Corneal water and electrolyte content following storage in moist chamber and MK medium. DAVID S. HULL, KEITH GREEN, AND KAREN BOWMAN.

Rabbit corneas were subjected to MK-medium or moist-chamber storage for periods up to 7 days. Sodium, potassium, and chloride concentrations and hydration were measured. The hydration of corneas stored in MK medium was less than that of those stored in moist chambers. The sodium concentration of MK corneas remained stable for up to 7 days; the potassium concentration, however, decreased with increasingly longer periods of storage. Chloride concentrations quickly equilibrated with the high chloride level of MK medium. The concentration of sodium and chloride in moist chamber-stored corneas fell with progressively longer storage periods. Potassium showed an initial decrease in concentration followed by an increase in concentration that paralleled, and equilibrated with, the increasing aqueous humor concentration.

Ionic concentration and hydration have been well documented in normal rabbit corneas. It was the purpose of this investigation to determine and compare the concentrations of sodium, potassium, and chloride, as well as the hydration, in rabbit corneas stored in MK medium and moist chambers for various periods of time.

Materials and methods. Albino rabbits weighing about 2 kilograms were sacrificed with an overdose of sodium pentobarbital. Eyes were enucleated and either stored, as the whole eye, in a moist chamber at 4° C, or the cornea with a 2 mm. scleral rim was removed from the eye and stored in a 20 ml. vial of Medium 199 with 5 per cent dextran (MK medium) at 4° C. After varying periods of time the corneas were removed, gently blotted with filter paper, and weighed in flasks that had been dried at 100° C. After drying at 100° C. for 24 hours, the corneas were again weighed after cooling for 30 minutes in a desiccator. After drying at 100° C. for 24 hours, the corneas were again weighed after cooling for 30 minutes in a desiccator and the dry weight and hydration calculated. One milliliter of 0.1N nitric acid was added and the corneas shaken for 24 hours for the purpose of electrolyte extraction. Chloride was determined on the acid extract with a Buchler digital chloridometer.

Sodium and potassium determinations were made with a Corning Model 450 flame photometer. Calculations at each time period were based on the data of four rabbits, which is presented as the mean ± standard error of the mean (± S.E.M.).

Results.

Hydration. Corneas stored in MK medium showed a gradual increase in hydration until, after 24 hours, a value was achieved which remained constant for up to 7 days (Fig. 1, A).

Moist chamber-stored corneas showed an increased hydration at 24 hours and continued to slightly increase in hydration over the next 6 days (Fig. 2, A). The 24 hour and 7 day hydrations of corneas stored in moist chambers were 84.2 ± 0.2 per cent and 86.7 ± 0.2 per cent, respectively. Corneas stored in MK medium showed hydrations of 79.8 ± 0.2 per cent at 24 hours and 80.5 ± 0.6 per cent at 7 days.

Sodium. Corneas stored in MK medium showed a maintenance of sodium concentration for storage periods of up to 7 days (Fig. 1, A). Moist chamber-stored corneas demonstrated a decreased sodium concentration, compared to normal, fresh corneas at 24 hours and 7 days (Fig. 2, A).

Potassium. Corneas stored in MK medium demonstrated a decrease in potassium after 1 hour, which continued to gradually fall with progressively longer periods of storage (Fig. 1, A).

Corneas stored in moist chambers showed a reduced potassium concentration at 24 hours (Fig. 2, A). This is similar to the concentration of potassium of corneas stored in MK medium for 24 hours. At 7 days, corneas stored in moist chambers showed a potassium concentration which had increased and was significantly greater than that at 24 hours. The increase paralleled that which occurred in the aqueous humor of the enucleated eye (Fig. 2, A and B).

Chloride. Corneas stored in MK medium demonstrated a rapid rise in chloride concentration at 1 hour, and at 6 hours had achieved a value close to that of the bathing solution (Fig. 1, A and B). The concentration remained constant and elevated for up to 7 days' storage.

Moist chamber-stored corneas showed decreasing chloride concentrations with progressively longer storage periods, which reflected the fall in concentration that occurred in the aqueous humor (Fig. 2, A and B).

Discussion.

Hydration. The hydration of fresh rabbit corneas (73.97 ± 0.22 per cent) compares favorably with that in previous reports. The hydration gradually increased to 79.8 per cent at 24 hours and remained essentially constant for up to 7 days. It is known that dextran enters the corneal stroma and within 24 hours an equilibrium is reached between about 15 per cent of the stromal water and the surrounding MK medium. Hence this
finding would be expected because of the osmotic effect of dextran in the ambient solution, causing an equilibrium hydration as a result of the balance of swelling and osmotic forces.

Sodium. Sodium concentrations of fresh rabbit corneas are in good agreement with those previously reported, and this concentration remained constant in MK-stored corneas for periods up to 7 days. It is to be expected that sodium levels in corneal stroma should be maintained at higher levels than the surrounding MK medium (Fig. 1, A and B) due to sodium binding by stromal acid mucopolysaccharide.

The decrease in sodium concentration of moist chamber-stored corneas (Fig. 2, A) probably reflects the dilution of stromal sodium as the cornea hydrates, with the consequent inhibition of a relatively dilute ambient solution. Previous work has shown that aqueous humor sodium concentration remains constant up to 4 hours post mortem at 3°C. In our study, aqueous humor sodium at 24 hours had fallen to 95 per cent of the concentration in fresh eyes and at 7 days had fallen to 84 per cent.

Potassium. Potassium concentrations for fresh whole rabbit corneas are in good agreement with those previously reported. The concentration of potassium falls after 1 hour of storage in MK medium, and continues to fall with longer storage periods (Fig. 1, A). Whether this represents rapid death of stromal cells with subsequent release of stromal potassium, or simple diffusion of potassium from the cells at low temperature, is not known. It is known that isolated stroma, when incubated in 0.9 per cent sodium chloride solutions, shows a complete loss of potassium within 1 hour. This finding was confirmed by the low level of stromal potassium in a group of de-epithelialized MK-stored corneas at 24 hours, which indicated that although potassium may be lost from the epithelium during storage, the contribution to the total potassium content by the epithelium is only about 25 per cent. In addition, the levels of potassium in fresh aqueous humor and fresh MK medium are similar (Figs. 1, B, and 2, B), so that the stromal potassium loss does not represent an attempt by stromal potassium to quickly equilibrate with the surrounding medium in a living preparation.

Moist chamber-stored corneas also demonstrated a diminution of stromal potassium at 24 hours (Fig. 2, A). The steadily increasing concentration of potassium in the aqueous humor with increasing storage time probably reflects the release of potassium from stromal cells, lens, and uveal tissue (Fig. 2, B) and confirms previous work which demonstrated similar changes over a considerably shorter time period. The increased level of potassium at 7 days in moist chamber-stored corneas over that at 24 hours parallels the rise in aqueous humor concentration of potassium (Fig. 2, A and B). Thus, the in-

Fig. 1. Sodium, potassium and chloride concentrations and hydration (± SEM) in (A) MK-stored corneas and (B) the corresponding MK solution.
increase is a purely passive one in response to ambient changes.

It is also of interest that the potassium concentration of human corneas behaved in a similar manner: fresh human (4 hours post mortem, \( N = 2 \)), 18.6 ± 0.9 (mean ± S.D.) milliequivalents per kilogram of water; 4 day storage in MK medium (\( n = 2 \)), 10.30 ± 1.70 mEq. per kilogram of water; 4 day storage in moist chamber (\( n = 4 \)), 13.24 ± 0.67 mEq. per kilogram of water.

Chloride. Chloride concentrations in fresh whole corneas are comparable to those previously reported.\(^1\) The rapid rise in corneal chloride concentration after 1 hour of storage in MK medium (Fig. 1, A) is due to the change in ambient solutions, that is, from the aqueous humor to the MK medium with its high chloride concentration (Fig. 1, B). Previous work has shown that chloride rapidly equilibrates with a bathing medium in vitro,\(^9\) and therefore this finding is to be expected.

Corneas stored in moist chambers showed a decreasing concentration of chloride at 24 hours, with lower levels at 7 days. This reflects the diminution in aqueous humor chloride concentration, with which the corneal stroma equilibrates (Fig. 2, A and B). The corneal chloride concentration, therefore, in all conditions of storage, reflects that of the surrounding ambient solutions.

Summary. MK-stored corneas maintain a lower hydration and higher sodium and chloride concentrations than do moist chamber-stored corneas. The higher potassium levels in moist chamber-stored corneas at 7 days are not due to a more favorable storage condition, but are rather due to inhibition of rising aqueous humor concentrations of potassium due to stromal, lens, and uveal cell death. In addition, the bathing of both corneal surfaces with a nutrient solution provides a medium superior to the stagnant aqueous of an enucleated eye.


Key Words: rabbit, cornea, tissue preservation, corneal preservation, corneal electrolytes, corneal hydration.

REFERENCES
An improved model of experimentally induced ocular hypertension in the rabbit.

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A model of experimentally induced ocular hypertension for the evaluation of antiglaucoma drugs in the unanesthetized rabbit is described. It is based on the intravenous infusion of suitable amounts of 5 per cent glucose solution, and advantageously substitutes the oral water load. The method is sensitive to drugs acting both on the outflow facility (2 per cent pilocarpine) and on aqueous humor formation (10 per cent ganciclovir).

In the search for improved medical therapy for glaucoma, new drugs must be tested experimentally in animals before any clinical trials in human beings are made. For practical and economical reasons the animal species most commonly used is the rabbit.

Unfortunately, the effect of many drugs on normal intraocular pressure is slight, so that their pressure-lowering properties may be missed or underestimated.

In the hope of obtaining more reliable results, many models of experimental ocular hypertension have been developed in the past, but most of them turned out to have important drawbacks and have been abandoned.

The requirements for a satisfactory experimental model of ocular hypertension are that it should (1) leave intact the ocular structures that respond to the action of the drug—for the study of drugs active on the outflow, the integrity of the outflow pathways is essential; (2) allow unanesthetized animals (therefore normally responsive to drugs and stimuli) to be used; (3) avoid trauma or excessive stimulation of the eye; and (4) be simple and easily reproducible.

It has been shown that it is possible to raise the intraocular pressure of normal rabbits by water load and that the ocular hypertension so produced is sensitive to drug action.1,2

Recently a good experimental model based on water loading and Mackay-Marg tonometry in awake rabbits has been advocated by Seidenhamel and Dungan.3 The model was demonstrated to be sensitive to epinephrine and suitable for testing the effect of antiglaucoma drugs. Nevertheless it requires the rather troublesome procedure of administering large amounts of water to the animals via orogastric gavage.

Bietti4 has demonstrated that in human beings the conventional water load can be advantageously replaced by the intravenous infusion of suitable amounts of 5 per cent glucose solution. The glucose is quickly removed from the circulation, and a lowering of the serum osmolarity ensues, which in turn produces a rise of intraocular pressure. This method has the advantage of being simpler and more reproducible than the water-drinking test, and individual variations due to differences of water absorption from the intestinal tract are avoided.

Since for reliable tonometry in awake rabbits, it is advisable to keep the animals as quiet and unfrightened as possible, avoiding excessive manipulation and stimulation, we thought of adapting this method to the rabbit with the aim of using it as a model of experimentally induced ocular hypertension for the evaluation of antiglaucoma drugs.

**Methods.** The experiments were performed on albino rabbits of both sexes, weighing 2 to 3 kilograms. The intraocular pressure (IOP) was measured with a Mackay-Marg electronic tonometer, after surface anesthesia (0.4 per cent benoxinate [Novesine]). The tip of the tonometer was moistened with mineral oil to avoid corneal abrasion.

IOP elevation was obtained by rapid infusion of 5 per cent glucose solution through a 20-gauge needle in the marginal vein of the ear. The amounts injected were 5, 10, and 15 ml per kilogram of body weight and the infusion was accomplished in all animals within 20 seconds.

**Results and discussion.** Immediately after the end of the infusion the eye pressure increased in all animals, reaching its maximum level between 5 and 10 minutes and returning to pretreatment levels within 40 minutes. The values of IOP increase were dependent on the amount of solution infused: the administration of 15 ml per kilogram produced an increase of about 12 mm. Hg, whereas with 10 and 5 ml per kilogram, a still

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