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Water Permeability of Normal and Pathological Lake Trout Corneas.* (31300)

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Allison(1-3) reported that lake trout are susceptible to sunburning and that they also develop corneal and lenticular lesions when reared in a hatchery environment. Hoffert and Fromm(4) have described a corneal lesion which occurs in lake trout from the Harrietta, Mich., hatchery and have categorized the symptoms of the lesion into stages. The purpose of this paper is to present data on the water permeabilities of lake trout corneas representative of the stages as described by Hoffert and Fromm(4). We also investigated the roles of the endothelial and epithelial layers of the cornea in the maintenance of the permeability characteristic of normal or non-pathological corneas.

Materials and methods. The 2- to 4-year-old lake trout *(Salvelinus namaycush)* used in these experiments were provided by the Michigan Conservation Department from their hatchery at Harrietta, Mich. In the laboratory they were kept in fiberglass lined tanks at 9°C under conditions of 15 hours light and 9 hours darkness each day.

A technique for holding trout corneas *in vitro* between 2 chambers in such a manner as to allow the fluid bathing each surface to be regulated has been described(5). In all experiments chemically defined tissue culture media (TC 199, Difco Laboratories, Detroit, Mich.) was used to bathe the endothelial surface of corneas and tap water was used to bathe the epithelial surfaces. Depending upon which direction water movement was to be investigated, 0.2 ml of tritiated water (0.1 mc HTO/ml) was added to either the 3 ml of TC 199 or the 3 ml of tap water. Four hours later, 20 lambda samples of the 2 media were taken using disposable micropipettes (Drummond Microcaps), and transferred to glass vials containing 15 ml of a liquid scintillation counting solution. That part of each cornea which was exposed to the 2 media was dissected free and also placed in 15 ml of counting solution. This solution contained a primary scintillator (5 g PPO; 2,5-diphenyloxazole), a secondary scintillator (50 mg α-naphthylphenyloxazole), and a solvent system of 80 g naphthalene plus dioxane to make 1 liter. Since dioxane is a good dehydrant it

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TABLE I. Permeability Constants, Tritiated Water Content and Hydration of Normal and Pathological Lake Trout Corneas.

Stage of disease	No. of corneas	Permeability constant ($cm^3/cm^2/hr$)	Corneal HTO content (CPM/mg)	Wet wt of cornea (mg)
Normal	6	.16 ± .02*	535	27.2 ± .9*
1	8	.20 ± .02	577	27.1 ± .6
2	9	.23 ± .03	725	29.3 ± .6
3	6	.17 ± .02	676	38.8 ± 5.1
4	3	1.46 ± .02	738	43.1 ± 8.4
1-a	8	.22 ± .02	978	25.6 ± 1.8

* Standard error of mean.

removed the water and HTO from the corneas thus enabling the activity within the corneas to be assayed. A Tricarb Liquid Scintillation Spectrometer 314A was used to assay the radioactivity in the samples. Each sample was counted 3 times for 10 minutes at a machine efficiency of 19% for tritium and the average number of counts/minute was corrected for background activity. Permeability constants (K_p) were calculated as follows:

$$\ln \frac{2C_1 - C_o}{C_o} = -2\frac{K_p A}{V} t$$

$$\text{or} \quad \frac{C_1}{C_o} = \tfrac{1}{2}\left\{1 + e^{-\frac{2K_p A}{V} t}\right\}$$

where C_1 and C_0 = final and initial concentration of tritiated water in the labelled compartment; A = surface area (0.28 cm^2); V = volume (3 ml) and t = time (4 hours).

In studies of the corneal layers, corneas were denuded with a kerotome knife and histological sections were made to verify that the epithelium and/or endothelium were completely removed by this procedure.

Results. In all experiments the solution which bathed the endothelial surfaces of the corneas was very nearly equal osmotically (about 290 mOs/l) to trout plasma whereas that bathing the epithelial surface was tap water. Thus, in every case there existed across the cornea an osmotic gradient of some 6.6 atmospheres favoring an osmotic movement of water through the cornea to the solution bathing the endothelial surface.

With the exception of the values for Stage 3 those given in Table I indicate that as the corneal lesion progresses from Stage 1 through Stage 4, there is a tendency for the permeability of the cornea to increase. Viewed in the light of previous histological work these results were not at all surprising. In all of these experiments tritiated water was placed in the epithelial or tap water compartment and permeability constants were calculated on the basis of appearance after 4 hours of HTO in the originally unlabelled compartment.

Data presented in Table II show that the movement of tritiated water in either direction across the healthy lake trout cornea is equal irrespective of the existing osmotic gradient. There was a slightly greater increase in the permeability when the epithelia were removed than when the endothelia were removed. When both layers were removed the permeabilities were significantly increased above the value for intact corneas but were not significantly different from the permeabilities of corneas with the epithelia alone removed.

TABLE II. Permeability Constants for Water Transfer and Corneal Tritiated Water Content of Normal and Denuded Lake Trout Corneas *in vitro*.

Condition of cornea	No. of data	Permeability constant ($cm^3/cm^2/hr$)	Corneal HTO content (CPM/mg)
(A. With tritiated water in epithelial compartment at start of experiment)			
Normal	6	.16 ± .02*	535
Epithelium removed	6	.52 ± .06	603
Endothelium "	6	.37 ± .02	651
Both layers "	8	.53 ± .03	827
(B. With tritiated water in endothelial compartment at start of experiment)			
Normal	5	.16 ± .01	916
Epithelium removed	6	.47 ± .03	386
Endothelium "	4	.34 ± .06	570
Both layers "	6	.53 ± .03	410

* Standard error of mean.

The tritiated water content of the corneas was determined and the concentrations of HTO in the corneas are expressed as CPM/mg of cornea. Data for normal intact corneas in the 2 experimental situations, A and B as given in Table II, indicate that the epithelium offers a greater resistance than the endothelium to entry of water into the cornea. With the epithelia removed, the A-condition corneas showed expected high concentrations of HTO. The B-condition corneas had less HTO than normal controls presumably because of a greater loss of HTO into the epithelial compartment. We are unable to explain the high HTO content of A-condition corneas with the endothelium removed. As expected, B-condition corneas with the endothelium removed had higher concentrations of HTO than those with the epithelium removed. With both layers removed the B-condition corneas had less HTO than A-condition corneas again presumably due to a greater loss of HTO into the epithelial compartment by the former. With a single exception these data are consistent with the idea that corneal water is much more freely exchangeable with water in the endothelial than with water in the epithelial compartment.

Discussion. Stage 1 of the lake trout corneal lesion as described by Hoffert and Fromm(4) is characterized by keratectasia, an increased anterior chamber and aqueous humor volume, and a possible thinning of the central area of the cornea. The movement of water across the cornea at this stage is not significantly different from that across normal corneas (Table I). Stage 2 corneas are characterized by a marked keratoconus-keratoglobus with pronounced changes in optical transparency. There are some degenerative changes in the epithelium including erosion of the superficial epithelium. It is at this stage that the effect of osmotic pressure apparently comes into play resulting in a great influx of water into the cornea causing it to become opaque. Various degrees of ulcerative keratitis characterize Stage 3. In this stage one also observes a prominent erosion and thinning of the corneal epithelium, an irregular thickening of Bowman's membrane, and a localized hyperplasia of the epithelium. The cornea shows a significant hydration and an increase in overall thickness. Values in Table I indicate that more water gets into the cornea at this stage but less gets through into the endothelial compartment, hence the permeability constant is lower than that for the preceding stage. Permeability constants for Stage 4 corneas have little meaning since these corneas are characterized as having total perforations in their central areas. Solutions in the immediate vicinity of both surfaces of Stage 4 corneas contained considerable quantities of HTO which probably accounts for the high HTO content of these corneas. Stage 1-a may be a continuation of Stage 1 or it may represent a second form of the onset of the lesion. In this stage the anterior chamber depth is normal and the cornea has a normal radius of curvature but it takes on a milk cloudiness which may be diffuse or discrete. The normal cuboidally arranged basement epithelial cells are disorganized and the epithelium does not remain attached to its base membrane. It is assumed that the increased permeability and hydration observed in Stage 1-a corneas is associated with this disruption of the basement layer of the epithelium.

We believe that as long as the epithelium and Bowman's membrane remain intact there is only a limited movement of water into or across the lake trout cornea. Some of the changes noted in the permeabilities of pathologic lake trout corneas can be explained on the basis of destruction of the anatomical barriers to water transfer which are normally present in intact, healthy tissue. It is most probable that there are alterations in the metabolism of the pathologic corneas which may also affect their water permeability. Smelser and Chen(7) report that the corneal epithelium of carp is rich in phosphatase and that this enzyme(s) may be important in the maintenance of normal corneal hydration. We have positive histochemical evidence of the presence of various enzyme systems indicating that the epithelium of lake trout corneas is quite active metabolically.

Data presented in Table II indicate that the permeability of normal lake trout corneas is the same irrespective of whether the values

are based on the transfer of tritiated water from the epithelial to the endothelial bathing media or vice versa. These results are similar to what we have previously observed with corneas of fresh-water rainbow trout. Permeability constants for both species of fish are of the same order of magnitude and are much lower than published values for corneas of terrestrial animals. Once again we are forced to believe that what we have measured represents an exchange of water across the cornea and not a net flow of water from one side to the other.

The data for mammalian corneas presented by Donn *et al*(6) are probably most comparable to those given here for trout although conditions were different in the two experiments. Their experiments with rabbit corneas were conducted at 20-25°C and the solutions (identical except for the presence of HTO in one) bathing both surfaces of the corneas were equal osmotically and were renewed by perfusion. In our procedure a small recirculated volume of fluid bathed each surface of the cornea. They applied a slight hydrostatic pressure to the endothelial surface, whereas no hydrostatic pressure difference was maintained in our experiments. In the calculations of permeability constants, neither research group took into account the specific activity of corneal water. The assumption was made in both cases that the amount of HTO which appeared in the originally unlabelled solution represented the transfer of a certain volume of water as determined by the initial radioactivity of the labelled solution.

Donn *et al*(6) reported that the movement of water across the rabbit cornea was equal in either direction. The permeability constants for intact rabbit corneas were some 300% higher (0.47 *vs* 0.16 $cm^3/cm^2/hr$) than our values for lake trout corneas. After removal of the epithelium, endothelium or both layers of the fish cornea the rate of transfer of water across the cornea increased. These denuded corneas had permeabilities comparable to those for intact rabbit corneas. Based on the permeability constants given in Table II, we have concluded that some resistance to water transfer across the fish cornea is offered by the endothelium but that the major resistance resides with the epithelium. With the exception of a single datum (A-condition, endothelium removed) the values for the tritiated water content of the corneas after removal of different layers are consistent with the above conclusion. Donn *et al*(6), on the other hand, showed that when the epithelium or the endothelium of the rabbit cornea is removed, the flux of water does not change greatly, indicating that these layers do not offer a large resistance to the passage of water.

Summary. The water permeability of lake trout corneas, *in vitro,* was found to be the same whether the data are based on the transfer of tritiated water from the epithelial to the endothelial bathing media or vice versa. This occurred despite the presence of an osmotic gradient which favored movement of water through the cornea to the solution bathing the endothelium. Permeability constants for normal and denuded corneas indicate that the endothelium offers some resistance to the transfer of water into or across the fish cornea but the major resistance to this movement is provided by the epithelial layer. Correlations between water permeability and histopathological condition of diseased corneas are noted.

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