cryoextracted lenses transferred to physiologic saline for brief periods of time. The deleterious effect of saline on cryoextracted lenses (Fig. 4), but not surgically excised lenses, is apparent from cation measurements and the fact that cryoextracted lenses exposed to saline accumulate ^{86}Rb to a significantly lesser degree (33%) than when exposed to TC199.

While the results of this investigation do not provide an explanation for the observed changes in cryoextracted lenses augmented by saline, it is likely that the lack of calcium in the saline solution for even brief periods of time may have contributed to the irreversible damage. It is well-known that in normal lenses the absence of calcium from culture medium during extended culture alters membrane permeability to cations.[12] Perhaps wound healing, to the extent that it occurs in the cryoextracted lens, is a process that requires a minimum amount of calcium in the culture medium. To test this possibility, several experiments were conducted in which the lens potentials were monitored during addition of calcium (1 mM) to the saline solution bathing cryoextracted lenses. The results showed that the presence of calcium increased the degree of recovery in lens potentials from -33 ± 5 mV to -51 ± 4 mV (n = 3) during subsequent culture at 37 for 1 hour, although no further improvement was evident.

Two conclusions regarding further experimentation with cultured human lenses emerge from the present study. First, when cryoextracted lenses are the only source available, short-term storage of lenses in calcium-free saline should be avoided in favor of a complete tissue culture medium such as TC199 to minimize the extent of irreversible damage. Second, while the observed differences in flux measurements in cryoextracted cataractous lenses and clear eye bank lenses used in this study are difficult to interpret, other physiologic information might still be obtained, provided that culture periods are limited to short durations.

Key words: cryoextraction, lens, cataract, injury, potential

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Na,K-ATPase In Simulated Eye Bank and Cryoextracted Rabbit Lenses, and Human Eye Bank Lenses and Cataracts

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In the rabbit, cryoextraction of the lens and subsequent storage in Tyrode's solution did not alter the Na,K-ATPase activity from that determined in immediately excised rabbit lenses. Similarly, the procedures employed with the rabbit eye to simulate collection and storage of normal human eyes (eye banking) had no effect upon the Na,K-ATPase activity of the lens. These results permitted the investigation of human lenses with the knowledge that measured Na,K-ATPase activity had not been altered grossly by any manipulation procedures. Analysis of Na,K-ATPase activity in 44 eye bank lenses, 14 primary nuclear cataracts, 11 primary cortical cataracts, 18 primary posterior subcapsular cataracts, and

31 mixed cataracts revealed no significant difference in the enzyme activity between these groups. Similarly, there was no correlation between electrolyte levels and Na,K-ATPase in a further 18 mixed cataracts. It is concluded that, despite an often pronounced electrolyte imbalance, human cataract can develop without significant alteration in Na,K-ATPase activity. Invest Ophthalmol Vis Sci 24:1534–1538, 1983

The electrolyte and water balance of the crystalline lens is determined largely by an active sodium pump and the passive permeability characteristics of the lens cell membranes. The characteristic cation transport enzyme, Na,K-ATPase, is resident primarily in the lens epithelium, strategically located just beneath the anterior lens capsule.[1] There is also evidence for a functional Na,K-ATPase associated with the membranes of the lens fibers lying deeper within the lens cortex.[2] Although the characteristics of the cation pump and the permeability of the lens membranes have been largely elucidated for the normal mammalian lens, the course of events that lead to derangement of lens electrolyte balance in many human cataracts is not understood. It is known that in human cataracts, involvement of the cortical region of the lens generally is associated with increased sodium and calcium levels, while the potassium level is reduced; there is a concomitant increase in lens hydration. On the other hand, pure nuclear cataracts generally exhibit minimal changes in electrolyte content and hydration is not increased.[3] The electrolyte balance of primary posterior subcapsular cataract is not well documented, but one might presume that electrolyte imbalance might be quite localized and, thus, analysis of a whole lens might not reveal gross electrolyte changes.

Human lens electrolyte imbalance in cataractogenesis could result from diminution of the active Na/K pump or from altered membrane permeability properties. Direct evaluation of membrane permeability properties in human lenses is fraught with so many difficulties that the results are necessarily equivocal. As a result, investigations have focused upon the cation pump aspect of the problem by determining the activity of Na,K-ATPase in human cataractous lenses. In certain experimental and hereditary cataracts in animals, lens opacification has been linked to a decrease in lens Na,K-ATPase activity.[4–6]

Studies of the human lens Na,K-ATPase have produced conflicting reports. Friedburg[7] reported an increase in epithelial Na,K-ATPase in senile cataractous lenses, while Gupta and Harley[8] and Nordmann and Klethi[9] reported a decrease in this enzyme. Auricchio et al[10] reported a decrease in Na,K-ATPase activity in human lenses with advanced opacities, in comparison with cortical and nuclear cataracts at an earlier stage of development. In a recent paper, Pasino and Mar-

aini[11] reported essentially no change in Na,K-ATPase activity in human senile cataract.

There are perhaps many reasons why the above studies of Na,K-ATPase activity in human senile cataract have been inconclusive. In several of the cited studies, the number of lenses examined was very small. Additionally, different methods of human lens acquisition and subsequent handling would be expected to affect the data.

The purpose of the present study was therefore twofold. Animal experiments were designed to evaluate Na,K-ATPase activity in lenses subjected to the manipulative procedures and time delay typical for the collection of eye bank lenses and cryoextracted human lenses. Na,K-ATPase also was measured in a larger population of human lenses: 44 eye bank lenses and 74 classified cataractous lenses. In a further 18 cataractous lenses, an analysis of lens electrolyte levels with respect to Na,K-ATPase activity was made.

Materials and Methods. Animal experiments: New Zealand strain albino rabbits were killed by an overdose of sodium pentobarbital. Rabbits were divided into six groups in accordance with the following procedures:

1. Immediate (control): Immediately after death the eyes were enucleated and the lens dissected from the globe by a posterior approach. The lens was then frozen in CO_2/ethanol and processed for the Na,K-ATPase assay (see below).

2. Refrigeration in situ, 4 hours: To simulate time delay in the eye banking procedure, the dead rabbit was kept at 4 C for 4 hours prior to enucleation, lens dissection, and Na,K-ATPase assay.

3. Refrigeration in situ, 8 hours: To simulate further time delay in the eye banking procedure, the dead rabbit was kept at 4 C for 8 hours prior to enucleation, lens dissection, and Na,K-ATPase assay.

4. Eye bank simulation: To mimic an entire eye banking procedure, the dead rabbit was kept at 4 C for 4 hours; the eyes then were enucleated and placed in a moist chamber at 4 C for 19 hours. The lens subsequently was dissected out, frozen, and assayed for Na,K-ATPase activity.

5. Isolated lens/storage in Tyrode's: To simulate the time delay and treatment during photography, immediately after death, the lens was dissected out and placed in 10 ml of Tyrode's solution (for composition, see below) for 2 hours at 4 C. The lens was then frozen for Na,K-ATPase assay.

6. Cryoextracted lens/storage in Tyrode's: Immediately postmortem, with the eye still in situ, the cornea and iris were removed and chymotrypsin (Catarase, 1:5000 dilution) was applied to the exposed lens for 3 minutes. The lens then was extracted using a cryoprobe, and the lens then was transferred to 10 ml of
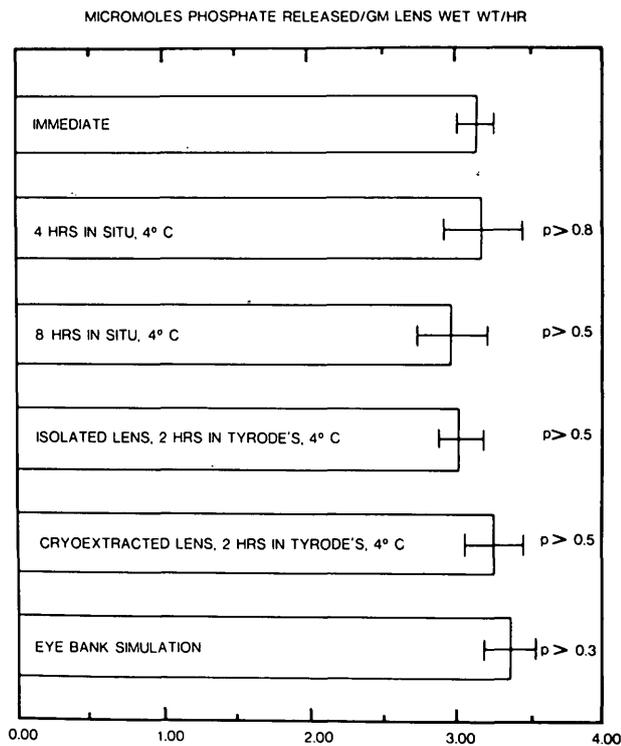
MICROMOLES PHOSPHATE RELEASED/GM LENS WET WT/HR

Fig. 1. The Na,K-ATPase activity in the rabbit lens. Lenses were processed immediately for the enzyme assay or subjected to the various manipulations as shown in the figure and described in detail in the Methods section. The bars represent the standard error of the mean of at least five experiments. There is no significant difference between the Na,K-ATPase activity in the immediately processed lenses and that in any of the other groups; P values are given on the figure.

Tyrode's solution for 2 hours at 4 C prior to freezing and assay. This procedure was designed to simulate cryoextraction and subsequent handling of the human cataractous lens.

Human lenses: Lenses were obtained from eye bank eyes made available through the Colorado Eye Bank. These eyes had been enucleated within 4 hours of death and had been stored for 4 to 48 hours prior to processing for these experiments. After dissecting the lens from the globe, the lens was placed in Tyrode's solution and photographed. The lens then was frozen for Na,K-ATPase assay. Eye bank lenses judged to have any cataractous involvement were not used in this portion of the study.

Cataractous lenses were obtained intact at the time of surgery; all lenses were cryoextracted. The lenses were placed in Tyrode's solution for up to 2 hours at 4 C, during which time they were identified by the pathologist and then photographed for classification. Subsequently, lenses were frozen for Na,K-ATPase assay.

Cataractous human lenses were classified as primary cortical, primary nuclear, or primary posterior subcapsular. Lenses that were judged to have no primary

changes were classified as mixed. Classification of lenses was achieved by examination of projected color slides by two experienced viewers who made their assessment independently. In addition, information was available from slit-lamp examination prior to surgery. Lenses with broken capsules were discarded.

Tyrode's solution: For both rabbit and human lens, the solution contained NaCl, 145 mM; KCl, 6 mM; HEPES, 10 mM; D-glucose, 5.5 mM; $CaCl_2$, 2.4 mM; and $MgCl_2$, 1.2 mM. The pH was adjusted to 7.4.

Na,K-ATPase assay: Lens Na,K-ATPase determinations were made using the colorimetric phosphate assay technique of Bonting, Simon, and Hawkins[12] as modified by Neville et al.[2] In brief, 100 μl of lens homogenate, containing approximately 20 mg of tissue were incubated for 60 minutes with 400 μl of buffer containing $MgCl_2$, 3 mM; KCN, 1 mM; KCl, 5 mM; NaCl, 116 mM; Trizma, 24 mM; EGTA, 1 mM; and ATP, 3 mM at pH 7.4. Under the conditions employed, enzyme activity was related linearly to the amount of lens homogenate used and the time of incubation. ATPase activity was expressed as micromoles of phosphate released per hour per gram wet weight of lens. Sodium-potassium-stimulated ATPase was defined as the difference between ATPase activity determined in the presence and absence of 10^{-4} M ouabain. Ouabain at 10^{-4} M was shown to maximally inhibit Na,K-ATPase. In both human and rabbit lens, the Na,K-ATPase was approximately 40% of the total ATPase activity.

Electrolyte analysis: Lenses were dried and then digested in 30% nitric acid. The lens digest was diluted appropriately for analysis of sodium, potassium, and calcium by atomic absorption spectrophotometry.
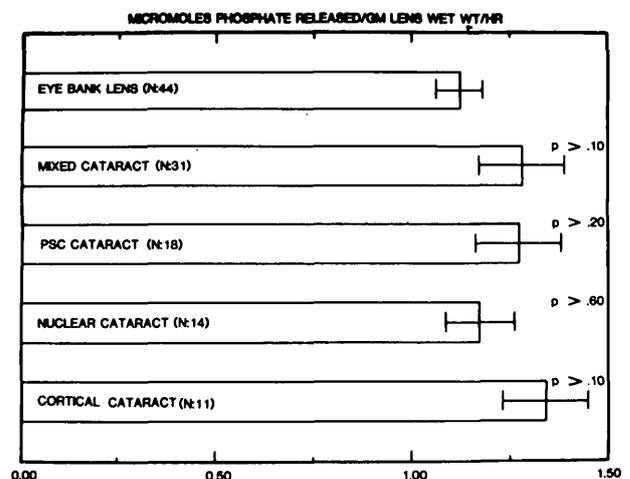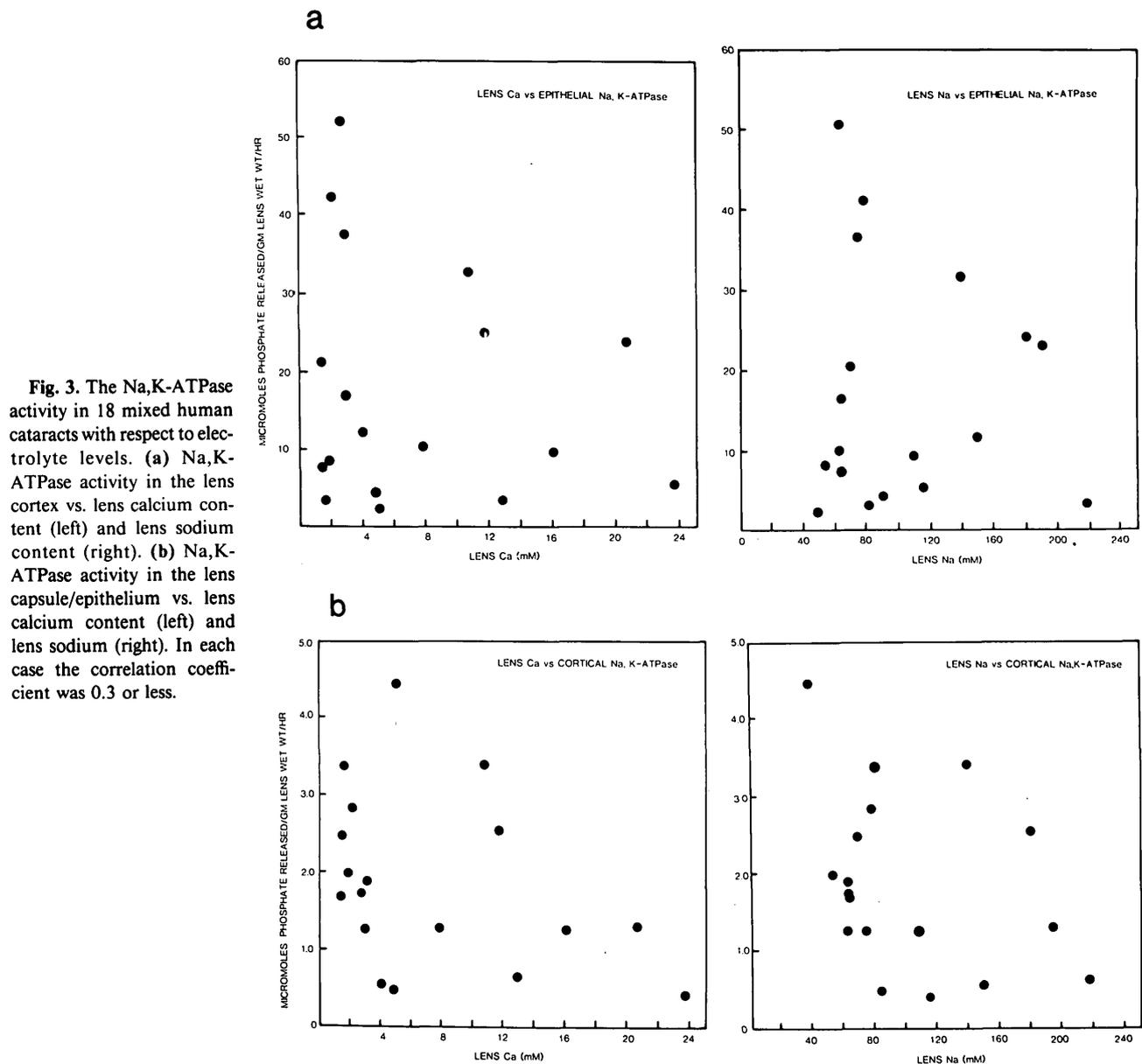

Fig. 2. The Na,K-ATPase activity in human eye bank lenses and classified human cataractous lenses. The bars represent the standard error of the mean. The number of lenses is indicated on the figure. There is no significant difference between the Na,K-ATPase in the eye bank lenses and that in any of the groups of cataractous lenses.

a

LENS Ca vs EPITHELIAL Na, K-ATPase

LENS Na vs EPITHELIAL Na, K-ATPase

b

LENS Ca vs CORTICAL Na, K-ATPase

LENS Na vs CORTICAL Na,K-ATPase

Fig. 3. The Na,K-ATPase activity in 18 mixed human cataracts with respect to electrolyte levels. (a) Na,K-ATPase activity in the lens cortex vs. lens calcium content (left) and lens sodium content (right). (b) Na,K-ATPase activity in the lens capsule/epithelium vs. lens calcium content (left) and lens sodium (right). In each case the correlation coefficient was 0.3 or less.

Results. *Na,K-ATPase activity in rabbit lenses:* The Na,K-ATPase activity in lenses dissected and processed immediately after killing the rabbit was 3.09 ± 0.15 (6) μmoles PO_4/hour/g wet weight tissue. This value was used as a control in the comparison of Na,K-ATPase activities obtained from lenses that had been subjected to a variety of manipulative procedures. Leaving the lens within the eye in situ at 4 C for 4 or 8 hours had no demonstrable effect on Na,K-ATPase activity. Similarly, the activity of this enzyme remained unchanged following the procedure to simulate eye banking.

Exposure of the freshly isolated lens to chilled Tyrode's solution for a period of 2 hours did not affect lens Na,K-ATPase activity. Likewise, the simulated cryoextraction procedure, including exposure to chymotrypsin and chilled Tyrode's solution, did not alter

the activity of this enzyme. These data are shown diagrammatically in Figure 1.

Na,K-ATPase activity in human lenses: Forty-four eye bank lenses and 74 cryoextracted cataractous lenses were assayed individually for Na,K-ATPase activity. Classification led to the identification of 14 primary nuclear, 11 primary cortical, 18 primary posterior subcapsular (PSC), and 31 mixed cataracts.

The Na,K-ATPase data generated from these lenses are given in Figure 2. The Na,K-ATPase activity of eye bank lenses was 1.12 ± 0.06 (44). None of the four categories of cataractous lenses was found to have Na,K-ATPase activity that differed significantly from the eye bank lens value. The absolute values of Na,K-ATPase activity in human lenses are within the range of previously reported values (see Introduction).

Since there was an overabundance of mixed cata-

racts, an additional series of experiments was performed with 18 such lenses. Prior to assay, a lens was separated into capsule/epithelium and two halves of cortex. Na,K-ATPase activity was measured in the capsule/epithelium and in one-half of the cortex. The other half cortex was analyzed for electrolyte content. The data obtained (Fig. 3) showed no correlation between electrolyte levels and Na,K-ATPase activity in either the capsule/epithelium or in the cortex.

Discussion. The intent of most laboratory studies on the human lens is to compare a given aspect of the normal lens with that same aspect in a cataractous lens. However, normal lenses are obtained most commonly from eye bank eyes while cataractous lenses are obtained following surgical intracapsular extraction, usually by cryoextraction. These very different procedures could potentially alter the parameter of lenticular metabolism under investigation. In previous studies of human lens Na,K-ATPase, this possibility had not been evaluated. The results of the animal experiments performed in this study demonstrate, with little doubt, that the manipulative procedures used to simulate human eye bank lens and cataractous lens collection and handling, in fact, did not significantly influence the Na,K-ATPase activity. Consequently, we were able to evaluate Na,K-ATPase activity in both human eye bank and human cataractous lenses with the cautious belief that the enzyme activity determined had not been altered grossly by the eye banking procedure or by cryoextraction and the subsequent manipulations.

The Na,K-ATPase data obtained from human lenses demonstrated no difference between eye bank lenses and any of the four cataract classification groups examined. Thus, even lenses with severe cortical opacification could not be distinguished from nuclear cataracts on the basis of Na,K-ATPase activity. This finding is in agreement with the Na,K-ATPase data of Pasino and Maraini,[11] who also demonstrated no difference between ^{86}Rb uptake in clear eye bank lenses and cataractous lenses. These authors suggested normal function of the cation pump in cataractous lenses.

It is well-known that substantial derangement of electrolyte balance accompanies cortical involvement in cataract.[3] Thus, from the present experiments, it would appear that the electrolyte balance of the lens can deteriorate without significant changes in Na,K-ATPase activity. Auricchio et al[10] similarly showed no relationship between human cataractous lens sodium levels and Na,K-ATPase activity. In the present study, also, no clear correlation was found between cortical or epithelial Na,K-ATPase and the level of sodium or calcium in the same lens. High levels of sodium are indicative of advanced osmotic type cataracts, yet the data showed no trend for reduced or increased Na,K-ATPase activity in these cases.

Since Na,K-ATPase activity appears to be unaltered in senile cataract, one might hypothesize that the primary event leading to cation imbalance is a change in lens cell membrane permeability. On the other hand, it is possible that Na,K-ATPase, although present in the cataractous lens, is unable to express itself as a cation translocation mechanism because of the influence of other biochemical changes associated with cataract. The finding of unaltered ^{86}Rb uptake in cataractous lenses[11] speaks against this latter hypothesis.

Key words: human lens, cataract, rabbit lens, Na,K-ATPase, electrolytes

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