
Osmotic pressure and ion movements in and out of the vitreous space after open-sky vitrectomy were studied in the in vivo eyecup of rabbits. The osmotic pressure of several hypo-osmotic solutions rose over a period of time by the penetration of ions from the surrounding tissue; increases in the concentration of sodium, chloride, potassium, and calcium were documented. Appearance and disappearance of ions were independent of the total osmolarity and depended on the concentration gradient for each ion.

Vitrectomy with artificial perfusates changes the chemical and physiologic conditions of the vitreous space, resulting in alteration of retinal and optic-nerve activity. Some changes are transient and some are permanent. We observed that electroretinograms (ERGs) from an in vitro preparation of rabbit retinas were severely diminished in amplitude when the control perfusate was replaced by normal saline, balanced salt solution (BSS), or allied artificial solutions and that some retinal functions were lost permanently even when other incubating conditions were ideal. This is a reasonable observation, considering that the ionic composition of an artificial solution is far from that of the normal vitreous body. On the other hand, vitrectomy with the same perfusates may be characterized by excellent recovery of visual function. Our hypothesis is that the destructive effect of artificial perfusates can be moderated by the presence of retinal and uveal blood circulation in an in vivo eyecup. Ion movement from the uvea and retina into the vitreous space would be expected to modify the inadequate composition of the artificial perfusates. In this report, osmotic pressure and ion movement in and out of the vitreous space after open-sky vitrectomy were studied.

Material and methods. Eighty adult albino rabbits weighing approximately 3 kg were used. In vivo eyecup preparations were made as follows: With the animal under general anesthesia (urethane by abdominal administration of 1.0 gm/kg body weight) a double fixation ring modified from Flieringa-LeGrand type for rabbits was sewn into the episclera to prevent collapse of the eyecup. The cornea was removed, and the iris was cut at the root. Under an operating microscope, an intracapsular lens extraction was followed by an open-sky vitrectomy, performed with a Kaufman Type II Vitrector. The eyecup was filled to the ciliary body with 0.9 ml of fluid. The volume of the vitreous cavity, as measured by aspiration of fluid, decreased to approximately 0.6 ml after 90 min, primarily because of choroidal edema.

For the first part of the experiment the eyecup was perfused with the solutions under study and ERGs were recorded. With use of Ames' solution, the a-wave and b-wave of the eyecup ERG were held constant for 2 to 3 hr. Each eyecup was rinsed twice with experimental solution before the start of equilibration experiments and was used for a series of measurements when the preparation was in good condition. All experiments ended within 100 min.

After the intended incubation time, the fluid in the eyecup was removed and osmotic pressure was measured by an osmometer (OSA-21; Nippon Jerrel Ash). Sodium, potassium, calcium, and magnesium concentrations were determined by a flame or flameless atomic absorption spectrophotometer (Model AA-780; Nippon Jerrel Ash). Absorptions at 5890, 7464.9, 4226.7, and 2852.1 Å were used for sodium, potassium, calcium, and magnesium, respectively. Chloride concentration was measured by the method of Cotlove with a chloridometer (Buchler Instruments, Fort Lee, N. J.). Standards were prepared with NaCl for sodium and chloride, KCl for potassium, CaCl₂·2H₂O for calcium, and MgSO₄ for magnesium. The ERGs in Fig. 1 were recorded between an active electrode immersed in the eyecup and a reference electrode on the ear. The amplifying, recording, and stimulating system was the same as that reported previously. The ERG amplitude was measured from the bottom of the a-wave to the peak of the b-wave. Compositions of solutions used in this study were as follows (mM/L): physiological saline (normal saline), 154.0 Na and 154.0 Cl; BSS (Alcon Laboratories, Inc., Fort Worth Tex.), 143.9 Na, 10.1 K, 3.27 Ca, 1.48 Mg, 129.1 Cl, 28.6 acetate, and 5.78 citrate; S-MA₂ (Senju Pharmaceutical Co. Ltd., Osaka, Japan), 145.7 Na, 4.8 K, 1.22 Ca, 1.2 Mg, 129.1 Cl, 25.0 HCO₃, 1.2 SO₄, 4.41 acetate, 3.4 citrate, and 8.33 glucose; Ames' solution, 143.7 Na, 3.6 K, 1.15 Ca, 1.20 Mg, 125.4 Cl, 22.6 HCO₃, 1.20 SO₄, 0.5
Fig. 1. A, ERGs recorded after substitution for the control medium by physiological saline (left column), BSS (middle column), and S-MA2 (right column). After complete substitution the medium was perfused at a rate of 9 ml/min. Numbers on the left of each column represent elapsed time after substitution. B, Time course of the b-wave amplitude from in vivo eyecups perfused with the different solutions. Responses in normal saline were severely damaged, and the control medium was restored after 40 min with partial recovery. Each point represents the mean of five experiments, vertical lines indicate 1 S.D.

Results

ERG amplitude when the eyecup was filled with artificial solutions. ERG amplitude was observed to be constant for over 120 min in trials with the control solution. The effects on ERG amplitude of perfusing the eyecup with different artificial solutions are shown in Fig. 1. Physiological salt solution decreased the ERG amplitude but to a lesser extent than occurred in the in vitro study. BSS and S-MA2 brought about only a small change, in
Fig. 2. Osmotic pressures in the vitreous space, filled at time zero with sucrose of 100, 150, 200, 250, and 300 mOsm/kg. Each point represents the mean of 10 experiments; vertical lines indicate 1 S.D. The rate of increase was highest in sucrose of lower concentrations.

Fig. 2. Osmotic pressures in the vitreous space, filled at time zero with sucrose of 100, 150, 200, 250, and 300 mOsm/kg. Each point represents the mean of 10 experiments; vertical lines indicate 1 S.D. The rate of increase was highest in sucrose of lower concentrations.

contrast to the corresponding in vitro studies, where severe damage to the ERG was observed. We considered that the prominent difference in retinal function between the in vivo and in vitro eyecup might have come from in vivo modification of the perfusate by the intact retinal and choroidal circulations. Thus the following experiments were carried out.

Equilibration of solution in the vitreous space. The eyecup was filled with various concentrations of sucrose (100, 150, 200, 250, and 300 mOsm/kg), and the time course of the osmotic pressure was followed for each concentration. As shown in Fig. 2, the osmotic pressure rose immediately, with the rate of increase highest for media of low concentrations, and it approached a plateau level over a period of time. The concentrations of some representative inorganic ions were serially determined during these osmotic experiments (Fig. 3). Sodium and chloride ions appeared rapidly in the vitreous cavity, and their rates of entry appeared to be independent of the initial concentration of sucrose. The sodium concentration increased from 0 to 75 to 105 mM/L, and concentration of chloride increased to over 50 mM/L in 30 min. Potassium and calcium concentrations also rose to values near the physiological level of the vitreous within 10 to 30 min. The appearance of sodium and chloride ions in the vitreous space accounted for the prominent elevation of osmotic pressure shown in Fig. 2. In addition, Figs. 2 and 3 illustrate that sucrose was continuously exiting from the vitreous space. If the concentration of sucrose remained constant, the osmotic pressure in the vitreous space would be higher than that shown in Fig. 2 because of the influx of sodium and chloride. This continual modification of ion composition in the vitreous space might explain the excellent ERG from the in vivo eyecup filled with artificial solutions.

Discussion. The present study showed rapid ionic and osmotic equilibration of the solutions infused within the vitreous space of the in vivo eyecup. However, we should note that these observations on the rabbit eyecup preparation cannot be directly compared to clinical closed vitrectomy for several reasons. (1) The eyecup allowed a more accurate and complete vitrectomy than is possible in the closed eye, and measured volumes of fluid could be completely and easily extracted for precise quantitative analysis. Open-sky vitrectomy is rarely used clinically, and it alters intraocular pressure, anterior hyaloid diffusion barrier, and blood-ocular barriers more than closed-eye vitrectomy. (2) The retinal vessels of the rabbit eye are poorly developed and are quite different from those of the human eye. (3) Preparing the eyecup disturbed the microcirculation of the anterior portion of the eye, and choroidal edema developed. (4) Since no outflow of fluid from the eyecup was observed during experiments, the ciliary body was probably damaged by surgical procedures, and ion and water secretion from it was modified. (5) In relation to ion analysis, contamination from the remaining vitreous body on the retinal surface could not be avoided. (6) The lens and iris were removed; thus normal ionic exchange from the lens surface or iris epithelium was omitted. The data must be interpreted in light of these limitations.

The initial level of osmolarity of sucrose minimally altered the rate at which ions appeared in the vitreous space. Ion appearance and disappearance were also independent from the total osmo-
larity in the vitreous space. Sodium, potassium, chloride, and calcium moved according to their own concentration gradient toward equilibrium. The origin of ions penetrating into the vitreous space in this study could not be identified. Investigation of this problem is necessary and experiments are now in progress. Our present feeling is that the posterior wall as well as the ciliary body has contributed to ionic and osmotic equilibration, especially under such an abnormal condition as open-sky vitrectomy. Ions might penetrate from the whole area of contact with the vitreous space by diffusion. Laties et al. have suggested that the blood-ocular barrier could be changed under such stress as acute glaucoma or hyperosmotic vascular perfusion. Traumatic injury to the eye or even cataract surgery may induce fluorescein leakage in the posterior pole, and thus open-sky vitrectomy is probably a sufficient stress to change the ocular barrier system. Disappearance of sucrose from the vitreous space should not occur ordinarily across a membrane barrier such as the pigment epithelium. However, it might have occurred in this study because of the high vitreal concentration gradient and damage to the blood-ocular barrier. The outflow of sucrose compensated for the increase in total osmolarity of the vitreous space induced by newly appearing sodium, chloride, and other important ions.

Our conclusion is that even if open-sky and almost-complete vitrectomy is performed and the vitreous cavity is filled with artificial solutions, the vitreous space will rapidly equilibrate ionically and osmotically with that of the surrounding tissues. Also, retinal function, which is compromised by an artificial solution, may recover when uveoretinal circulation is normal. However, this result does not deny that solutions as perfect as possible should be employed during surgery. Irrigation by inferior solutions can cause major changes in the retinal tissue, and some of these changes can be irreversible in vitro without circulation.

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Fig. 3. Time course of sodium and chloride (left scale), and potassium and calcium (right scale) concentrations in the vitreous space when filled at time zero with sucrose of 100, 200, and 300 mOsm/kg. Data for sodium and potassium represent the mean of 10 experiments; data for chloride and calcium represent the mean of five experiments. In the 200 mOsm/kg incubation, calcium was not measured. Vertical lines indicate 1 S.D. Note that the final sodium concentrations exceeded 100 mM/L.
Immunohistochemical comparison of ocular zonules and the microfibrils of elastic tissue. BARBARA W. STREITEN, PATRICIA A. LICARI, AMERICO A. MARUCCI, and ROBERT M. DOUGHERTY.

Antibody to bovine zonules raised in rabbits gave diffuse staining of zonular fibrils with prominent accumulations at 35 to 45 nm sites of overbanding by means of the indirect immunoperoxidase technique. Antibody binding occurred in both fresh and paraffin-embedded material and was not species specific. The same pattern of heavy antibody binding and 35 to 45 nm periodicity was found on the microfibrils of elastic tissue in human and bovine ciliary body, calf ligamentum nuchae, and chick aorta. This cross reaction is the first direct evidence of a structural similarity or identity between components of the zonules and the microfibrils of elastic tissue, correlating with similarities in their morphology and amino acid profiles. Interstitial collagen fibers appear to have a minimal quantity of antigenically similar material at cross-banding sites. These relationships may holp important clues to the site of systemic involvement in connective tissue diseases associated with lens dislocation. Zonular antibody should prove useful in investigating abnormalities of the zonular-elastic microfibrillar system and cross-banding sites.

The ocular zonules are unique fibers whose relationship to other structural fibrous tissues is still uncertain. Although originally thought to be collagenous like the adjacent vitreous, the zonules contain no hydroxyproline and are insensitive to collagenase. Morphologically, zonular fibrils most closely resemble the microfibrils of elastic tissue, which are fine fibrils surrounding the amorphous elastin-containing core of the elastic fiber. The amino acid profile of the zonules, including an unusually high cysteine content, also resembles that of elastic tissue microfibrils. To determine whether a structural relationship may exist between these two types of fibrils, they were compared immunohistochemically.

Materials and methods. Zonules were cut from fresh bovine eyes with the use of microinstruments under a dissecting microscope, carefully avoiding contamination with lens capsule and vitreous. Zonular antibodies were raised in 2.5 kg male New Zealand white rabbits by injecting a 1:1 mixture of zonules sonicated in 0.01M phosphate-buffered saline (pH 7.3) and complete Freund's adjuvant. The rabbits received 1 ml of the mixture divided among five sites subcutaneously at 2 week intervals for 8 weeks. Sera were then tested for the presence of antibody in Ouchterlony plates. Because zonules are insoluble without treatment, antigen for the reaction was prepared by reducing fresh zonules with 4M urea, 1% sodium dodecyl sulphate (SDS), and 0.04M dithiothreitol (DTT). The reduced zonular solution was then dialyzed against 0.04M DTT before immunodiffusion.

Tissues were examined for antibody binding by both light and electron microscopy, with peroxidase-labeled staphylococcal protein A as an immunoreagent. The protein A was conjugated by the periodate method. For light microscopy,