the rabbit but less extreme for the osmolarities used. In normal eyes (Fig. 9a) the outer segments were adherent to the RPE. In eyes that had been injected 2½ to 3½ hr previously with hyperosmotic solutions, the RPE showed damage to the microvilli and organelles (Fig. 9b). As in the rabbit, the intercellular tight junctions were not visibly affected.

Discussion

Osmotic detachment in the primate. Sanders and Peyman\(^1\) and Marmor\(^2\) showed that the injection of hyperosmotic solutions into the rabbit vitreous can cause detachment and degeneration of the retina. However, these findings are not automatically relevant for clinical ophthalmology because of the differences between the rabbit and primate eye in...
Osmotically induced retinal detachment

Fig. 6c. Rabbit RPE 25 min after midvitreal injection of 1600 mOsm mannitol. There is gross distension of the cells, fragmentation of the cytoplasm, disorganization of the apical microvilli, enlargement of the basal infoldings and rupture of the apical membrane, although tight junctions appear intact.

Estimates from rabbit experiments suggest that the apparent retinotoxicity of some currently used drugs may relate to osmotic rather than chemical effects. Unfortunately it is not easy to translate osmolarities which are toxic for the rabbit into corresponding values for the primate or man. On the basis of vitreous volume, one would expect that the small monkey eye would require a midvitreal injection of roughly twice (and man about four times) the number of milliosmoles than the rabbit eye to produce the same effect. This estimate holds to a rough degree, since detachments of similar scope were produced by midvitreal injections (0.05 ml) of 1200 to 1600 mOsm in the monkey and 800 to 1000 mOsm in the rabbit. In the rabbit, solutions of 500 mOsm or greater have the potential to detach the retina. It is possible that higher osmolarities can be tolerated in man if injected in small volume into the anterior vitreous, but the margin of safety is unknown. Clearly, the osmolarity of drugs should be considered in evaluating intravitreal injections, and care should be used to avoid the injection of concentrated solutions close to the retina.
damage. A major question in analyzing the anatomic data in this paper is whether visible anatomic changes account for the detachment, or whether the anatomic changes are a result of either the conditions which produce detachment or the detachment itself. The RPE villi were severely disorganized, and in the rabbit bullous cysts protruded between the cells after 10 to 30 min exposure to an intravitreal hyperosmotic solution—but morphologic changes were minimal during the first 2 min of exposure when the detachments actually began. Furthermore, in both rabbit and monkey the tight junctions appeared grossly intact (at least to low magnification TEM) even after long hyperosmotic exposures. Thus, the immediate loosening of the retina probably results either from a subtle degree of RPE dysfunction which is not associated with obvious histologic changes or from fluid pressure upon the retina by the osmotically induced flow of water toward the vitreous. The latter would have to occur across the intact RPE or through channels of molecular dimension.

It is of interest to compare these findings from the RPE with data from other epithelial tissues. Exposure of the mucosal surface of the toad urinary bladder to hyperosmotic solutions reduces the electrical resistance of the tissue, increases its ionic permeability, and induces the formation of cysts above and within the apical intercellular tight junctions.5, 6 Electron micrographs show that these cysts are formed from the apical projections of two cells, one on either side of the junction. The cysts observed in the present study appear similar, although we did not identify cell boundaries in the thin cyst walls. These cystic structures are clearly associated with water flow through the epithelium in the direction of the apical surface,6 but Bindslev et al.7 raised the question of whether they are a cause or effect of increased epithelial permeability. They found that water flow across the epithelium increased within a fraction of a second of passing an electric current across the tissue, but no cysts could be demonstrated even when the conductance change and water flow were at maximal levels 20 sec later. Thus they postulate that the primary increase in permeability involves subtle changes in the cell junctions or cell membrane and that the cysts are produced secondarily by the flow of water.

This explanation may apply to the RPE as well and would account for the relatively normal appearance of the cells during the early phases of osmotic detachment, as well as the later appearance of large cysts after a large volume of fluid has passed across the RPE. Further evidence that cysts are not required for an increase of epithelial permeability comes from experiments in which toad bladder7 or monkey RPE6, 9 were exposed to hypertonic solutions on the serosal (basal) side. Under these conditions, permeability is increased but there is neither the formation of intercellular cysts nor retinal detachment. Okinami et al.8 observed, as we have, that severe osmotic stress may cause the rupture of cells but does not split cells apart at the intercellular junctions. This observation may
have relevance for clinical disorders in which the blood-retinal barrier is disrupted at the level of the RPE.

So far we have considered the anatomic changes only as a sequel to osmotic stress, but some of them may relate more specifically to retinal separation. Both the normal rabbit and primate RPE show two types of microvilli: short ones which ensheath the rods and tall ones which ensheath the cones or conelike photoreceptors. Inahara noted that the distinction between the two types is lost 1 hr after experimental retinal detachment in the rabbit, and Kroll and Machemer found similar changes in the monkey eye 24 hr after surgical detachment. We have observed that the tall microvilli disappear and that all of the microvilli become disarrayed within 2 to 3 min of hyperosmotic detachment in the rabbit and at least within 3 hr in the primate. These microvillous changes could be a response to osmotic stress, but it seems more likely that retraction and collapse of the microvilli represents a rapid response of the RPE to loss of contact with the photoreceptor outer segments. If this is the case, then the RPE microvilli are not static appendages but are dynamic structures that can change configuration according to physiologic demand.

The adhesion between retina and RPE weakens precipitously after death and the RPE has been presumed to play an active role in maintaining retinal apposition. The RPE is obviously damaged after prolonged exposure to vitreal hyperosmolarity, but it is hard to specify which of the anatomic changes were critically cytotoxic and whether metabolic damage occurred early enough to contribute to the retinal separation. In this context, the possible artifact of postmortem damage must be considered. Rabbit eyes kept at 37° after death show swelling of the mitochondria and basal infoldings at 15 min and severe distension of these organelles after 1 to 2 hr. However, since the eyes in the present study were opened immediately after enucleation and placed directly into glu-
taraldehyde, fixation was very prompt and postmortem effects should be minimal.

The fact that the hyperosmotic agents we used did not all produce identical effects is of interest. Most of the differences reflect variability in injection site, diffusability of agents, etc., but some may result from pharmacologic effects of the agents used. For example, the severe denudation of the RPE by EDTA at lower osmolarities than the other agents suggests that calcium may have some role in the maintenance of the villous architecture. After hyperosmolar penicillin injections the RPE microvilli were matted diffusely over the cells, in contrast to the "tufted" appearance after NaCl or mannitol. Hyperosmolarity of the vitreous is sufficient to damage the RPE and retina but is not necessarily the only mechanism by which a concentrated agent can cause damage.

REFERENCES
Fig. 9b. Rhesus RPE after mannitol detachment (same eye as Fig. 4, b). The photoreceptors are detached, the RPE surface is flattened, the microvilli have lost their vertical orientation, the melanin granules are deep within the cytoplasm, and the mitochondria were severely swollen. However, the apical tight junctions (arrow) appear intact and the outer cell membrane shows no visible defects. (Magnification bar = 1 μm.)