Small nonrhegmatogenous retinal detachments (blebs) were made in Dutch rabbit eyes by injecting solution into the subretinal space. There was no difference in resorption time between blebs made with isotonic, hypertonic or hypotonic solutions. There was no difference in resorption time between solutions were made by producing two blebs of the same or different animals but varies between different retinal loci; comparisons between solutions were made by producing two blebs within the same eye, each containing a different solution.

To change the rate at which subretinal fluid is resorbed across the RPE, one may either alter metabolic parameters that affect transport by the RPE, or alter the physical factors that affect resorption such as osmotic or hydrostatic pressure. We have shown previously that metabolic inhibitors such as ouabain, acetazolamide, or cyanide can accelerate or slow down subretinal fluid resorption. In the present study, we examined the effects of osmolality both within the subretinal space and systemically. Systemic hyperosmotic solutions have been shown previously to open the tight junctions of the RPE reversibly, but little is known about the effects of osmotic changes on the resorption of subretinal fluid.

Materials and Methods. The experiments were performed on Dutch rabbits weighing 1.3 to 1.8 kg. Small retinal detachments were made in the avascular posterior pole, free from the medullary rays, by passing a micropipette through a limbal incision and across the vitreous to penetrate the subretinal space, into which an experimental fluid was injected. The methods of anesthesia and techniques of bleb formation and measurement have been described previously. We observed the blebs until 50% of their diameter had become reattached, and used this endpoint for comparative analysis. Bleb resorption time may vary between eyes of the same or different animals but varies less between different retinal loci; comparisons between solutions were made by producing two blebs within the same eye, each containing a different solution.
In the experiments on systemic osmolality, the solutions of 20% mannitol (1098 mosm) or distilled water were injected intravenously within 10 min of the time the bleb was made.

Fluorescein angiography was performed using a Topcon TRC-F3 camera. Tissues were prepared for scanning electron microscopy as previously described1,2 and examined with an ISI-40 scanning electron microscope. Osmolality (mosm/kg) was measured using a vapor pressure osmometer (Wescor 5100C); these values are nearly equivalent to osmolarity for dilute ionic solutions, but are more precise for proteinaceous fluids.

**Results. Osmolality changes within the subretinal space:** Blebs were made with hypertonic (430 mosm/kg) and hypotonic (160 mosm/kg) sodium chloride in the same eye (Fig. 1). The average resorption time for the hypertonic blebs was only 10% longer than that of the hypotonic ones, and both were similar to the range of average resorption times ±1 SD from 75 control blebs made with Hanks' solution. Considering the range of the data and the effect of bleb size on resorption time, we do not think these differences are significant.

Blebs also were made using solutions of sucrose, a substance which does not ordinarily penetrate cell membranes or tight junctions. Blebs made with hypotonic (160 mosm/kg) sucrose either resorbed more slowly than blebs filled with Hanks' solution or required times near the upper limits of normal (Fig. 2A). Blebs filled with hypertonic (430 mosm/kg) sucrose always resorbed much more slowly than Hanks' blebs of the same initial size (Fig. 2B), in part because the diameter of the hypertonic sucrose blebs increased by 10–66% over the first 30–60 min after bleb formation. Even taking these shifts in diameter into account, as done in Figure 2B, the hypertonic sucrose blebs often resorbed more slowly than normal. We occasionally observed that the retina became cloudy shortly after making blebs with hypertonic sucrose, but the tissue cleared by the time maximum bleb diameter was attained. Neither the change in size, nor the occasional retinal clouding, were observed with sodium chloride blebs (of any tonicity) or with hypotonic sucrose.

Fluorescein angiograms were performed within 10 min after the formation of blebs. There was no difference in leakage comparing blebs made with Hanks' solution to those made with either hypertonic or hypotonic sucrose solution. By scanning electron microscopy, the RPE surface under resorbed hypertonic or hypotonic blebs showed no obvious difference in appearance from the RPE under Hanks' solution blebs.

**Systemic osmolality changes:** The intravenous injection of 25 ml of 20% mannitol raised serum osmolality by 8–18 mosm/kg relative to the initial level. The resorption times, after mannitol, of blebs made with Hanks' solution are shown in Figure 3A, with the range of normal values ±1 SD shown for comparison. Most of the resorption times after mannitol fall below the normal range.

The intravenous injection of 25 ml of distilled water caused serum osmolality to decrease by 7 to 13 mosm/kg in 15 min. The osmolality returned to its initial level within 60 min. The resorption times of Hanks'-filled blebs after water injection did not differ significantly from normals.

Fluorescein angiograms made within 30 min after the injection of mannitol and bleb formation, showed no difference in leakage relative to controls (ie, animals given no mannitol). There also are no obvious differences in the appearance by scanning electron microscopy of the RPE surface under Hanks' blebs made in animals with or without systemic mannitol.
Discussion. Osmotic agents are well known for affecting fluid movement in the eye. Mannitol and other hyperosmotic agents have been used systemically to lower the intraocular pressure in glaucoma, or as a means of softening the vitreous preparatory to cataract surgery. Such treatment might, in theory, promote resorption of subretinal fluid, presuming that an osmotic difference could be generated between the choroid and the proteinaceous subretinal fluid, but there is little data on such treatment.\(^6\) If hyperosmotic solutions are injected into the vitreous, however, the retina rapidly detaches, presumably because of fluid drawn out of the choroid.\(^7\)

One might predict that blebs made with hypertonic fluid invariably would take much longer to resorb than blebs made with isotonic fluid because water drawn into the bleb would increase its volume. However, we found no effect on either bleb size or resorption time using hypertonic or hypotonic sodium chloride to make the blebs. We postulate that both water and sodium chloride exchanged freely between the surrounding tissues so that the osmotic differences inside the bleb equilibrated very quickly. These data also suggest that exposure to moderately hypertonic or hypotonic sodium chloride solution was not particularly disruptive or toxic to the RPE.

Blebs made with hypertonic sucrose behaved more in accordance with the prediction above. Sucrose should not cross through the RPE membranes and tight junctions and is also supposed to diffuse more slowly across the sensory retina than sodium chloride. Thus, to compensate for the hypertonic solute, water moved into the blebs and increased their size. We did not observe a decrease in size using hypotonic sucrose, but a small decrease would have been difficult to document in our experimental system since, we could not accurately measure bleb height. Furthermore, sodium and chloride may have diffused into the hypotonic sucrose blebs from the surrounding tissue and prevented any significant change in size. We have shown
previously that isotonic sucrose is absorbed much more slowly from the subretinal space than saline, presumably because the sucrose cannot be transported effectively across the RPE. This would explain why even hypotonic sucrose blebs in the present experiments resorbed more slowly than Hanks' blebs. The present data also are consistent with our observation that blebs filled with serum resorb more slowly than those filled with Hanks' solution, because protein, like sucrose, cannot easily cross the RPE. Others have found that rhegmatogenous detachments made with serum in the monkey decrease 10 times more slowly than those made with Ringer's solution.

In previous experiments, we found that hyperosmolarity of the vitreous not only caused retinal detachment but morphologic changes in the surface of the RPE. However, fluorescein angiography and scanning electron microscopy of the bleb floor in the present experiments did not disclose any gross morphologic damage, either from hypertonic or hypotonic solution. Possibly the small size of the blebs allowed the osmolality gradients to equilibrate quickly enough to prevent serious and grossly visible damage. We cannot rule out, of course, that subtle physiologic damage could have affected resorption rate, but one might then have expected to see abnormal leakage of fluorescein on angiography or a more consistent result among the different hypertonic solutions that were used.

The systemic administration of a hyperosmotic solution might facilitate resorption not only by raising the osmotic pressure in the choroid but also by opening the tight junctions of the RPE. The latter would reduce resistance to diffusion and flow and, thus, facilitate osmotic resorption. However, the solutions used by others to produce blood-retinal barrier breakdown were of higher osmolality and were perfused arterially; we do not know whether our more gentle perfusion would produce the same effect. We could not demonstrate any increase in fluorescein leakage by angiography in these experiments, such as we observed after mechanical, photothermal or chemical damage to the RPE, but fluorescein angiography is not the most sensitive test for slight degrees of barrier damage and we cannot rule out a subtle opening of the tight junctions. Although hypertonic mannitol clearly increased resorption, the converse experiment of making the serum hypotonic did not affect the resorption rate. Possibly the degree or duration of hyposmolality was insufficient to change the resorption rate measurably.

Systemic mannitol may have other effects that could, in theory, have influenced our results. Mannitol may lower intraocular pressure (which would slow down resorption if anything), but in these experiments, we did not repressurize the globe after making the scleral slit and the intraocular pressure was always very low.

Mannitol can reduce systemic blood pressure, increase retinal oxygen tension and increase the ERG c-wave, but the magnitude of these effects and their potential effect on the RPE function is not known. The resorption of subretinal fluid is sensitive to oxygenation and RPE metabolism, but we doubt that the limited osmotic load of these experiments would generate metabolic changes sufficient to overwhelm the more direct effects of osmotic pressure. Histologically, the effect of systemic mannitol on the RPE is less than that on the ciliary body. The dosage of mannitol in these experiments (about 3.3 g/kg) is roughly twice that which is commonly used clinically (1–2 g/kg).

Our data suggest that the systemic administration of hypertonic solutions might help facilitate the resorption of subretinal fluid. However, this effect would be reduced when the subretinal fluid contains a high concentration of protein and is nearly isosmotic with the choroid; hyperosmotic injections would not be of lasting benefit in a clinical situation unless the source of subretinal fluid were eliminated.
Key words: retinal pigment epithelium, retinal detachment, subretinal fluid, blood–retinal barrier, osmolality

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