Electrolyte Content of the Corneal Stroma of the Bullfrog[1] (34396)

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In recent years there has been an interest in ion transport mechanisms in the cornea and the relationship between these mechanisms and the maintenance of corneal transparency (1). In the bullfrog cornea, Zadunaisky (2) found an active Cl^- transport mechanism and a transcorneal potential difference (PD) with the aqueous side positive. These findings were confirmed by Ploth and Hogben (3). Candia *et al.* (4) studied the electric profile of the isolated frog cornea with microelectrodes and reported that the stroma is negative to both outer surfaces. They proposed that the active Cl^- mechanism, which transports Cl^- in the direction of aqueous to tears, is located in the endothelium and results in an increase in Cl^- in the stroma, and that the passive movement of Cl^- and other ions across the epithelium is responsible for the epithelial PD. Their suggestion stresses the need for knowledge of the ionic composition of the corneal stroma of the frog. For this and other reasons which are given in the "Discussion," we determined the Na^+, K^+, and Cl^- content of the bullfrog corneal stroma and aqueous humor and these results are presented below.

Methods. Frogs (*Rana catesbeiana* purchased from Lemberger, Oshkosh, Wisconsin, and kept at room temperature) were pithed, samples of aqueous humor were obtained, the corneas were removed and placed in tared crucibles, and the wet weights were obtained. The corneas were dried at 105° for at least 24 hr before obtaining the dry weights. In the past, determinations of Na^+, K^+, and Cl^- have not been made on the same sample of tissue. It would be of considerable advantage to be able to determine all three of these ions on the same sample of tissue. After trying a variety of procedures we found the following method to be satisfactory. After obtaining the dry weight, 0.5 ml of 4.0 *M* LiOH were added to the crucibles and the tissue was digested at 70° for 2 hr, following which 0.5 ml of 4.0 *M* HNO_3 were added and the resultant mixture was diluted to an appropriate volume with distilled water. A flame photometer (Eppendorf), not using an internal lithium standard, was used for the Na^+ and K^+ determinations. Standard K^+ and Na^+ solutions were prepared in the same way as for tissue digestion (LiOH plus HNO_3). Determinations of Na^+ and K^+ in aqueous humor were made both directly and after the addition of LiOH and HNO_3 and there was no significant difference between the two procedures. Chlorides were determined with the Cotlove chloridimeter. In some corneas the epithelium was removed by scraping it off the intact globe and the resulting stroma plus endothelium was used for the analyses. In other corneas, a Ringer's was dripped over the cornea during the scraping of the epithelium. The composition of the Ringer's was (m*M*): 100 Na^+, 4 K^+, 1 Ca^{2+}, 0.8 Mg^{2+}, 81 Cl^-, 25 HCO_3^-, and 0.8 SO_4^{2+}. Some corneas in which the epithelium was removed were fixed in neutral formalin and stained with hemotoxylin and eosin. Microscopic examination revealed that only small remnants of the epithelium remained.

Results. The results are presented in Table I. The endothelium was still present in both the intact corneas and in those with the epithelium removed. Since the endothelium is only several microns thick and represents such a small fraction of the stromal volume

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TABLE I. K^+, Na^+, and Cl^- of Cornea and Aqueous Humor.[a]

Type of expt.	$\frac{WW}{DW}$	% H_2O	K^+ (meq/kg of H_2O)	Na^+ (meq/kg of H_2O)	Cl^- (meq/kg of H_2O)
Intact corneas	6.3 ± 0.2 $n = 5$	84.1 ± 0.5 $n = 5$	37 ± 2 $n = 5$	118 ± 7 $n = 5$	86 ± 5 $n = 5$
Stromas	6.6 ± 0.2 $n = 5$	84.7 ± 0.6 $n = 5$	20 ± 1 $n = 5$	128 ± 8 $n = 5$	86 ± 4 $n = 5$
Stromas + Ringer's	8.8 ± 0.6 n = 3	88.6 ± 0.8 $n = 3$	9 ± 2 $n = 3$	121 ± 3 $n = 3$	82 ± 1 $n = 3$
Aqueous humor			2.44 ± 0.08 $n = 16$	100 ± 1 $n = 16$	82 ± 1 $n = 16$

[a] $\pm$ = Standard error of the mean; n = number of experiments; WW = wet weight; DW = dry weight; stromas = cornea with epithelium scraped off; stromas + Ringer's = corneas with Ringer's dripping over cornea during removal of epithelium.

(about 1%) its ionic content could not significantly influence the results. Therefore the corneas after removal of the epithelium would be expected to give an accurate picture of the average ionic concentration of the stroma. However, during removal of the epithelium it is possible that there would be some diffusion of ions between the "epithelial scrapings" and the stroma. Therefore experiments were performed in which Ringer's was dripped over the corneas during the scraping.

The average $[Cl^-]$ for the four sets of determinations in Table I ranged from 82 m*M* for the aqueous humor to 86 m*M*. On the basis of histological sections, the volume of the epithelium is about 10% of the total corneal volume so that if the epithelial cells contained no Cl^-, the Cl^- of the stroma (assuming no loss of Cl^- from the stroma into the epithelial scrapings) should be about 8 m*M* greater than that of the intact corneas. It is apparent that the average [Cl] of the stroma is not markedly different from that of the aqueous humor. The Na^+ data show that the average Na^+ concentration for all three sets of data on the corneas is significantly greater than that of the aqueous humor (Student's *t* test, $p<0.01$). The stromal $[Na^+]$ is about 30% greater than that of the aqueous humor. The average $[K^+]$ of the intact cornea is greater than that after removal of the epithelium and both values are considerably greater than that of the aqueous humor. During scraping of the epithelium from the cornea it is possible that some K^+ diffuses from the epithelial scrapings into the stroma and that the 20 m*M* K^+ found for the stroma is higher than the normal stromal content. In the experiments in which the Ringer's was dripped over the cornea during the removal of the epithleium the $[K^+]$ was 9 m*M*. In these experiments the WW/DW significantly increased ($p<0.01$), hence a decrease in the K^+ concentration would be expected as a result of the imbibition of Ringer's solution which contained only 4 m*M* K^+. Calculations on the assumption that no stromal K^+ is lost during the imbibition of the Ringer's solution yields a value of 12 m*M* K^+ for the stroma. Therefore the stromal $[K^+]$ lies somewhere between 12 and 20 m*M*.

Discussion. Originally Candia *et al.* (4), assumed that the Cl^- transport mechanism was located in the endothelium. Recently they have changed their position and now believe that the Cl^- mechanism is located in the epithelium (Zadunaisky, personal communications). On the basis of the present results, it would appear that there is no substantial chloride gradient between the aqueous humor and the stroma which is compatible with work from our laboratory in which we have found clear evidence that for the bullfrog the PD is zero across the endothelium [(5) and unpublished results].

In studies on the frog gastric mucosa under conditions of anoxia and with bathing media containing no K^+ we found (unpublished

results) that the $[Na^+]$ was significantly greater than that of the ambient fluids. It is possible that under these conditions Na^+ accumulates in cells and/or the $[Na^+]$ of extracellular fluid is not the same as the ambient (or not the same as plasma for *in vivo* conditions). It is not feasible in the case of the gastric mucosa to obtain significant amounts of connective tissue free of smooth muscles or mucosal cells so as a first approach to this problem we decided to study the ionic composition of the corneal stroma, a tissue that is primarily composed of connective tissue. It is of interest that the sodium concentration of the stroma is significantly higher than that of the aqueous humor. This finding indicates that the $[Na^+]$ of extracellular fluids may be higher than that of the ambient fluid and the assumption that they are equal may lead to error in estimating the intracellular $[Na^+]$ of tissues.

Summary. The Na^+, K^+, and Cl^- content of aqueous humor of the bullfrog is 100, 2.4, and 82 meq/kg of water, respectively. The $[Na^+]$ and $[K^+]$ of the corneal stroma are significantly higher than those of the aqueous humor while the Cl^- content of the stroma is essentially the same as that of the aqueous humor.

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