

Mechanisms of Subretinal Fluid Resorption in the Cat Eye

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Small, non-rhegmatogenous retinal detachments (blebs) were made in cat eyes by injecting fluid into the subretinal space, and the time course of fluid resorption was monitored. Blebs made with Hanks' solution over the pigmented RPE resorbed 22% faster than those over the tapetum. Blebs made with a non-ionic solution (isotonic sucrose) took 43% longer to resorb than those made with Hanks' solution, and blebs containing 3×10^{-3} M sodium cyanide took 32% longer than controls. These results suggest that active ionic transport is involved in the absorption of subretinal fluid in the cat, as it is in the rabbit. Oncotic pressure in the choroid may also contribute to resorption, because blebs made with autologous serum took roughly 3 times longer to resorb than those made with non-proteinaceous Hanks' solution. The retinal vascular system does not appear to contribute, since the resorption time was similar for Hanks' blebs made under normal retina and those made under ischemic retina (produced by occluding retinal branch arteries with argon laser photocoagulation or endodiathermy). Invest Ophthalmol Vis Sci 27:1560-1563, 1986

In our previous studies on subretinal fluid resorption in the rabbit, we found that both metabolic activity of the retinal pigment epithelium (RPE) and choroidal oncotic pressure contribute to the resorption of fluid from under experimental non-rhegmatogenous retinal detachments.¹⁻⁴

We suspect that similar mechanisms exist in the human eye, judging by the fact that subretinal fluid can be absorbed very quickly from under a rhegmatogenous retinal detachment after sealing the retinal break.⁵⁻⁶ However, the human eye differs from the rabbit eye in that it has an intrinsic retinal vasculature which leaks fluid in various pathological conditions and, in theory, could also help to resorb fluid.

In this paper, we report on the mechanisms of subretinal fluid resorption in an animal which has an intrinsic retinal vasculature, the cat. Our primary concerns were whether metabolic transport was involved and whether the intrinsic retinal vessels contributed to the removal of fluid.

Materials and Methods

These investigations adhered to the ARVO Resolution on the Use of Animals in Research. Cats weigh-

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ing 2.1-5.8 kg were anesthetized with 35 mg/kg pentobarbital administered intraperitoneally. The pupils were dilated with two drops of 1% cyclopentolate and 10% phenylephrine. The outer canthus was incised, and some of the lateral orbital bone removed to expose the temporal scleral surface, in which a 3 mm slit was made meridionally 5 mm posterior to the limbus. The basic technique for making detachments was identical to that used for rabbits.^{1,2,4} In brief, a glass micropipette with tip diameter 30-40 μ m was advanced under microscopic observation through the scleral slit and across the vitreous to penetrate the sensory retina. Fluid was injected slowly into the subretinal space by air pressure to create detachments (blebs) with diameter 2.5-4.0 mm. The blebs were observed every 15-20 min until 50% of the initial diameter was reattached (resorption time). Bleb diameters were measured with a grid scale attached to the eyepiece of the microscope. After withdrawal of the micropipette, the scleral slit was closed with 6.0 nylon sutures. To eliminate variability in resorption time between different eyes, comparisons were always made between different kinds of blebs made in the same eye. Control blebs were filled with Hanks' solution (402S, Grand Island Biological, Grand Island, NY); experimental blebs contained either 280-290 mosm/kg sucrose solution, or Hanks' solution plus 10^{-3} M sodium cyanide.

To investigate the role of the intrinsic retinal vessels in subretinal fluid resorption, arterial branches in one quadrant of tapetal retina were closed either by endodiathermy (Mentor, Hingham, MA) or by argon laser photocoagulation using multiple applications of 100 μ m spots at 100-200 mW and 0.5-1.0 sec. Within 2

hr after closing the vessels, one bleb was made in the ischemic area, and another in the normal area.

After the reattachment of blebs, eyes were enucleated for histological observation. Eyes were cut in half, the posterior eye-cups were immersed in the modified Karnovsky's fixative (1% formaldehyde, 1.5% glutaraldehyde in Cacodylate buffer), and the sensory retina was carefully peeled from the RPE. After remaining in the fixative overnight, the tissues were processed for scanning electron microscopy.

Results

The cat retina seemed to have greater adhesivity than rabbit retina, judging by the difficulty of starting blebs; thus, we were concerned that either RPE microvilli or outer segment tips might be ruptured during separation. Scanning electron micrographs of the RPE and retinal surface showed that, in more than 90% of blebs, the surface structure of both photoreceptors and RPE was preserved with minimum damage, in both the tapetal and non-tapetal areas. The few blebs in which RPE microvilli or outer segments were damaged were recognizable in vivo by an altered appearance of the detached retina, and data from these blebs were not used.

Figure 1 compares the resorption times of blebs filled with Hanks' solution over the tapetal and non-tapetal areas of the cat fundus. The resorption times are plotted against bleb diameter, which can influence the rate of resorption.^{2,4} In general, blebs made over pigmented RPE resorbed somewhat faster than those made over the tapetum. If these data are averaged together, the average resorption time from the blebs over the non-tapetal area was 22% shorter than that over the tapetum (Table 1). Since the posterior pole, which is tapetal in the cat, is more easily reached with our methods than other parts of the fundus, we used only tapetal blebs for the experiments that follow.

To study the role of metabolic transport in adsorbing subretinal fluid, one or two control Hanks' blebs were made in one quadrant of the fundus, and, in another quadrant of the same eye, blebs were made with either

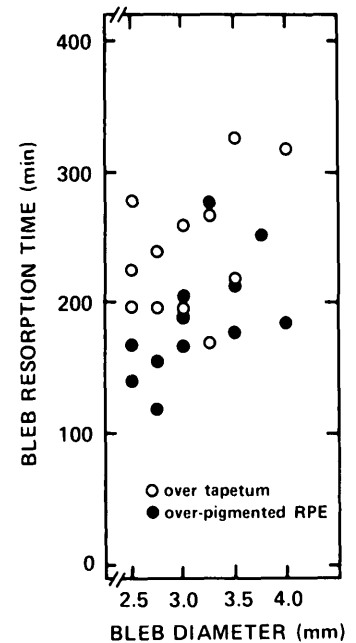


Fig. 1. Effect of tapetal vs non-tapetal location on the resorption times of blebs made with Hanks' solution.

Hanks' solution plus 3×10^{-3} M sodium cyanide or isotonic sucrose. Figure 2 (left) shows that the blebs containing cyanide resorbed more slowly than controls. If the data are averaged together, the resorption time after cyanide was 32% longer (Table 1). Even greater differences were observed between blebs filled with isotonic sucrose and Hanks' controls (Fig. 2, right). Sucrose is a non-ionic substance that does not cross cell membranes, and, thus, renders ineffective the component of subretinal fluid movement that depends on active ionic transport. On the average, the sucrose blebs took 43% longer to resorb than the controls (Table 1).

We found very striking differences in resorption time between blebs made with autologous serum and Hanks' solution. The serum blebs typically persisted for hours after the control blebs were resorbed (Fig. 3). The average resorption time of the serum blebs was 257% of the control value (Table 1).

To produce retinal ischemia, arterial branches were closed by laser photocoagulation or endodiathermy.

Table 1. Significance of experimental data

	Average bleb diameter (mm)*			Average bleb resorption time (min)		
	Control†	Experimental	Significance‡	Control†	Experimental	Significance‡
Blebs over pigmented RPE	3.04	3.13	N.S.	240.6	187.1	$P < 0.05$
Cyanide-filled blebs	2.84	2.91	N.S.	198.5	262.9	$P < 0.05$
Sucrose-filled blebs	2.90	3.04	N.S.	268.6	386.3	$P < 0.01$
Serum-filled blebs	2.88	3.13	N.S.	218.5	561.7	$P < 0.01$
Blebs under ischemic retina	3.08	3.03	N.S.	268.3	262.1	N.S.

* Bleb diameters should not differ significantly if control and experimental blebs are to be comparable.

† Control blebs were over tapetal RPE and filled with Hank's solution.

‡ Significance of the difference between control and experimental values. N.S. = not significant.

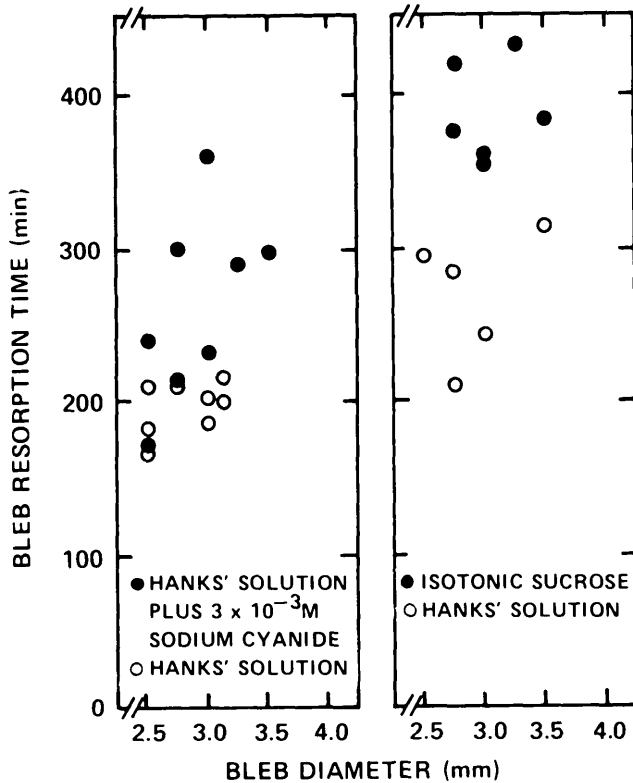


Fig. 2. Effect of inhibiting metabolic transport on bleb resorption times, relative to the resorption of Hanks' solution in the same eye. Left, Addition of 3×10^{-3} M sodium cyanide. Right, Replacement of Hanks' solution with isotonic sucrose.

The sealed vessels usually showed no flow of blood throughout the experiments, although very slow segmental blood flow was sometimes observed in the late stage of bleb resorption. The ischemic tapetal quadrant sometimes showed a bluish color, compared to the normal yellowish cast of the tapetum. The resorption times from blebs made with Hanks' solution under

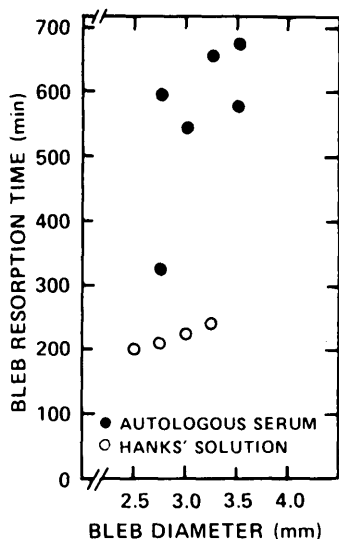


Fig. 3. Resorption times of blebs made with autologous serum relative or Hanks' balanced salt solution within the eye.

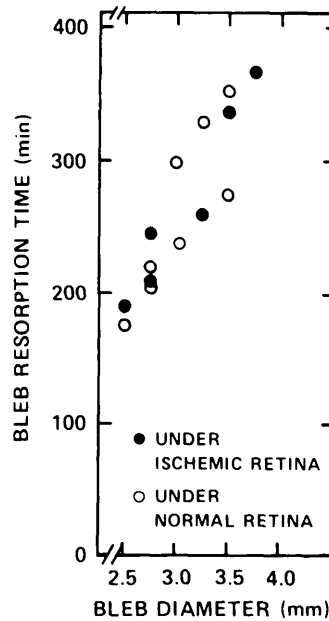


Fig. 4. Effect of ischemic vs normal retina on resorption times of blebs made with Hanks' solution.

normal or ischemic retina are plotted in Figure 4, showing that there was no consistent difference between them (Table 1).

Discussion

In general, blebs made with Hanks' solution in the cat eye resorbed with a time course similar to that for blebs in the rabbit eye.^{1,4} Although our retinal detachments were made by penetrating the sensory retina, the small holes seem functionally insignificant.⁷ We found that blebs made over the tapetal area resorbed more slowly than those over pigmented RPE, for reasons that are not clear. In the tapetal area, fine capillaries lie between the tapetum and RPE, but the sinus-like dense meshwork of choroidal vessels which underlies pigmented RPE is missing. The RPE cells are very thin over the tapetal capillaries, and are taller where directly attached to the tapetal cells. They also lack basal infoldings.⁸ These structural characteristics may cause differences in RPE metabolism or in physical factors, such as outflow resistance.

Blebs filled with a non-ionic solution (sucrose) or containing a metabolic inhibitor (cyanide) took significantly longer to resorb than control blebs. These results suggest that active ionic transport is important to the resorption of subretinal fluid. If resorption were purely passive, then sucrose, which does not cross the RPE barrier and cell membranes,^{9,10} should have been absorbed nearly as fast as saline. Then why do sucrose blebs resorb at all? Because there is no barrier to diffusion into the vitreous space,¹¹ so that sucrose leaves and sodium chloride enters the bleb to equilibrate the concentrations and facilitate eventual transport across the RPE. Sodium cyanide blocks metabolic ion trans-

port and accompanying fluid movements, which shows normally a net flow directed to the choroidal side.¹² These results in the cat eye are similar to those from the rabbit, in which active transport across the RPE has been shown to contribute strongly to the absorption of subretinal fluid into the choroid.¹⁻³

The high oncotic pressure of the choroid is also a significant driving force for the removal of subretinal fluid.¹³ Filling a bleb with serum cancels this oncotic difference, and the effect should be reflected in a longer resorption time. In the rabbit, over normal RPE, the difference of resorption times between serum blebs and Hanks' controls is about 50%; when the RPE barrier has been damaged, so that Hanks' solution can enter the choroid more rapidly, the difference increased to roughly 1,000%.⁴ We postulate that, in normal eyes, effects of choroidal oncotic pressure are limited by the high resistance barrier of the RPE; thus, metabolic ionic transport is required to fully dehydrate the subretinal space.⁴ In our cat experiments, the resorption times of serum blebs over apparently normal RPE were about 300% that of Hanks' controls, a difference much larger than in the rabbit. The reasons for this species difference are obscure. Possibly, our bleb-making procedure damaged the RPE barrier in the cat, despite our failure to see damage in the scanning electron micrographs; possibly, there are anatomical or physiological differences in the tapetal RPE of the cat relative to the RPE of the rabbit.

The intrinsic retinal vessels form a part of the blood-retinal barrier. Under normal conditions, retinal extracellular fluid should contain little protein, and it is damage to this barrier that allows leakage of proteinaceous fluid with resultant retinal edema or separation. The intrinsic retinal vessels are presumed to help clear pathologic intraretinal fluid, but their role in the absorption of subretinal fluid is uncertain. Toris and Pederson injected horseradish peroxidase into the vitreous of eyes with chronic rhegmatogenous retinal detachment, and found that the tracer collected around retinal vessels, staining their basal lamina.¹⁴ These results raised the possibility of fluid absorption by the retinal vessels, but did not allow estimation of the amount of water movement. The vasculature of the cat retina is similar to that of the human retina, in that it extends to the inner border of the outer plexiform layer.¹⁵ However, the resorption time for blebs filled with Hanks' solution was not noticeably altered by occluding the retinal arteries. This suggests that the retinal vascular system does not play an important role in the

resorption of subretinal fluid, which, therefore, must occur primarily across the RPE.

Key words: retinal detachment, retinal pigment epithelium, subretinal fluid, ionic transport, retinal vasculature

Acknowledgments

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