IN VITRO CORNEAL ION AND WATER MOVEMENT IN THE RAINBOW TROUT, SALMO GAIRDNERI

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ABSTRACT

IN VITRO CORNEAL ION AND WATER MOVEMENT IN THE RAINBOW TROUT, SALMO GAIRDNERI

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Cation concentration and water content were determined in the normal rainbow trout cornea. Mean values for sodium were 108.9 ± 3.5 mEq/l, and for potassium, 5.8 ± 0.6 mEq/l, while water content was 80.1 percent. Anatomically, mammalian and teleost corneas were compared; rainbow trout corneas possessed a thicker epithelium and a very predominant Bowman's membrane, while Descemet's membrane and endothelium were very vestigial.

An in vitro recirculation method was used to measure ion and water movement across the cornea in various osmotic gradients. The in vitro cornea is slightly permeable to both water and ions, probably related to the anatomical makeup of the cornea. Corneal ion concentration varied inversely with corneal hydration, and data indicated that chloride ions behaved in a passive manner.

The teleost cornea possesses all of the characteristics of a metabolically active system, suggesting a sodium pump to help maintain proper ionic composition of the interstitial fluid bathing the collagen fibers of the stroma.

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By

Henry Francis Edelhauser

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Whatever contribution this thesis makes toward man's scientific knowledge is dedicated to my beloved father, the late Henry F. Edelhauser, Sr., whose guidance throughout life has made this work a reality.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

APPENDIX

INTRODUCTION

Since the control of the sea lamprey in 1957, the production of lake trout (Salvelinus namaycush) has increased in Michigan hatcheries. Concurrently with this increased rearing of lake trout, diseases have become evident. One of these resembles the development of a cataract and results in permanent damage to the eye (Allison, 1963). The pathological progression of this disease is: keratoconus and/or keratitis, keratomelasia, rupture, and fibrotic infiltration. Changes in permeability and hydration of the cornea could be important early symptoms of this disease.

The subject of swelling and transparency of aquatic corneas has received little attention. Physiologically, the fish cornea is of interest since ecological distribution of aquatic vertebrates have necessitated adaptations to waters of extremely varied osmotic and ionic concentration. The cornea of the fresh water fish is constantly in contact with a hypotonic solution, whereas sea water is hypertonic to tissue fluids of marine fish.

The cornea of the teleost is unique for physiological investigation because its area is remote from a blood supply and because its surfaces are readily accessible. Thus, the cornea is well suited for in vitro investigation of salt and water permeability. It is the movement of water and ions across corneal boundaries which determine whether the cornea will be clear or opaque.

The study of the fresh water teleost cornea may uncover new mechanisms which will aid in understanding mammalian corneal physiology and establish normal values in which the diseased teleost cornea can be compared.

The basic purpose of this study was to investigate the response of the fresh water teleost cornea in vitro to different osmotic conditions. The recirculating apparatus utilized in the experiment provided an excellent opportunity to study in vitro corneal permeability to water and salts. The aspects of corneal physiology considered were: permeability and concentration within the cornea of sodium, potassium and chloride ions as well as water in various osmotic gradients, the effect of corneal hydration, and the anatomical localization of regions in the cornea which act as barriers to the movement of these substances.

MORPHOLOGY AND ANATOMY OF THE TELEOST CORNEA

Basically the teleost cornea resembles those of other vertebrates (Figure 1) and can be divided into five layers (Figure 1--top to bottom): (1) epithelium, (2) Bowman's membrane, (3) stroma, (4) Descemet's membrane, and (5) endothelium. Figure 2 illustrates the cornea of a rabbit which structurally looks quite similar to the trout cornea; however, there are differences, and this differentiation is one of the factors distinguishing corneas whose epithelium is in contact with air from those exposed to water. The essential differences are: (a) relative cell sizes in the corneal epithelium of the teleost are larger and the cells are more compact than those in the mammal; (b) there is a well-developed Bowman's membrane in the teleost, whereas in the rabbit it is not apparent; and (c) no Descemet's membrane or a continuous well-developed endothelium could be demonstrated in the teleost such as occurs in the mammal.

Smelser and Chen (1954) and Smelser (1962) reported that Bowman's membrane was apparently absent in the carp but well developed in the primitive elasmobranch. In rainbow trout, Salmo gairdneri (Figure 3), the epithelium consists of many tightly packed cells, and Bowman's membrane is well developed. Even though Bowman's membrane is considered to be modified stroma, it may aid in maintaining transparency of the aquatic cornea by inhibiting the influx of water.

The stroma makes up most of the thickness of the cornea in both teleosts and mammals. It is divided into sheets of collagenous material with the stromal lamellae lying parallel to the surface. The gaps in the lamellae in Figures 1, 2, and 3 are fixation artifacts.

Figure 1

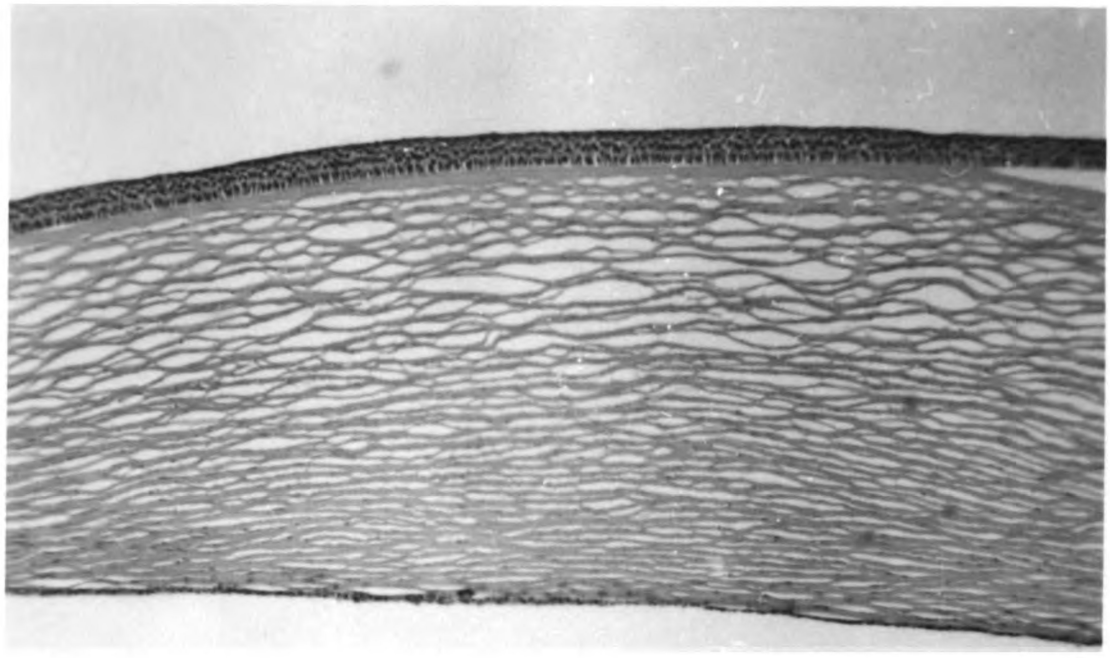
Rainbow Trout Cornea XS

(H X E 50 X)

Figure 2

Mammalian Cornea XS

(H X E 50 X)



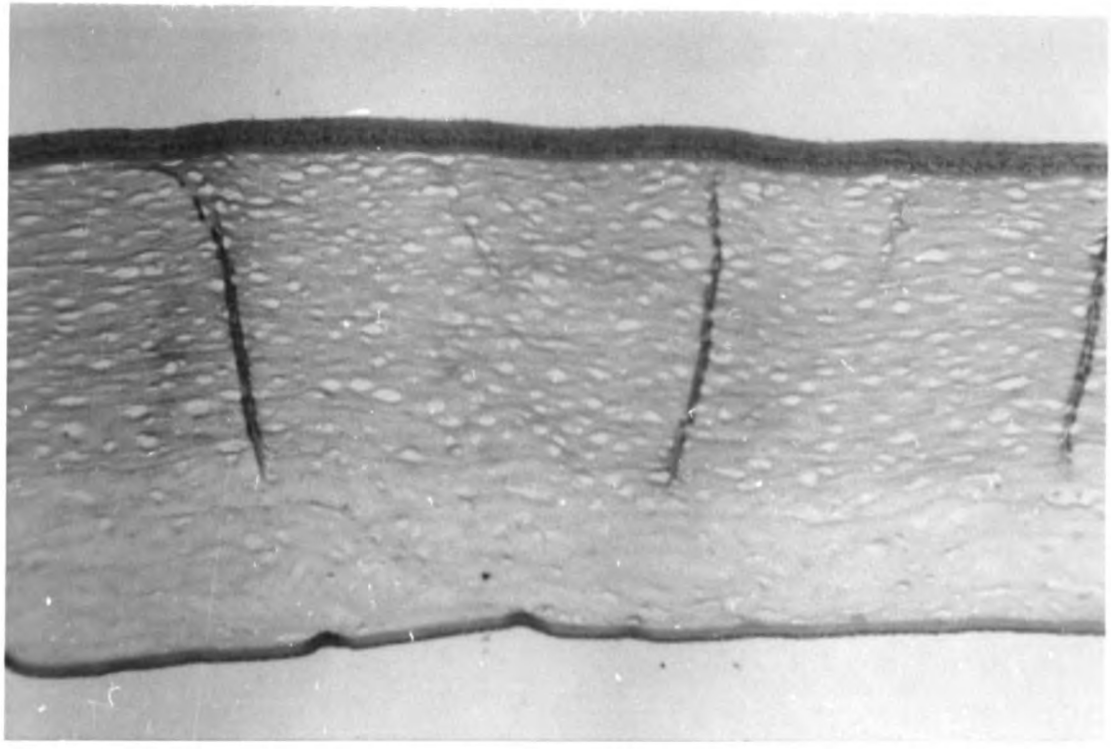
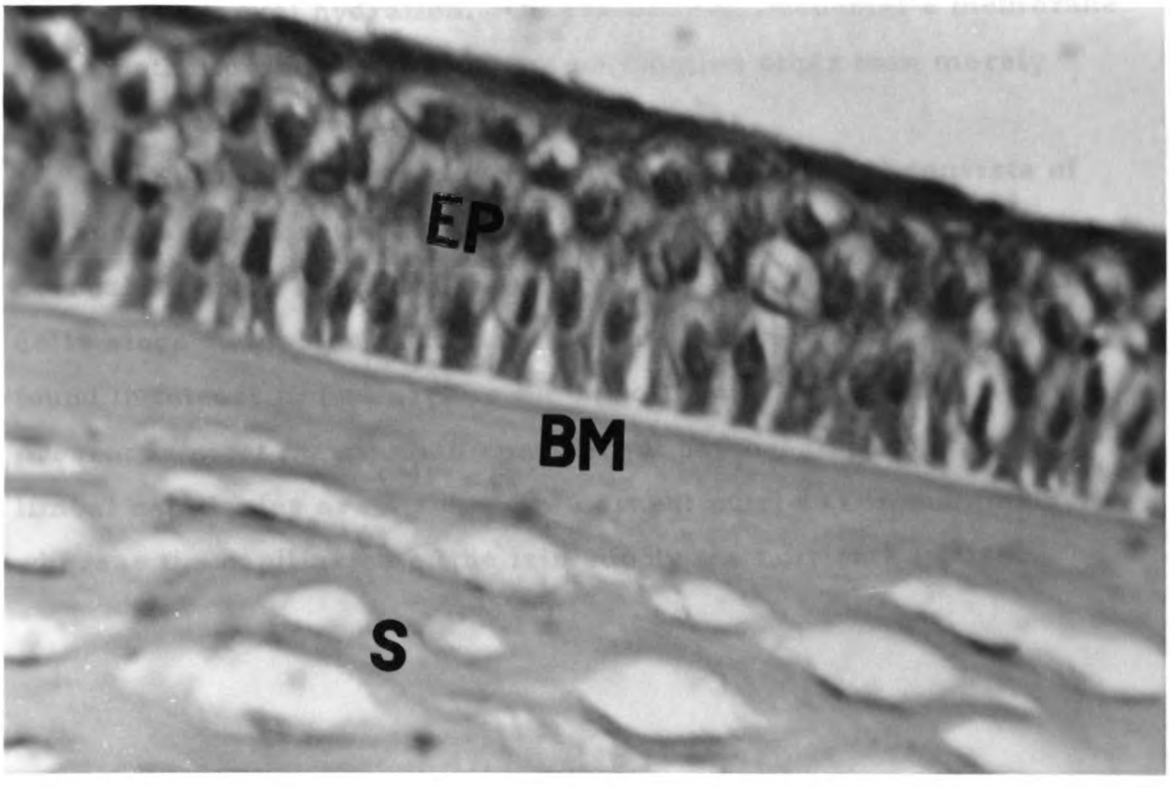


Figure 3

Cross Section Rainbow Trout

Epithelium, ep; Bowman's membrane, bm; Stroma, s.

(H X E 400 X)


EP
BM
S

Descemet's membrane, which is so prominent in mammalian species but not always present in teleosts, lies on the inner surface of the stroma bounded by the endothelium internally. It is the endothelium in mammalian species according to Donn (1962) which holds the key to corneal hydration. Others believe Descemet's membrane and endothelium in the teleost has no function other than merely separating the cornea from aqueous humor.

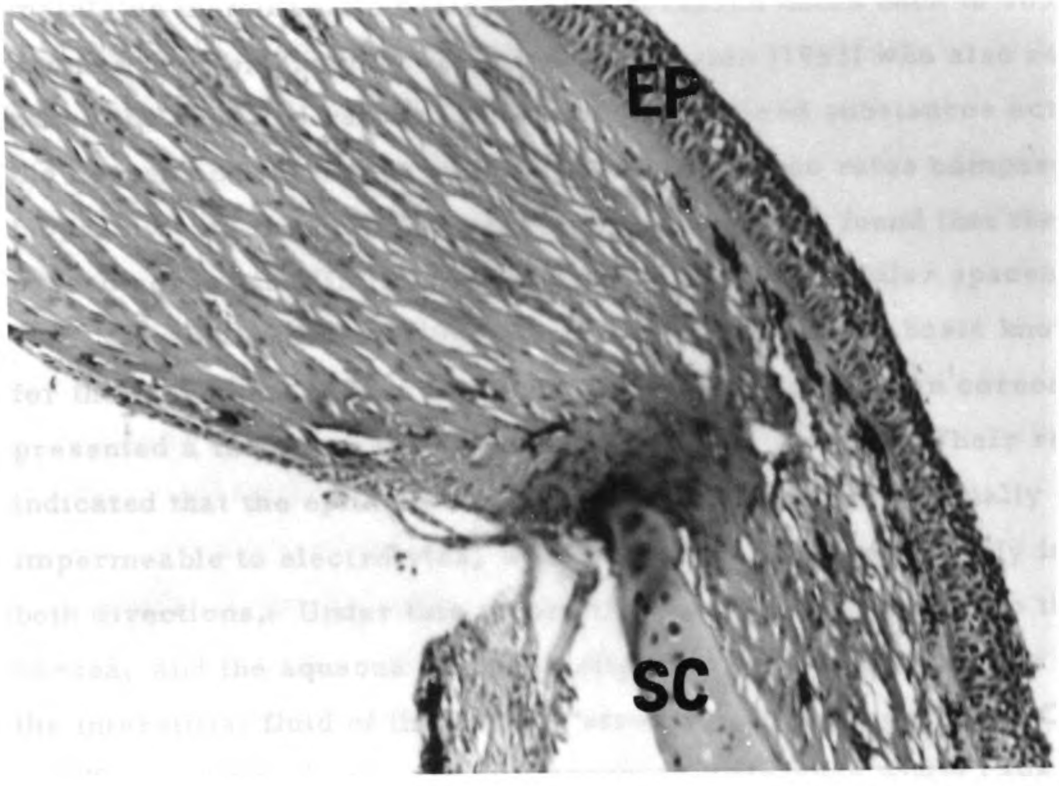
The limbal area or peripheral cornea (Figure 4) consists of the annular ligament, cartilaginous sclera, and the outer and inner corneal layers. The outer corneal layer consists mainly of epithelial cells since Bowman's membrane tapers to nothing. One feature found in teleost limbal area is the lack of capillaries which are present in most mammalian species. It has been speculated that limbal capillaries are a source of corneal nutrients in mammals, whereas the capillaries of the iris supply the nutrients in fish.

Figure 4

Limbal area, Rainbow Trout Cornea

sclera, sc; epithelium, ep.

(H X E 100 X)


EP
SC

LITERATURE REVIEW

The literature on the permeability of the cornea has focused mainly on mammalian species. This research dates back to 1853 and has been extensively reviewed by Maurice (1953) who also compared the reported rates of penetration of ionized substances across the cornea and its layers with that of Na^{24}. These rates compared favorably with the previous research; however, he found that the ions passed across the limiting membranes via extra-cellular spaces.

Cogan and Kinsey (1942a) correlated most of the basic knowledge for the movement of ions and hydration of the mammalian cornea and presented a theory on the deturgescence of the cornea. Their results indicated that the epithelium and the endothelium were virtually impermeable to electrolytes, whereas water could pass freely in both directions. Under this theory the tears are hypertonic to the cornea, and the aqueous humor is slightly hypertonic to plasma and the interstitial fluid of the corneal stroma. Thus according to Cogan and Kinsey (1942a), the osmotic pressure difference would cause water to be constantly drawn out of the cornea. Permeability was also analyzed by Cogan and Kinsey (1942b) by scraping away the various limiting layers (epithelium and endothelium) of the cornea. Hence, permeability was determined for each of the corneal layers.

Many of the early investigations on corneal permeability have been of little significant value since the experimental conditions were poorly regulated (Maurice, 1953). Permeability criteria have utilized one of the following techniques (Davson, 1962); (a) applying the test substance to the epithelium surface, if there is an increase in the concentration of this substance in the stroma and aqueous humor

penetration had occurred. Criticisms of this technique are that the epithelium cells may be damaged, thereby yielding an increase in permeability or the limbal circulation may remove the test substance; (b) injecting the test substance into the blood and tracing the migration of this substance through the aqueous humor, stroma, and epithelial surface of the cornea; (c) injecting a test substance into the stroma and measuring the amount in the aqueous humor. This method may lead to possible endothelium damage. Potts et al. (1954) found little difference in the amount of Na^{24} in the aqueous humor if the layer was intact or not; (d) in vitro techniques utilized by Klein et al. (1938) and Cogan and Kinsey (1942b) where excised corneas were placed between two solutions and water and ion movement determined.

Donn et al. (1959) devised a method whereby excised mammalian corneas would yield values for the permeability of the limiting layers of the cornea comparable to those found in vivo. This in vitro technique was more versatile than using the intact eye since both corneal surfaces are freely accessible. With this technique Donn et al. (1959) suggested the occurrence of active transport of sodium across the epithelium. They also determined that the electrical resistance of the corneal epithelium was around 5,000 ohms/cm^2.

Recently Donn (1962) reviewed data on the movement of solutes and water across the corneal boundaries, concluding that the stroma was the main corneal barrier. At the same time Donn et al. (1962) used tritiated water as a tracer and found that the amount of water moving across the cornea was the same, whether the flux was measured from aqueous humor to tears or from tears to aqueous humor.

Schwartz et al. (1954), Davson (1955), Harris and Nordquist (1955), and Langham and Taylor (1956) implied that if metabolism was impaired in the mammalian cornea, swelling or hydration resulted. Hence, the

dehydrating mechanism was one of active transport of water across the external cell layers rather than passive osmosis. Specifically, water is probably pumped out of the cornea or drawn out osmotically by the tears or aqueous humor.

Dohlman and Anseth (1957) concluded that the dehydrating forces were of importance in the maintenance of normal corneal hydration. Later Dohlman et al. (1962) indicated that it was the normal tendency of the corneal stroma to swell; therefore, the swelling pressure was bearing outward on the limited membranes. Swelling pressure energy of the cornea can be measured as the sum of the activity of stromal polyelectrolytes and polysaccharides. The corneal stroma is normally in the compressed state, maintained by a negative fluid pressure controlled by the active transport of water and ions across the cellular layers. Anseth and Dohlman (1957) suggested that intraocular pressure acting against the cornea would balance to a certain extent its natural swelling pressure.

In view of the evidence presented that corneal hydration is linked with metabolism, Harris (1957) suggested the idea of a water pump within the corneal tissue which maintains corneal hydration. Normal dehydration states of the cornea result from an active transport of water from the cornea in equilibrium with the migration of cations to or from the cornea via passive diffusion. Recently Harris (1962) correlated the factors influencing corneal hydration and investigated these factors in three different experimental situations: (a) hydration of excised pieces of cornea; (b) corneal hydration in the isolated but intact eye maintained in a moist chamber; and (c) corneal swelling in situ.

Evidence indicates that corneal hydration is prevented by the movement of fluid (water and ions) across the endothelium, but what constitutents of this fluid are actively secreted, remains to be answered.

Potts (1962) summarized the concept of corneal hydration and corneal transparency, concluding that results in this area still await the synthesizer.

Mammalian corneal physiology has created much interest due to its clinical application. On the other hand, aquatic corneal physiology has generated very little interest. Wall (1942), Smelser and Chen (1954), and Varbec (1959) have mainly studied the teleost cornea from an anatomical point of view.

Smelser (1962) was the first to indicate that probing into lower animals, the teleosts, marine and fresh water, plus the elasmobranchii, might give rise to information to aid in understanding mammalian corneal hydration and transparency. The corneas he studied with respect to corneal hydration led him to the conclusion that the system which keeps the teleost cornea dehydrated and transparent is passive. This probably depends upon the colloid-osmotic pressure of the aqueous humor rather than by active pumping, dependent upon the metabolic activity of the epithelium or endothelium as is typical of the mammal. The elasmobranch cornea behaved differently from either of the teleosts and mammals. It is not hydrophilic, and was not found to swell in any of the media studied.

MATERIALS AND METHODS

Experimental Animals

Rainbow trout, Salmo gairdneri, were selected as experimental animals because they are closely related to the lake trout, Salvelinus namaycush, which are affected with this eye disease. Rainbow trout, available from the Michigan Department of Conservation Wolf Lake Hatchery, ranging from 7 to 12 inches (2-3 years old), were transported from the hatchery in a galvanized metal tank lined with non-toxic paint and fitted with an agitator for aeration. They were held in a constant temperature room in 50-gallon fiberglass-lined wooden troughs under continuous illumination at 12 $\pm$ 2^{o} C. and fed commercial trout pellets ad libitum. Water was changed tri-weekly and aerated 24 hours before fish were placed therein. It was found that approximately one week was required for the trout to adjust to this new environment and resume normal feeding.

Principles of Method

An in vitro method was devised, which permitted serial samples to be taken from the solutions bathing both surfaces of an excised cornea without disrupting the cornea itself. At the end of the investigation the exposed cornea was still intact, enabling final analysis of the tissue.

Description of Apparatus

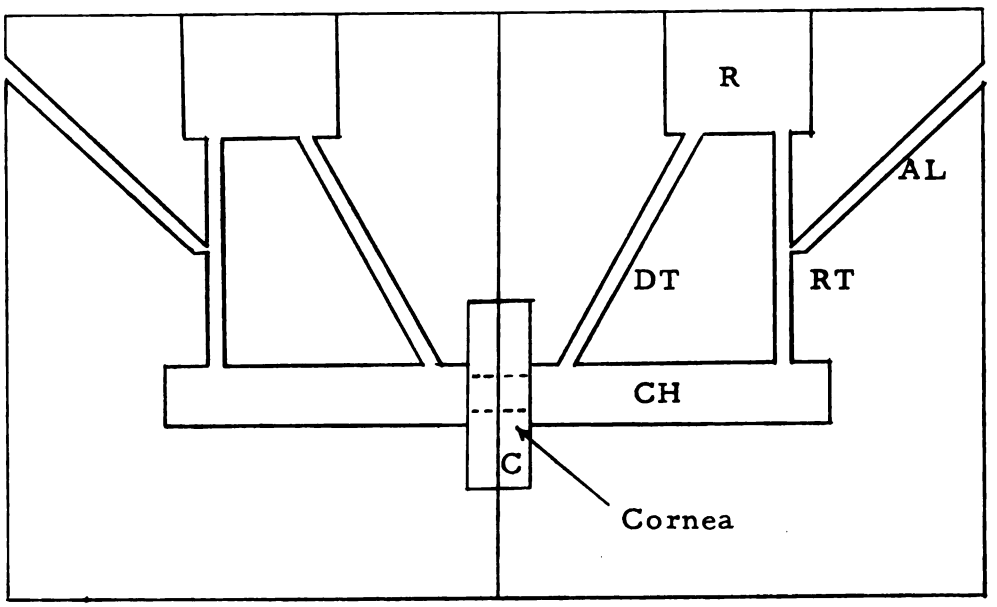
The clamps to hold the excised trout cornea (Figure 5) without slipping were constructed of either stainless steel or lucite. By tightening the six screws evenly, equal tension could be maintained around

the perphery of the cornea. The clamps had a lumen diameter of 6 mm, providing an exposed surface area of 28.3 mm^2. Histological sections of the previously clamped corneas (Figure 6) show that clamping caused little damage to the central areas. (Note normal epithelium, stroma, and endothelium, and compare this with the area that has been compressed with the clamp.) By clamping the cornea in this manner, only the thin, central portion is exposed for the permeability studies, and the thick perpheral portion is clamped.

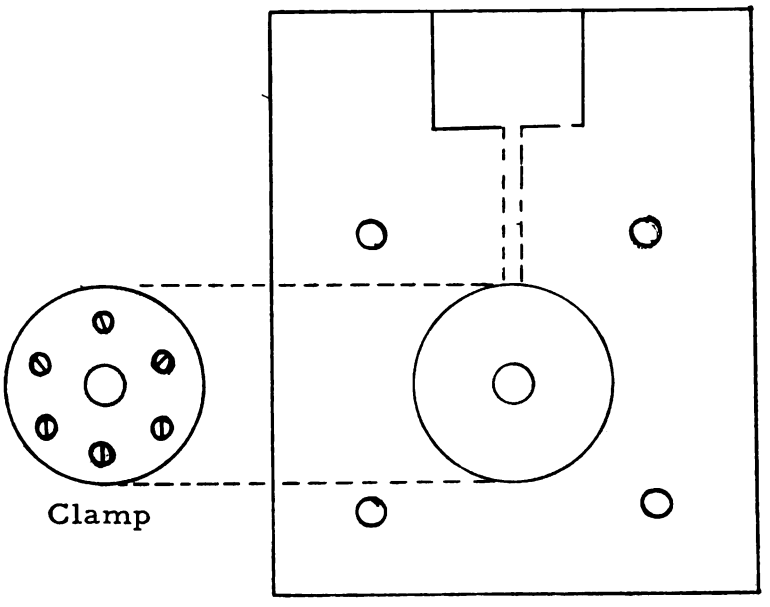
Two lucite blocks, each with a recirculation network, were designed to hold the clamped cornea in a leak-free system (Figure 5). The minimal recirculation fluid volume was 2.0 ml.; maximal volume, 5.0 ml. Each clamped cornea was placed between the blocks, and the blocks were bolted together, media added, and recirculation was via an air lift siphon. The air lift siphon was operated by a gas mixture (compressed air and 95% oxygen and 5% carbon dioxide). This gas was mixed in a wash bottle converting dry gas to moist gas. The pH was maintained by regulating the amount of compressed air and 95% oxygen and 5% carbon dioxide. Phenyl red was used as the indicator. It was found that 95% oxygen and 5% carbon dioxide at 13° C. could not be used due to the high solubility of CO_2 at such a low temperature. The pH of the media was maintained at 7.0 by diluting the gas mixture with air. This air lift siphon provided adequate circulation and mixing of the bathing media.

Temperature of the circulating fluids, blocks, and cornea were maintained at 13° C. by conducting the experiments in a constant temperature room. Caps on the reservoir helped reduce evaporation losses.

Figure 5. Corneal clamp and recirculation apparatus for trout cornea (actual size).



Side View



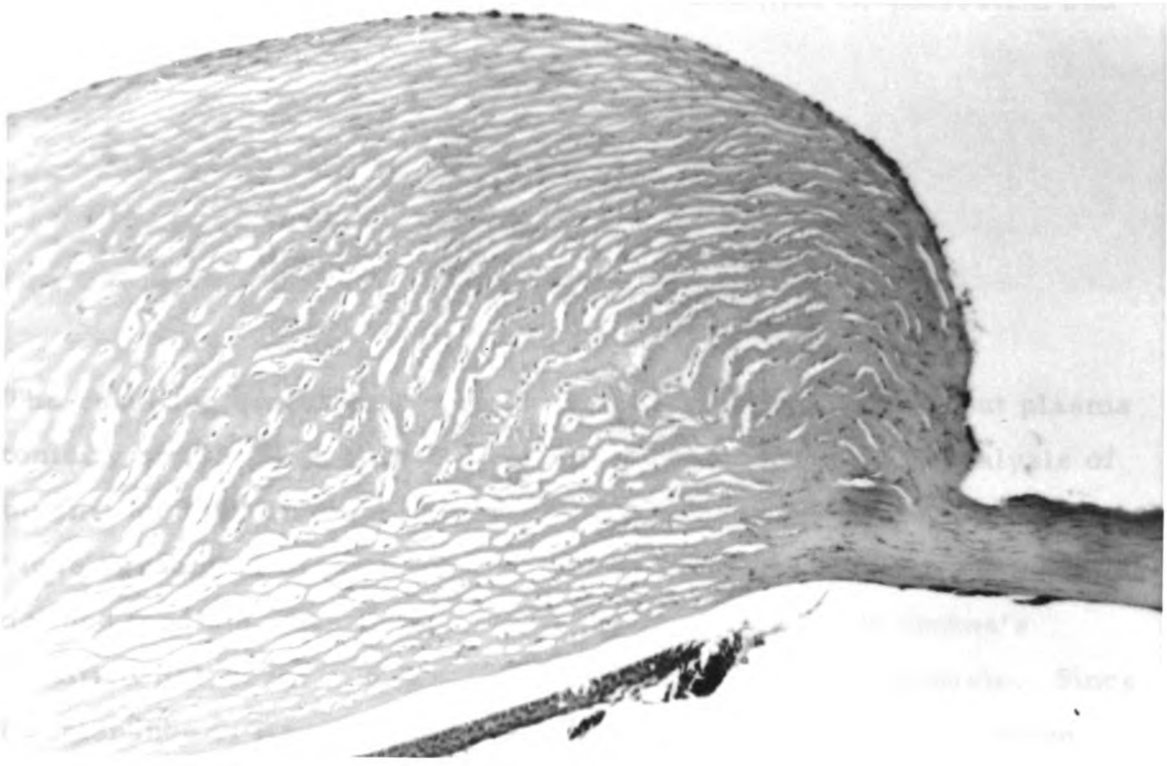
End View

R = Reservoir
CH = Chamber
DT = Delivery Tube
RT = Recovery Tube
AL = Air Line
C = Clamp

Figure 6

Rainbow Trout Cornea Clamped

(H X E 50 X)



Tissue and Experimental Media

Chemically defined tissue culture medium 199 without phenyl red (Difco Laboratories, Detroit, Michigan) was used to simulate aqueous humor. To each 100 ml. of 199 medium 0.5 ml. of a 1% phenyl red solution was added plus 50 IU penicillin G. potassium and 0.1 mg. streptomycin.

199 medium

mOs/l	272 ± 1 (SE)
Na	131 mEq/l
K	5.4 mEq/l
Cl	132 mEq/l

The resulting osmolarity compared closely with rainbow trout plasma tonicity of 293 (SD ± 5) mOs/l (n=6). Flame photometric analysis of plasma and 199 medium Na^+, K^+, and Cl^- concentrations also indicated close agreement of the principal solute content. Although 199 chemically defined medium was slightly hypoosmotic (5 mOs/l) to Stokes's Modified Rainbow Trout Medium (1962), it was more complete. Since maintenance of tissue viability was the main concern, 199 medium was selected in preference to Ringers' solution. The other solutions used were: (a) 400 mOs/l sodium chloride solution (12.702 gms/l, H_2O); (b) aerated tap water (7-8 mOs/l); and (c) deionized water.

Preparations and Procedure

All preparations were conducted at a constant temperature. The trout were immobilized by a blow on the head, weighed, and corneas excised by cutting around the limbal area.

Immediately after the corneas were excised, they were placed in the clamp, which were mounted in the blocks; next the air lines were connected, and bathing fluids added. The fluids bathing both sides of the cornea were as follows:

Expt. No.	Nature of Fluid Bathing: Epithelial Surface	Endothelial Surface	Symbol
I	Tap water	TC 199 medium	O_T/Im_T
II	TC 199 medium	TC 199 medium	Om/Im
III	400 mOs NaCl sol.	TC 199 medium	O_S/Im_S
IV	Deionized water	TC 199 medium	O_D/Im_D

In experiments I and II, the volume of circulating fluid was 3.5 ml., whereas 4.5 ml. were used for experiments III and IV. Volumes small enough to indicate changes of solute concentrations are desirable as long as they are large enough to permit serial sampling and maintenance of a minimal circulating volume of 2.0 ml. The volume of the bathing fluids were initially measured with a syringe. A needle attached to the syringe was placed into the chamber via the delivery tube. This eliminated trapped air in the system. Prior to experimentation the bathing fluid was allowed to equilibrate to experimental temperature. The TC 199 medium bathing the endothelial surface was maintained at pH 7.0 with the gas mixture.

Compressed air was used to circulate the fluid bathing the epithelial surface, although this fluid was slightly alkaline to the inside surface, it simulated the environment of the trout. These air flows were used to activate an air lift siphon and maintain a constant concentration of dissolved gases.

From the time of clamping the cornea until recirculation within the blocks, approximately ten minutes elapsed. Time zero was recorded when the recirculation actually started.

In each experiment serial samples of 1/4 ml. were taken at the first and second hour, then at two-hour intervals up to eight hours. At the end of eight hours, the exposed surface of the cornea was weighed, dried, and tissue water determined. Finally, after ashing, cornea Na^+ and K^+ were determined. Serial samples of bathing fluids

were placed in glass vials and immediately stored in the freezer for future analysis of mOs, Na^+, K^+, and Cl^-.

Analytical Determinations

The corneal section for analysis was weighed and then dried at 95° - 100° C. to constant weight (48 hours). Percent dry weight was determined as follows: $\frac{\text{dry wt.}}{\text{wet wt.}} \times 100 = \%$ dry wt. After ashing in a muffle furnace at 600° C. for 2 1/2 hours, a 0.02% sterox SE in deionized water (Hartman-Leddar Co., Philadelphia, Pa.) solution was added to the ash to equal initial wet weight of the corneal section; then a 1:100 dilution was made using 0.02% sterox SE. The analysis reported here was of the entire corneal tissue, including both cellular and noncellular elements and data are expressed as cation concentration (mEq) per liter of corneal water.

The sodium and potassium ion content of bathing fluids and corneal tissue were measured on the Coleman model 21 flame photometer with a direct-reading scale. Standards were made from stock reagents (Hartman-Leddar Co., Philadelphia, Pa.) and diluted with deionized water plus sterox SE, yielding the final standard concentrations, 150 mEq Na^+/l and 5 mEq K^+/l in 0.02% sterox SE. Chloride determinations were made by the Schales and Schales (1941) method as modified by the Coleman ultramicro analytical program (Coleman Instruments, Inc., Maywood, Illinois).

RESULTS

Cation Concentration, Water Content, and Osmotic Gradients of the Normal Rainbow Trout Cornea

In Figure 7 the normal cation concentration (mEq/l) and water content of the cornea (assuming 1 gm. of tissue equals 1 ml.), aqueous humor, and lens of the rainbow trout eye are given with indicated standard errors. Values for corneal Na^+ and K^+ represent the mean of 16 determinations each and those for tissue water, aqueous humor, and lens are 31, 12, and 14, respectively. (Hoffert, 1964)

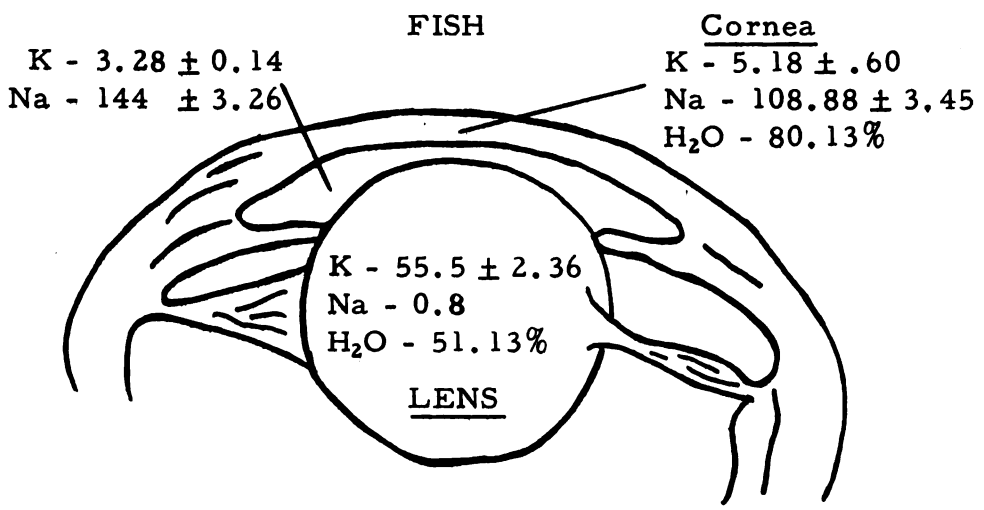


Figure 7. Normal cation concentration and water content of the cornea, lens, and aqueous of fish eyes with indicated standard errors.

These values compare favorably with those for mammalian corneas given by Harris (1955), who states that the cornea is primarily

a sodium tissue. The mammalian cornea is relatively isotonic to the fluids bathing the epithelium and endothelium, whereas in the teleosts tremendous chemical and osmotic gradients exist across the cornea.

For accurate comparisons of the ion and water movement within the cornea and bathing media, data is expressed as percent increase or decrease from the control values (statistical comparisons were calculated using the student's t test). For complete data the reader is referred to the Appendix. In order to obtain a meaningful picture of ion and water movement, one must consider simultaneously changes in all the parameters at one time which include potassium, sodium, water, and osmotic concentrations of the bathing fluids and cornea. It is in this way that a net ionic flux can be demonstrated. One point that should be emphasized is that the volume of the cornea is minute compared to the volume of the bathing fluids used for analysis; consequently, a small change in the concentration of ionic and osmotic concentrations of the bathing fluids will yield large changes in corneal values.

I. Tap Water: O_T/Im_T

(Table I, Figure 8)

Corneal hydration occurred rapidly during the first hour resulting in a decrease in the concentration of corneal potassium and sodium. Following this initial change corneal ionic concentration varied inversely with corneal hydration. The osmolarity of the endothelial media decreased whereas that of the tap water bathing the epithelium increased.

Table I. Experiment: O_T/Im_T. Water, Cation and Anion Movement Expressed as Percent Increase or Decrease from Control

	CORNEA			MEDIA						mOs/l	
TIME	WATER	Na^+	K^+	Im_T Na^T	O_T Na^T	Im_T K^T	O_T K^T	Im_T Cl^T	O_T Cl^T	Im_T	O_T
Control 0hr	80.15%	108.8	5.18	30.39	0.128	1.535	0.016	37.16	0.317	272	6.2
1hr	+8.36***	-17.28	-26.06	-1.59	+62.79	-1.24	+25.0	-2.56	+19.87		
2hr	+5.86*	-9.93	-2.32	-1.93***	+194.57	-1.37	+93.75	-2.56	+79.81		
4hr	+4.05	-5.79	+72.78*	-1.74*	+318.6	-0.59	+156.25	-1.10	+142.9***		
6hr	+5.55***	-20.04**	-24.71	-1.35*	+403.88	-0.78	+212.50	-2.21	+196.5***		
8hr	+7.49***	-35.20***	-77.80**	-1.12*	+489.15	+0.52	+300.0	-1.96	+250.16***	266	12.4

Media controls expressed as mEq/gm tissue water
\+ increase percent from control
\- decrease percent from control

* Significant from control at the 10% level
** Significant from control at the 5% level
*** Significant from control at the 1% level

Above data is plotted in Figure 8; each point represents an average of at least six determinations.

Figure 8

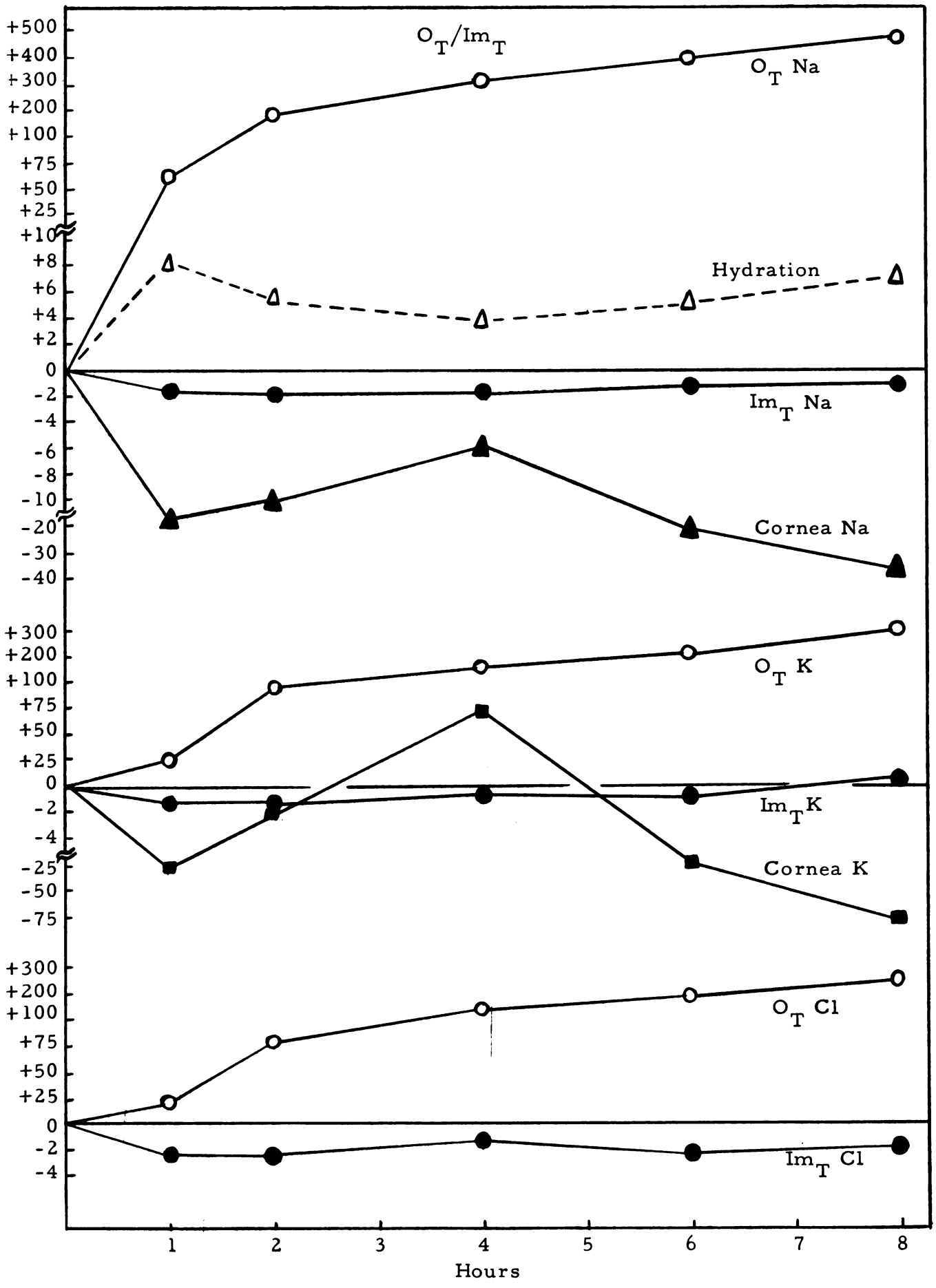
Water and Ion Movement

Expressed as Percent Change

Experiment: O_T/Im_T

(Scale at Left is Percent Increase

and Decrease From Control)


+500
+400
+300
+200
+100
+75
+50
+25
+10
+8
+6
+4
+2
0
-2
-4
-6
-8
-10
-20
-30
-40
O_T/Im_T
O_T Na
Hydration
Im_T Na
Cornea Na
+300
+200
+100
+75
+50
+25
0
-2
-4
-25
-50
-75
O_T K
Im_T K
Cornea K
+300
+200
+100
+75
+50
+25
0
-2
-4
O_T Cl
Im_T Cl
1
2
3
4
5
6
7
8
Hours

II. 199 Medium: Om/Im

(Table II, Figure 9)

Hydration of the cornea in this experimental series showed a gradual increase with time to 5 percent above normal at the end of eight hours. Concentration of corneal Na^+ and K^+ decreased initially during the period of equilibrium and increased until the greatest influx of water (4–5 hours) after which they decreased below normal. In the bathing media Na^+, K^+, and Cl^- decreased initially and remained below normal throughout the experiment. Thus, there was a steady influx of ions and water from the media into the cornea, except for the potassium on the inside media. After four hours it increased above normal and remained there. This may well be the critical point at this experimental hour which indicates tissue break-down. Following this tissue break-down all corneal ionic concentrations decrease relatively as a result of hydration.

III. Deionized Water: O_D/Im_D

(Table III, Figure 10)

Although the chemical and osmotic gradients are similar to those in the experiment with tap water, slight differences in the results were observed. Osmolarity of the endothelial medium decreased, and it increased in the deionized water bathing the epithelium. The speed and magnitude of ion changes in the media bathing the endothelium was much greater than in the experiment with tap water. However, sodium movement from the cornea tissue was only slightly detectable by sample analysis.

Table II. Experiment: Om/Im. Water, Cation and Anion Movement Expressed as Percent Increase or Decrease from Control

	CORNEA			MEDIA						mOs/l	
TIME	WATER	Na^+	K^+	Im Na	Om Na	Im K	Om K	Im Cl	Om Cl	Im	Om
Control 0hr	80.15%	108.8	5.18	38.39		1.535		37.16		272	272
1hr	+1.48	-5.14	-29.92	-2.13	-2.37**	-2.54	-2.86	-0.01	-4.57**		
2hr	+2.48*	+19.11**	+106.6***	-2.32***	-2.91***	-1.82	-1.63	+0.40	-4.04*		
4hr	+3.23**	+16.72*	+94.01***	-1.66	-1.43*	+3.65	-1.43	-1.68	-3.39**		
6hr	+7.45***	-10.38	-26.64	-1.5	-1.3**	+2.93	-1.49	-0.27	-4.06**		
8hr	+5.09***	-19.11	-65.63**	-0.8	-1.27	+3.64	-1.17	-1.83	-5.06***	274.4	268.5

Media controls expressed as mEq/gm tissue water
\+ increase percent from control
\- decrease percent from control

* Significant from control at the 10% level
** Significant from control at the 5% level
*** Significant from control at the 1% level

Above data is plotted in Figure 9; each point represents an average of at least six determinations.

Figure 9

Water and Ion Movement

Expressed as Percent Change

Experiment: Om/Im

(Scale at Left is Percent Increase

and Decrease From Control)

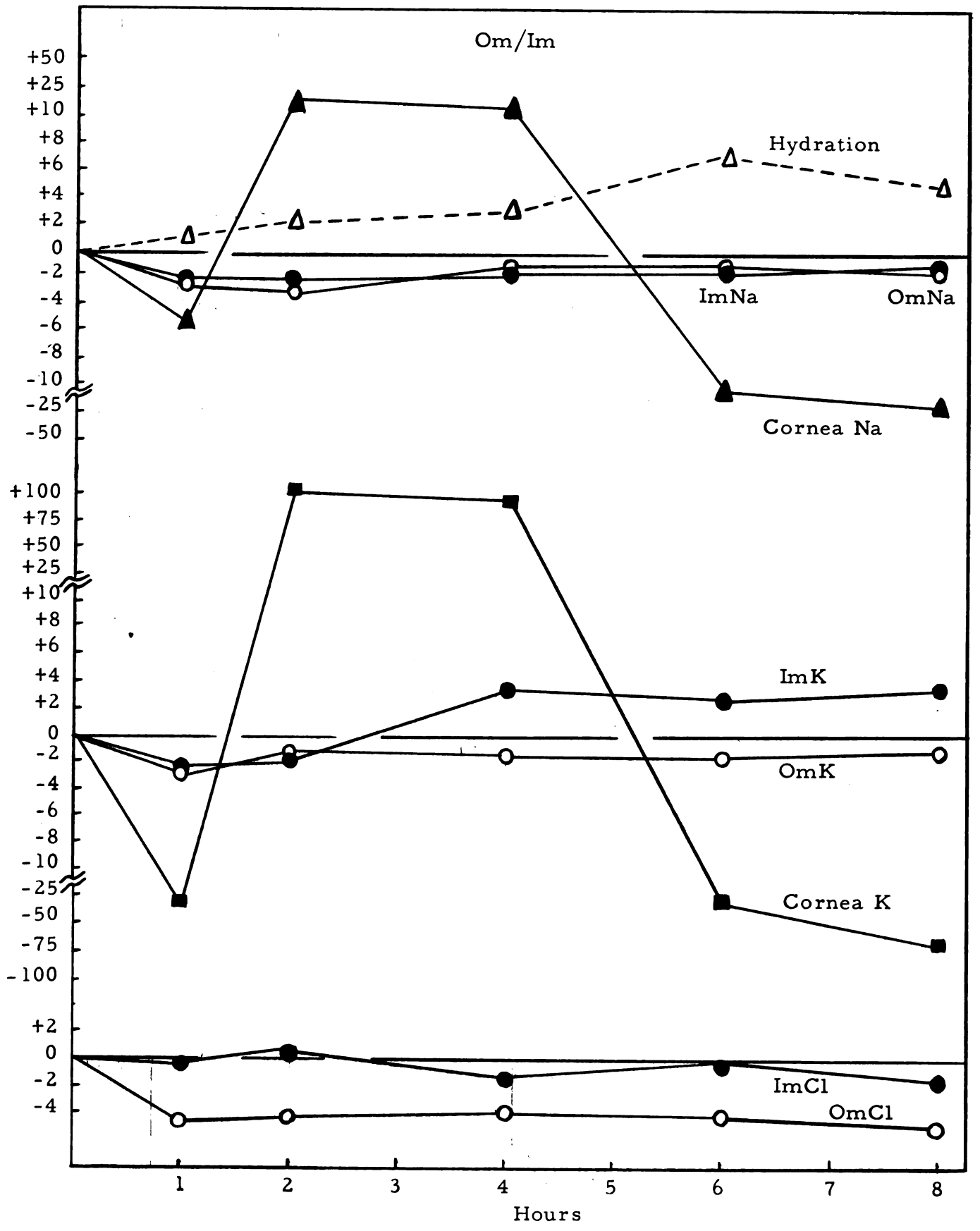

Om/Im
+50
+25
+10
+8
+6
+4
+2
0
-2
-4
-6
-8
-10
-25
-50
Hydration
ImNa
OmNa
Cornea Na
+100
+75
+50
+25
+10
+8
+6
+4
+2
0
-2
-4
-6
-8
-10
-25
-50
-75
-100
ImK
OmK
Cornea K
+2
0
-2
-4
ImCl
OmCl
1
2
3
4
5
6
7
8
Hours

Table III. Experiment: O_D/Im_D. Cation an Anion Movement Expressed as Percent Increase or Decrease from Control

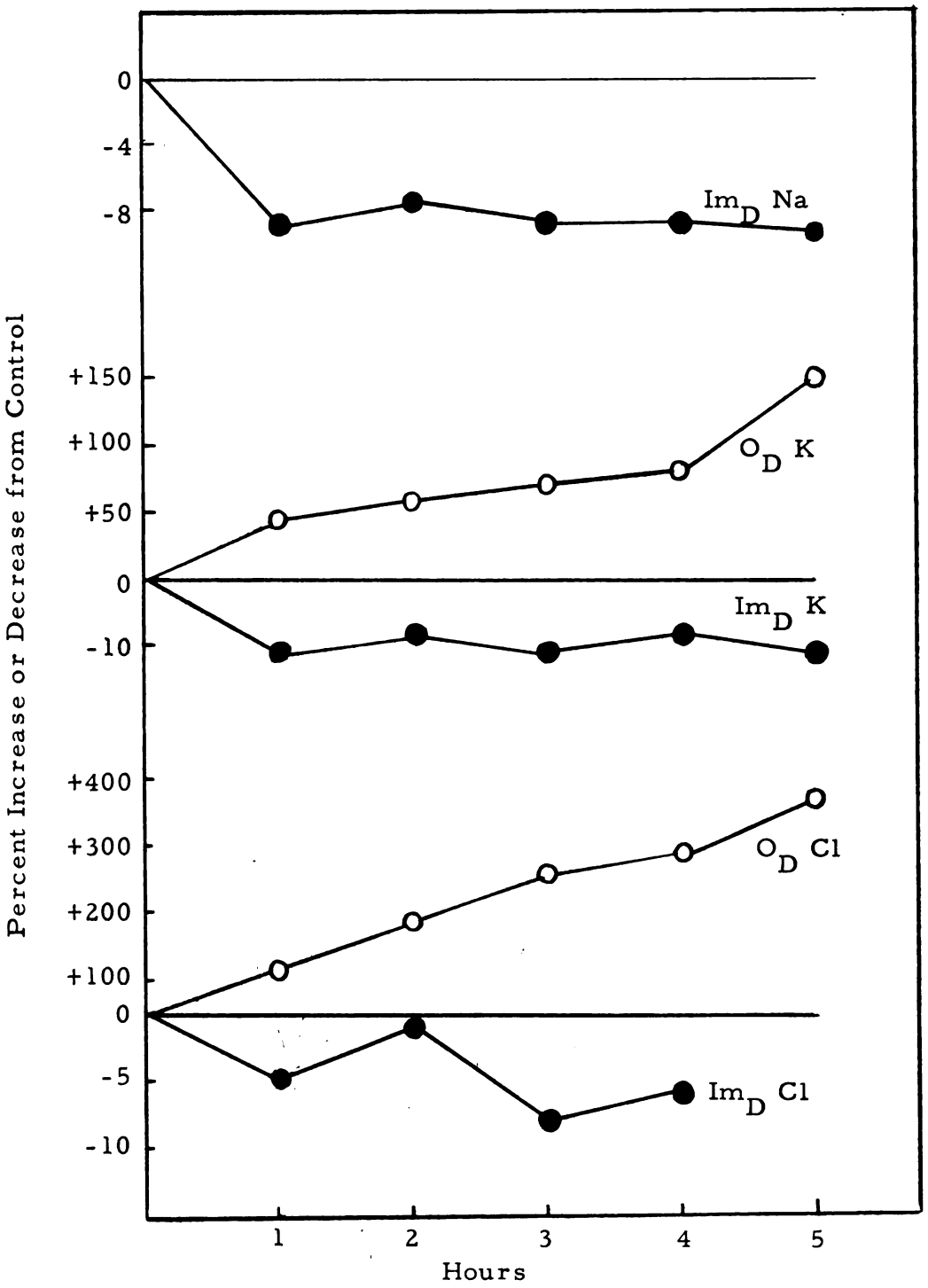
	MEDIA						mOs/l	
TIME	Im_D Na	O_D Na	Im_D K	O_D K	Im_D Cl	O_D Cl	Im_D	O_D
Control	47.1831		1.9225	0.19859	47.3592	0.4704	272	0
1hr	-8.823**		-10.764	+45.73	-4.951	+117.0		
2hr	-7.360**		-8.268	+60.80	-0.819	+189.36*		
3hr	-8.695**		-11.180	+73.37	-7.985	+259.57*		
4hr	-8.695**		-8.268	+81.91	-5.623	+291.49**		
5hr	-9.098**		-11.024	+151.26	------	+372.34	264	6

Media control expressed as mEq/gm control tissue water
+ increase percent from control
- decrease percent from control

* Significant from control at the 10% level
** Significant from control at the 5% level

Above data is plotted in Figure 10; each point represents an average of at least six determinations.

Figure 10. Experiment: O_D/Im_D - Ion movement expressed as percent change.



IV. 400 mOs/l NaCl solution: O_S/Im_S

(Table IV, Figure 11)

In this experiment the corneas were exposed to an osmotic gradient which ordinarily is not encountered by the fresh water teleost fish. This osmotic gradient enhanced the movement of water and ions into the cornea. Osmolarity slowly increased on the inside and decreased on the outside. Figure 11 illustrates that both ions and water move with the gradient from 199 medium into the cornea. Apparently, water moves through the cornea to the NaCl solution, and ionic movement is inhibited by the cornea. This could account for the decrease in osmotic concentration, implying that ionic movement is not as free as water movement. Therefore, corneal sodium increased throughout six hours. Corneas exposed to the same conditions for 10-20 hours show a 27 percent increase in corneal sodium ion concentration.

Table IV. Experiment O_S/Im_S. Cation an Anion Movement Expressed as Percent Increase or Decrease from Control

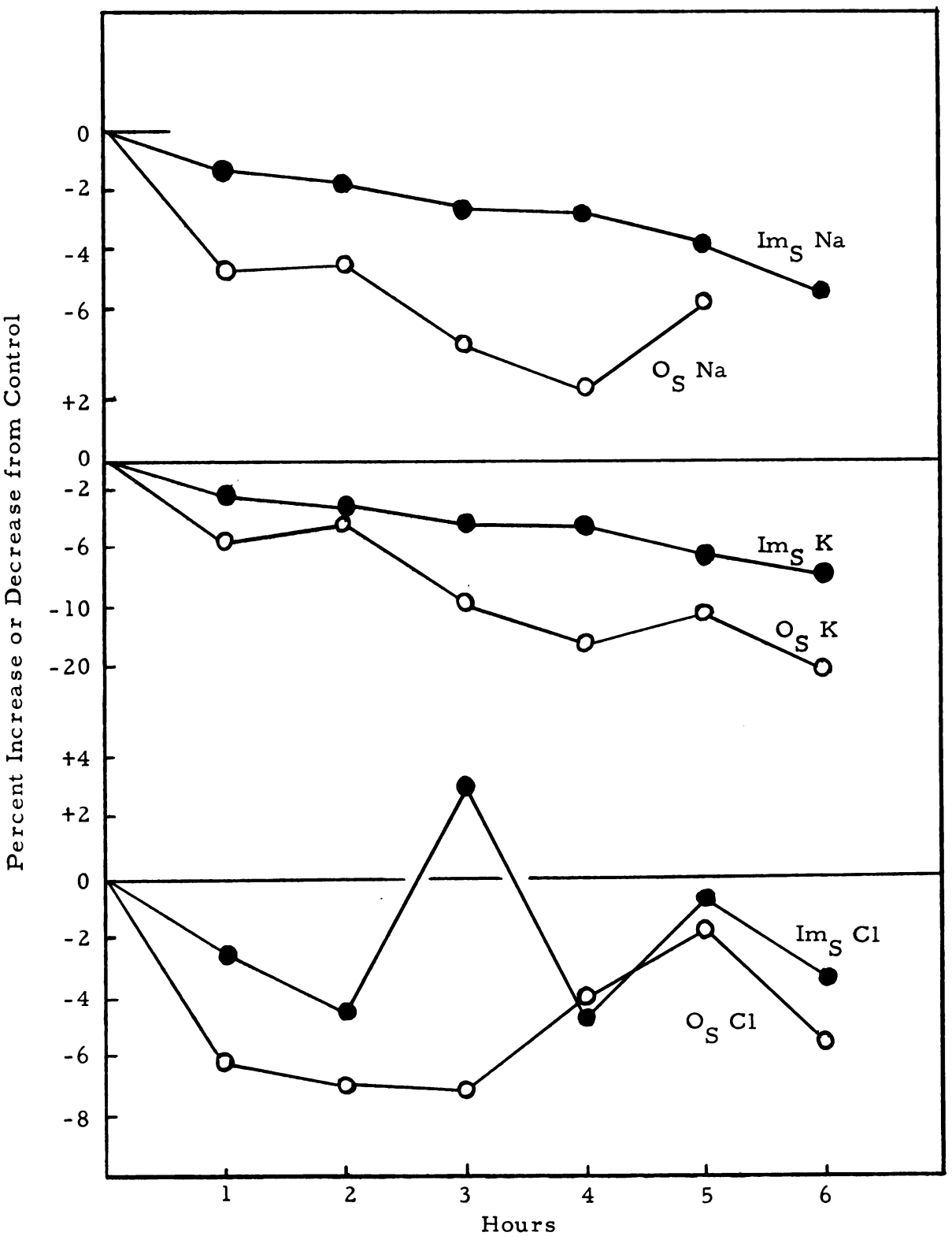
	MEDIA						mOs/l	
TIME	Im_S Na	O_S Na	Im_S K	O_S K	Im_S Cl	O_S Cl		
Control	47.1831	76.4577	1.893	0.2284	47.359	74.412	272	400
1hr	-1.135	-4.584	-2.165	-5.676	-2.552	-6.09		
2hr	-1.776	-4.457	-3.048	-4.365	-4.558	-6.950		
3hr	-2.613*	-7.148**	-4.701	-9.606	+3.127	-7.071*		
4hr	-2.757*	-8.611***	-4.355	-15.28	-4.706*	-3.980		
5hr	-3.79**	-5.728*	-6.6477	-10.92	-0.758	-1.897		
6hr	-5.346	------	-7.556	-20.09	-3.291	-5.525	280	394

Media control expressed as mEq/gm control tissue water
\+ increase percent from control
\- decrease percent from control

* Significant from control at the 10% level
** Significant from control at the 5% level
*** Significant from control at the 1% level

Above data is plotted in Figure 11; each point represents an average of at least six determinations.

Figure 11. Experiment: O_S/Im_S - Ion movement expressed as percent change.



DISCUSSION

In the normal environment of the fresh water teleost, the osmotic concentration is of the order of 10 mOs/l, whereas the concentrations of the aqueous humor is approximately 300 mOs/l. The cornea which lacks a mucus layer and scales of the other parts of the integument must withstand this tremendous osmotic gradient and does so presumably with a high degree of impermeability to both water and ions.

Anatomically, terrestrial and aquatic corneas are different in that corneas adapted to aquatic environments have a thick epithelium and Bowman's membrane. Aerial environmental corneas are faced with the problem of evaporation of interocular fluids, and they possess a well-developed functional endothelium and Descemet's membrane (Figures 1 and 2).

When the corneal epithelium was stripped from the intact rainbow trout eye and the fish was placed in tap water, swelling and opaqueness of the cornea resulted after one hour. When the endothelium was abrased after one week, the cornea was clear and transparent. Smelser and Chen (1954) reported that when the anterior epithelium was denuded in the carp cornea, it swelled; however, in the mammal the swelling was much less. Abrasion of the endothelium of the guinea pig cornea resulted in considerable hydration, but similar treatment to the carp resulted in only a limited amount of swelling.

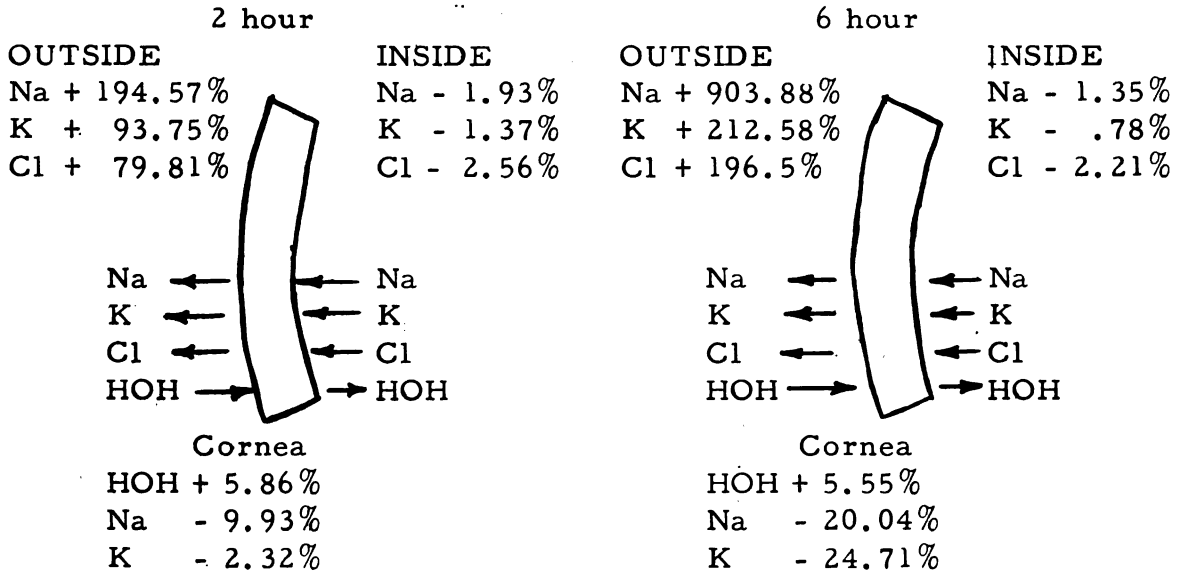
The high degree of impermeability of the fresh water teleost cornea is probably due to a thick epithelium and Bowman's membrane, and both are therefore of prime importance in maintaining the normal transparency of the teleost's cornea.

Effects of Tap Water

These experimental conditions best simulate the in vivo situation; however, there are two parameters not considered: (a) lack of hydrostatic pressure to simulate interocular pressure, and (b) 199 maintenance medium does not contain the mucopolysaccharides which enhance the viscosity and colloid osmotic pressure of the aqueous humor.

In the in vitro teleost cornea, water and ions permeate down a concentration gradient. Under experimental conditions, there was a steady outward flux of ions from the cornea into the tap water, concurrently with some corneal hydration. Replacement of water and ions, although not completely adequate to maintain normal corneal concentration, was from the 199 medium. Due to the absence of a well-developed endothelium and Descemet's membrane, it is assumed that materials are freely exchangeable between corneal interstitial fluid and the 199 medium.

The distribution of ions and water at two and six hours are shown graphically as follows:



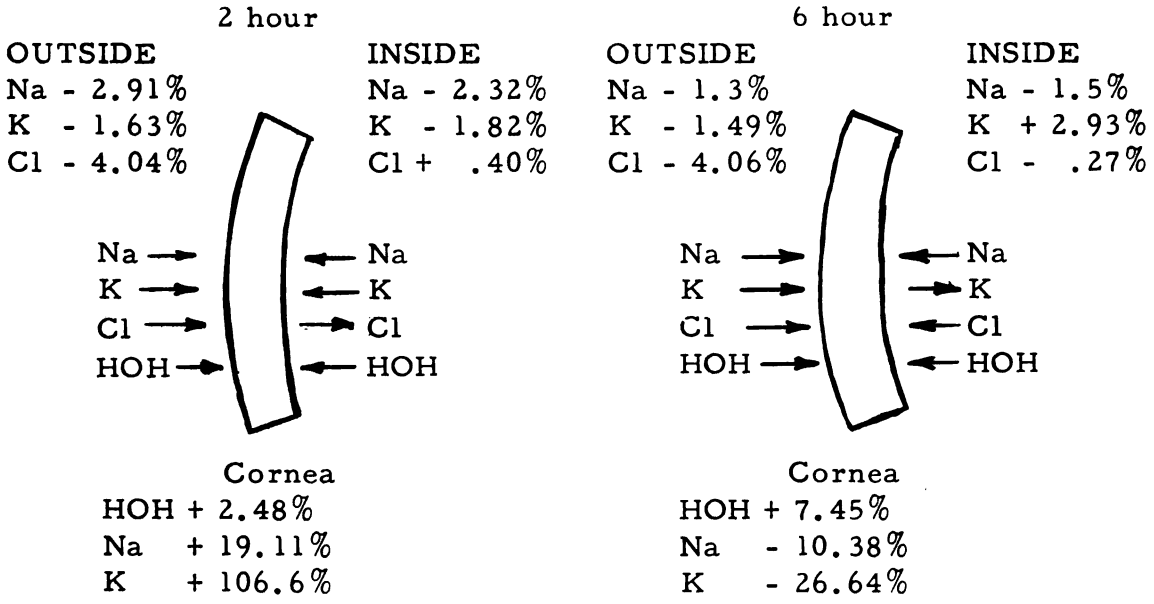
After two hours corneal hydration was only +5.86 percent compared to 26 percent reported by Smelser (1962) for corneas bathed on both sides with tap water. Hydration of the corneas, under our experimental conditions, appears to be minimized by loss of water across the endothelium to the 199 medium and by uptake of salts from the 199 medium. Ion concentrations in the tap water are doubled after six hours, a large increase which can be attributed to the low initial ion concentration. It is unlikely that the changes in ionic concentration of the cornea and fluids bathing the cornea are the result of water movement alone. Movement of chloride ions appears to follow the movement of sodium ion as previously shown by many investigators for other tissues (Davson, 1960).

With this in vitro preparation of the teleost cornea, the epithelium is to some extent permeable both to water and ions, but probably in the intact eye, it serves to retard the entrance of water. Also, the endothelium appears to be permeable to both ions and water. This agrees with Smelser (1962) who reported that the endothelium is most likely impermeable to high molecular weight constituents of aqueous humor but freely permeable to water and ions.

Effects of 199 Chemically Defined Media

The study of active ion movement within the cornea was approached by bathing both surfaces with 199 maintenance medium. After the equilibrium period the concentration of corneal sodium and potassium was found to increase whereas the concentration of both ions decreased in both bathing fluids. Since the sodium content of the cornea is 108 mEq/l and the 199 media is 132 mEq/l, there is an inward diffusion gradient. Although there is no further evidence for active transport, these results compare favorably with the data presented by Donn et al. (1959), who reported a sodium pump in the mammalian cornea;

however, the sodium moved inward, not outward. Directional movement of water and ions at the two and six-hour periods are shown graphically as follows:



Even though the osmotic pressure was equal on both corneal surfaces, corneal hydration gradually occurred throughout the eight hours. It is the tendency of excised corneas (mammalian and fish) to swell under isosmotic conditions. Ion and water movement at two hours was into the tissue; however, the four and six-hour samples indicated potassium moved out of the cornea via endothelium surface. Concurrently, corneal hydration increased maximally and relative cornea ion content decreased. This severe sodium-potassium shift in the cornea occurs when there is rupture of the rudimentary Descemet's membrane and endothelium layer.

There may be a possibility of an active sodium pump within the fish cornea as suggested by these experiments. The sodium pump would help insure proper ionic composition of the interstitial fluid bathing the collagen fibers of the stroma, thus enabling the proper

bonding and orientation of these macromolecules. Ions can diffuse inwardly through the epithelium in the in vitro state, and Smelser and Chen (1954) have reported that the epithelium is rich in glycogen and phosphatase which strongly suggests that it is a metabolically active tissue able to preserve the corneal ions. Hoffert (1964) has shown that oxygen consumption and glucose utilization of the trout cornea is high compared to mammalian corneas. All of these parameters are characteristics of an active system so there is the possibility that an inward pumping mechanism is present, which would help preserve the ionic environment of the stroma. In the absence of more conclusive experimental data, it would be unwise to extend this concept any further.

Effects of Deionized Water

Although the data in Figure 10 is not as inclusive as the previous figures, it does provide a good comparison with the tap water experiment where the chemical gradients are almost the same. With these experimental conditions, there was a steady outward flux of corneal ions into the deionized water and a constant inward water flux. The percent ionic decrease within the 199 medium in this experiment is comparatively greater than in tap water. Results from this work suggest that in some way the ions in the tap water are functional in altering the corneal permeability for Na^{+}, K^{+} and Cl^{-}. The moderate amount of Ca^{++} available in tap water (2.93 mEq/l) would be sufficient to demonstrate this control. The effect of calcium in the water appears to be similar to that observed by Phillips et al. (1955, 1958) and Hunn (1963) who reported that the uptake of certain ions were related inversely to the level of calcium in the medium. Therefore, increased permeability due to low calcium may lead to an increased water influx through the cornea, as is the case with deionized water.

A higher level of calcium on the epithelium of the cornea (tap water) will effect the permeability of the cornea by decreasing the permeability--hence inhibiting the movement of ions across the epithelium. This phenomena is common in biological membranes (Davson, 1960).

Effects of 400 mOs/l Saline Solution

A reverse osmotic gradient for the rainbow trout cornea resulted in an outward flux of water and ions from both the cornea and 199 medium. This outward flux can be attributed to the osmotic gradient. There is indication that the water movement was much greater and not inhibited by the corneal barriers including the epithelium and Bowman's membrane since ion concentration decreased in both bathing media but the osmolarity decreased on the outside as a result of water addition. Therefore, the ions were concentrating within the cornea as the result of lagging behind the movement of water. Under these conditions of reversed osmotic gradients, a fish eye would become dehydrated, and one would expect an altogether different type of specialization if this were a marine teleost.

These studies of the trout cornea in vitro indicate that the cornea is relatively impermeable to both water and salts. The fresh water fish has an intraocular pressure of approximately 5 mm Hg which, coupled with the colloid osmotic pressure, tends to counteract the flow of water into the cornea. These experiments also suggest the presence of a sodium pump which aids in maintaining the sodium content of the cornea. Smelser (1962) postulates that corneal ions increase the compactness of the charged polymers of the corneal mucopolysacchairdes which make up the corneal stroma, and that any ionic shift could lead to a disruption of the normal corneal homeostasis.

With these permeability studies of the rainbow trout cornea providing the normal corneal ionic and osmotic parameters, it should be possible to compare the diseased fish cornea in relation to these parameters.

SUMMARY AND CONCLUSIONS

The investigation of the ionic composition of the normal teleost cornea and the permeability of the in vitro teleost cornea of the rainbow trout Salmo gairdneri has shown the following:

1. The normal cation concentration of sodium and potassium ion in the fish cornea is 108.9 ± 3.5 mEq/l and 5.8 ± 0.6 mEq/l, respectively, while the water content is 80.1 percent.
2. Anatomically the rainbow trout cornea possesses a thicker epithelium and a more prominent Bowman's membrane than those found in mammalian corneas. Descemet's membrane and the endothelium appear more vestigial than their mammalian counterparts.
3. The cornea in vitro shows a limited permeability to both water and ions. Corneal ion concentration varied inversely with corneal hydration.
4. There are indications that the trout cornea possesses a Na pump which would enable it to maintain itself as a high sodium tissue. The flux of Cl ions appears to be passive.
5. Data presented here are consistent with the idea that the endothelium and Descemet's membrane show a limited permeability to water and ions but are impermeable to macromolecules of the aqueous humor. Also the low permeability of the epithelium and Bowman's membrane plus the colloid osmotic pressure of the mucopolysaccharides and the interocular pressure act to maintain the intact cornea relatively impermeable to water and ions.

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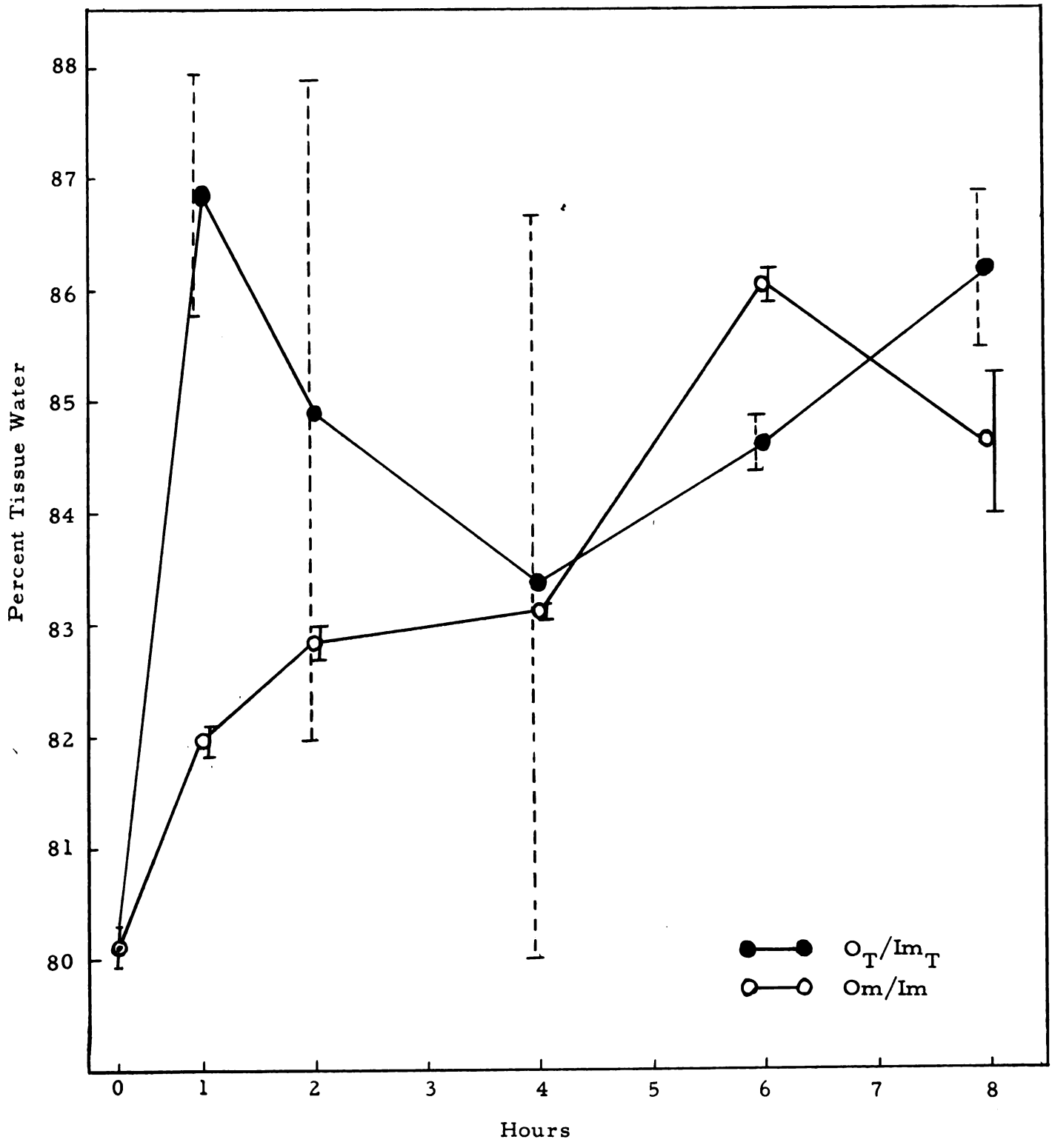
APPENDIX

Each Point Represents At Least Six Determinations

I - Standard Errors

Figure A. Percent tissue water; experiments: O_T/Im_T and Om/Im.



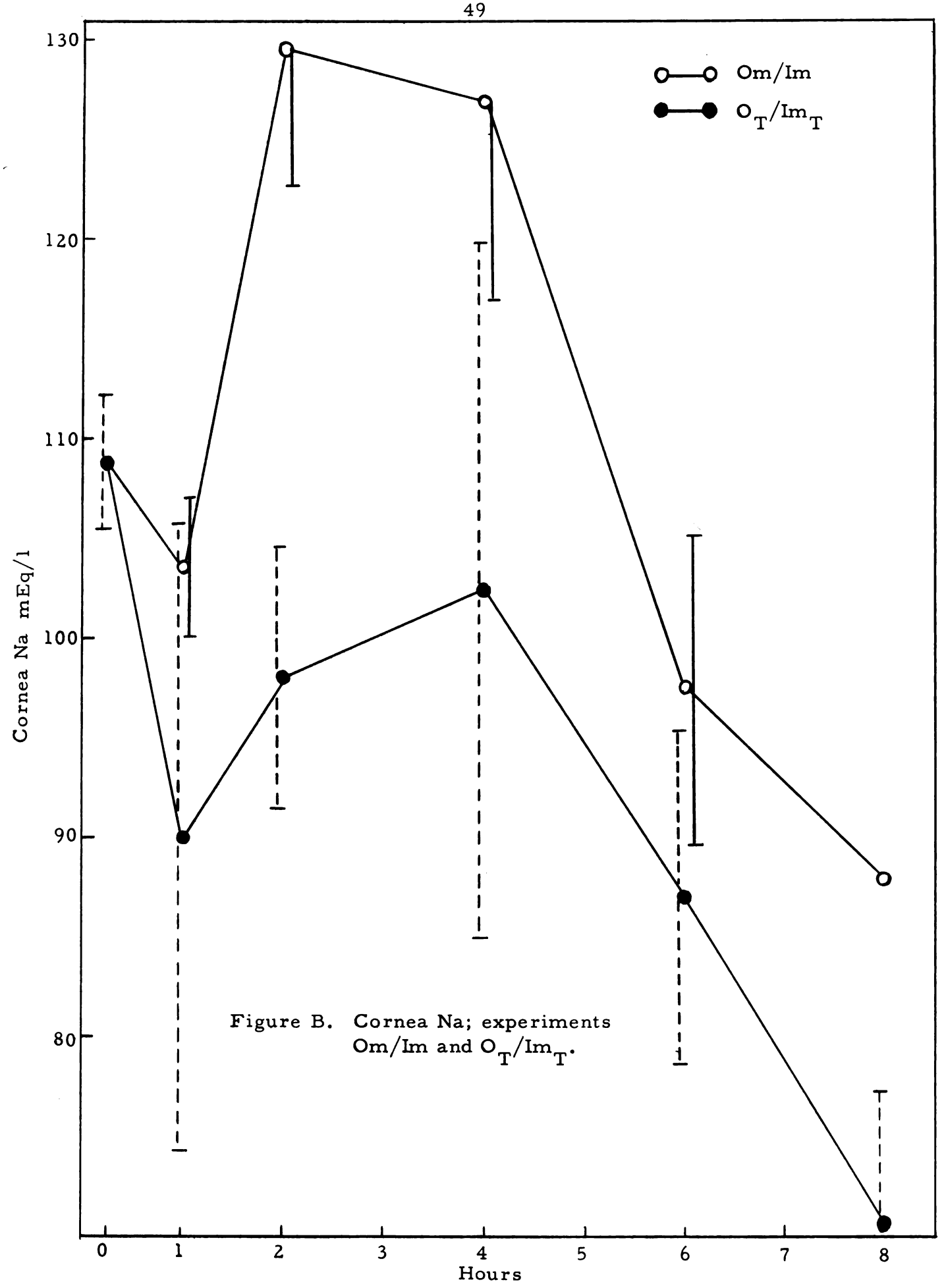


Figure B. Cornea Na; experiments Om/Im and O_T/Im_T.

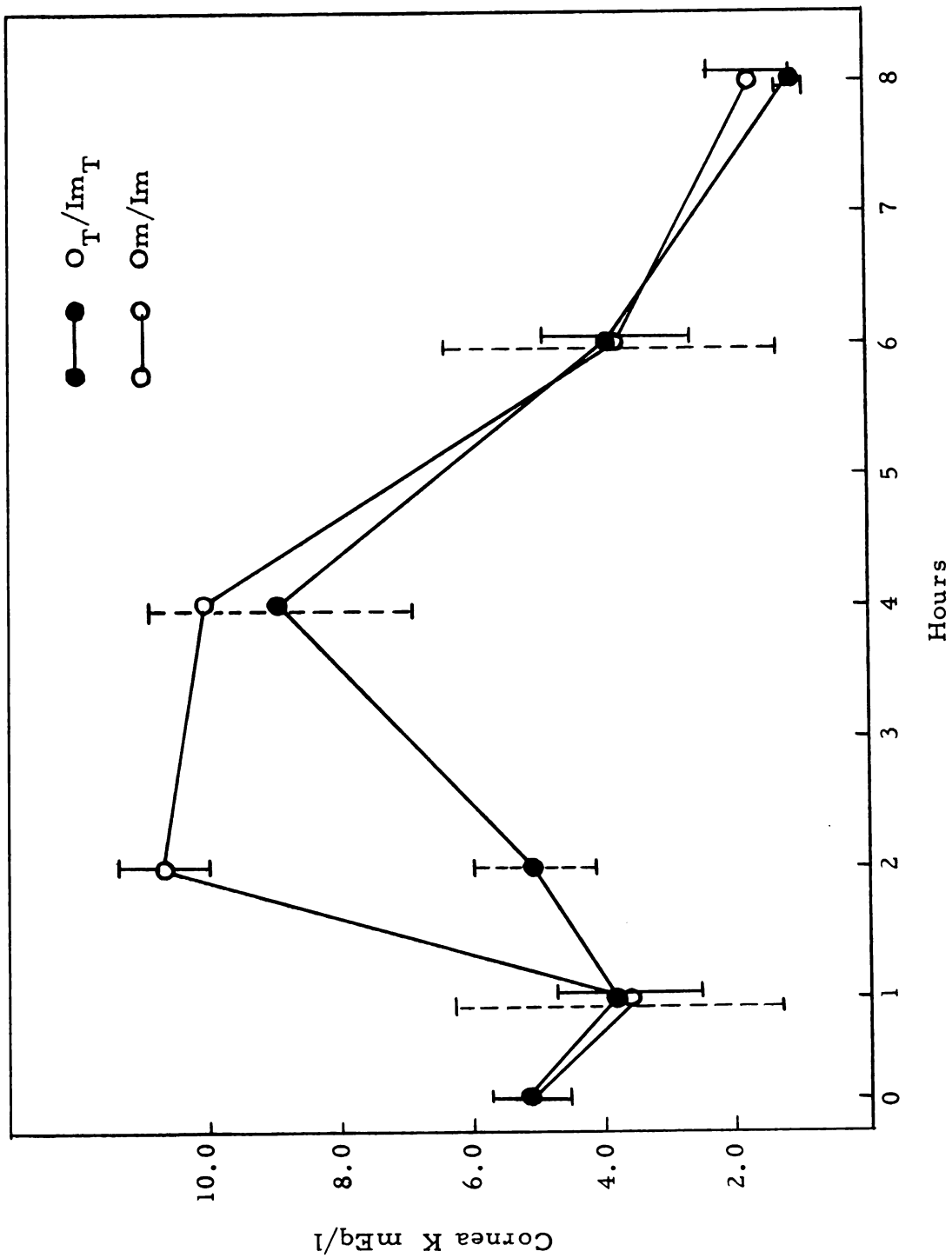


Figure C. Corneal K; experiments: O_T/Im_T and Om/Im.

Figure D. Media Na; experiment: O_T/Im_T.

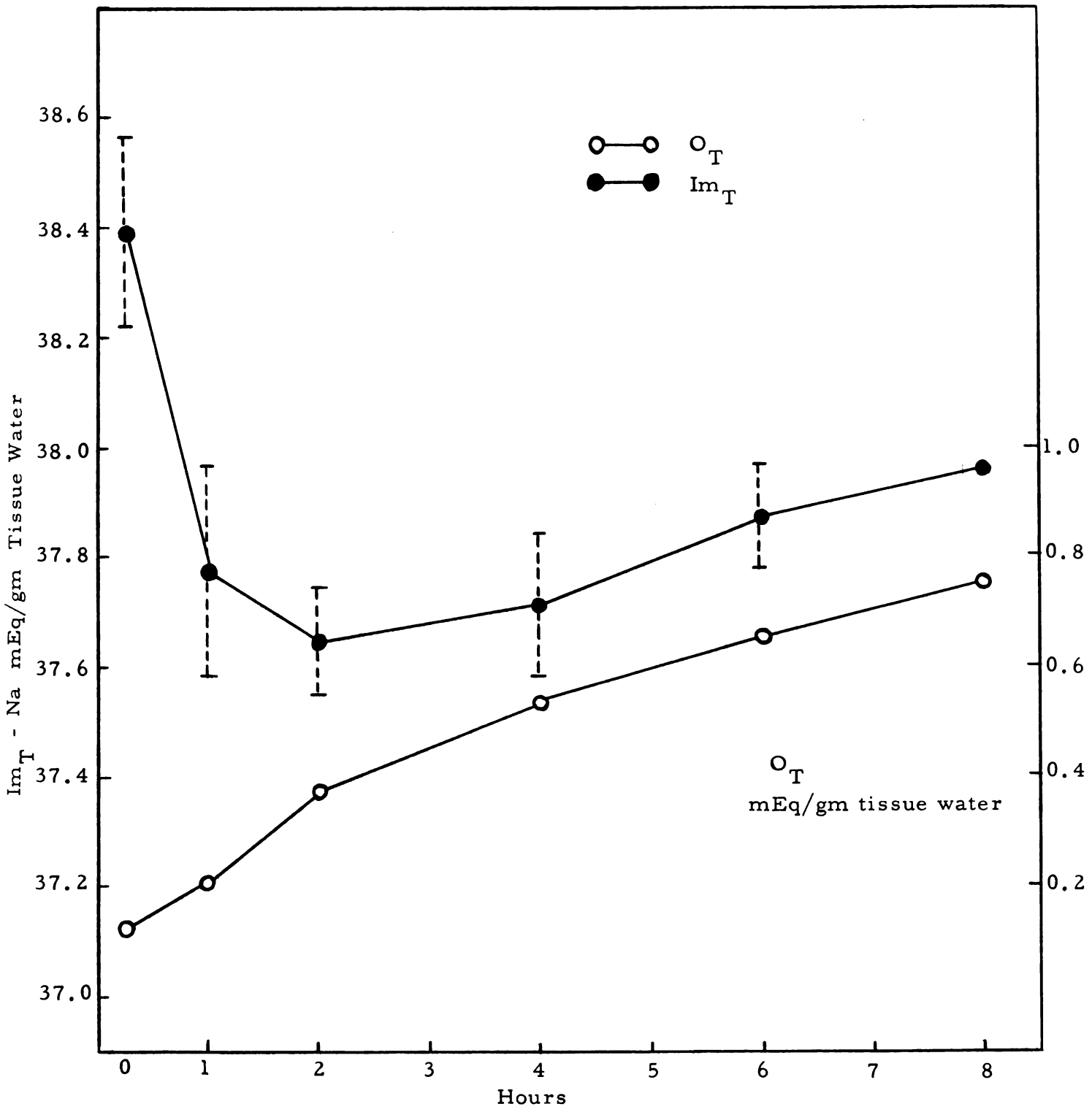


Figure E. Media Cl; experiment: O_T/Im_T.

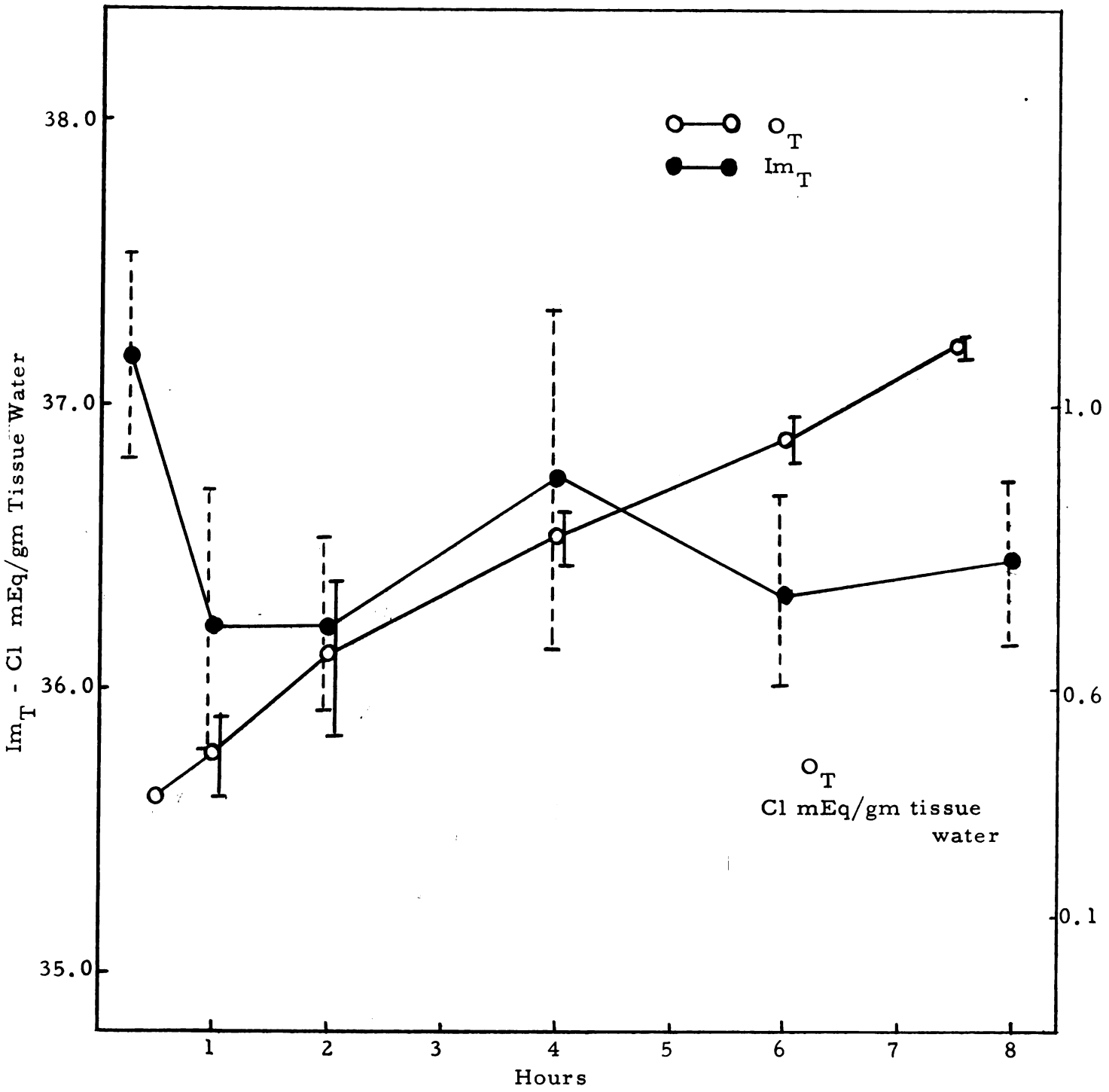
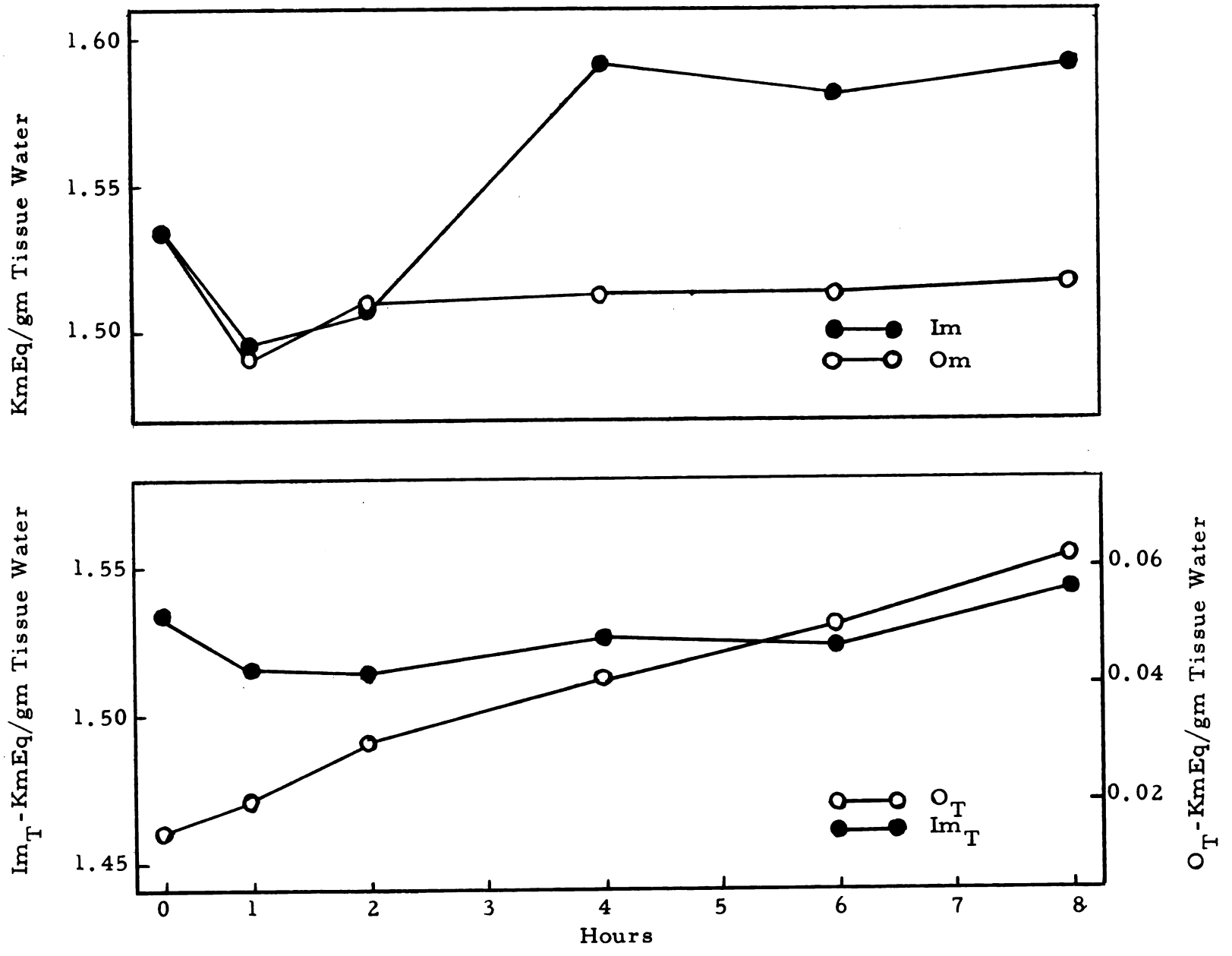


Figure F. Media K; experiments: O_T/Im_T and Om/Im.



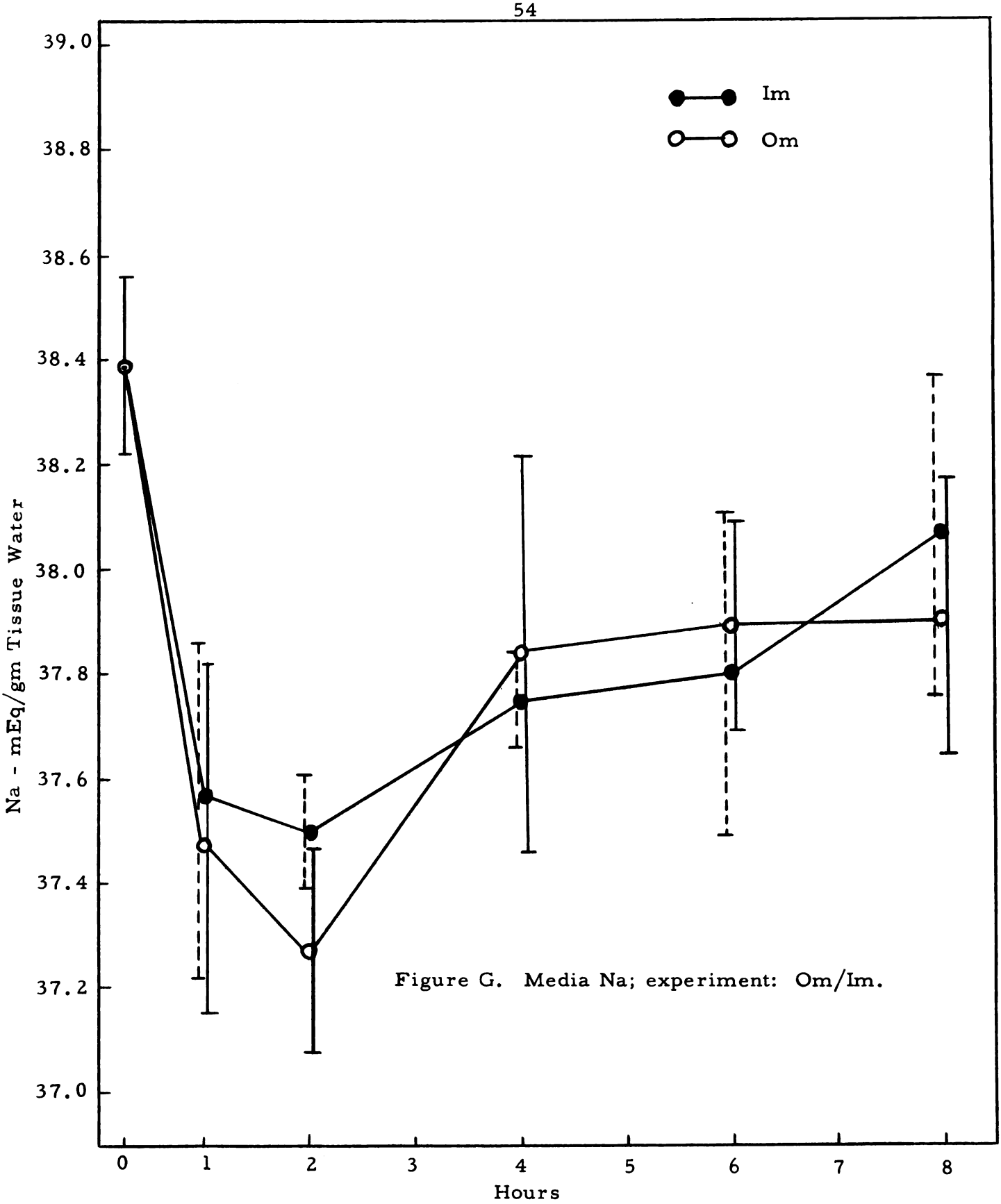


Figure G. Media Na; experiment: Om/Im.

37

36

35.0

Cl mEq/gm Tissue Water

Figure H. Media Cl; experiment: Om/Im.

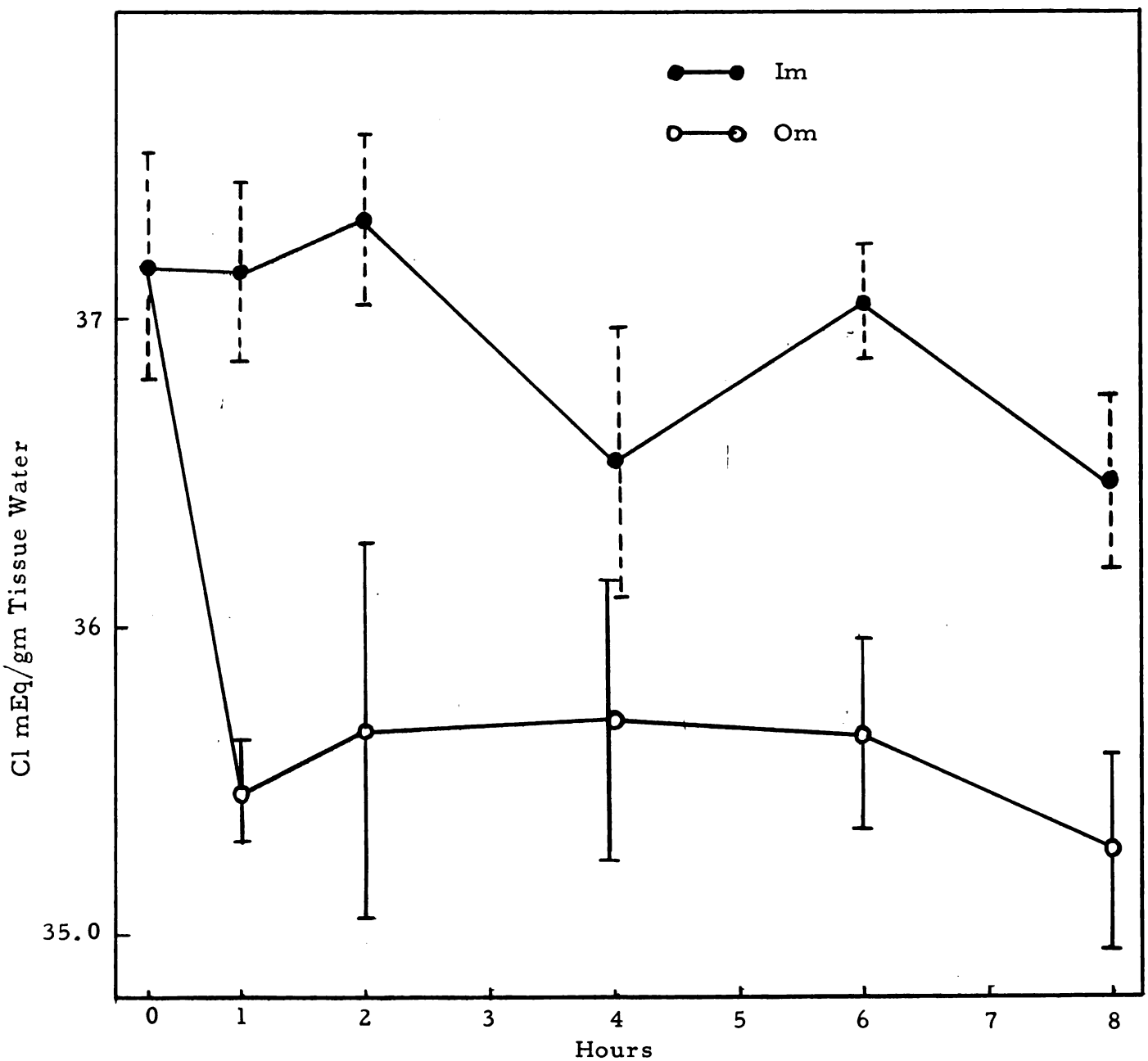


Figure I. Media Na; experiment: O_D/Im_D.

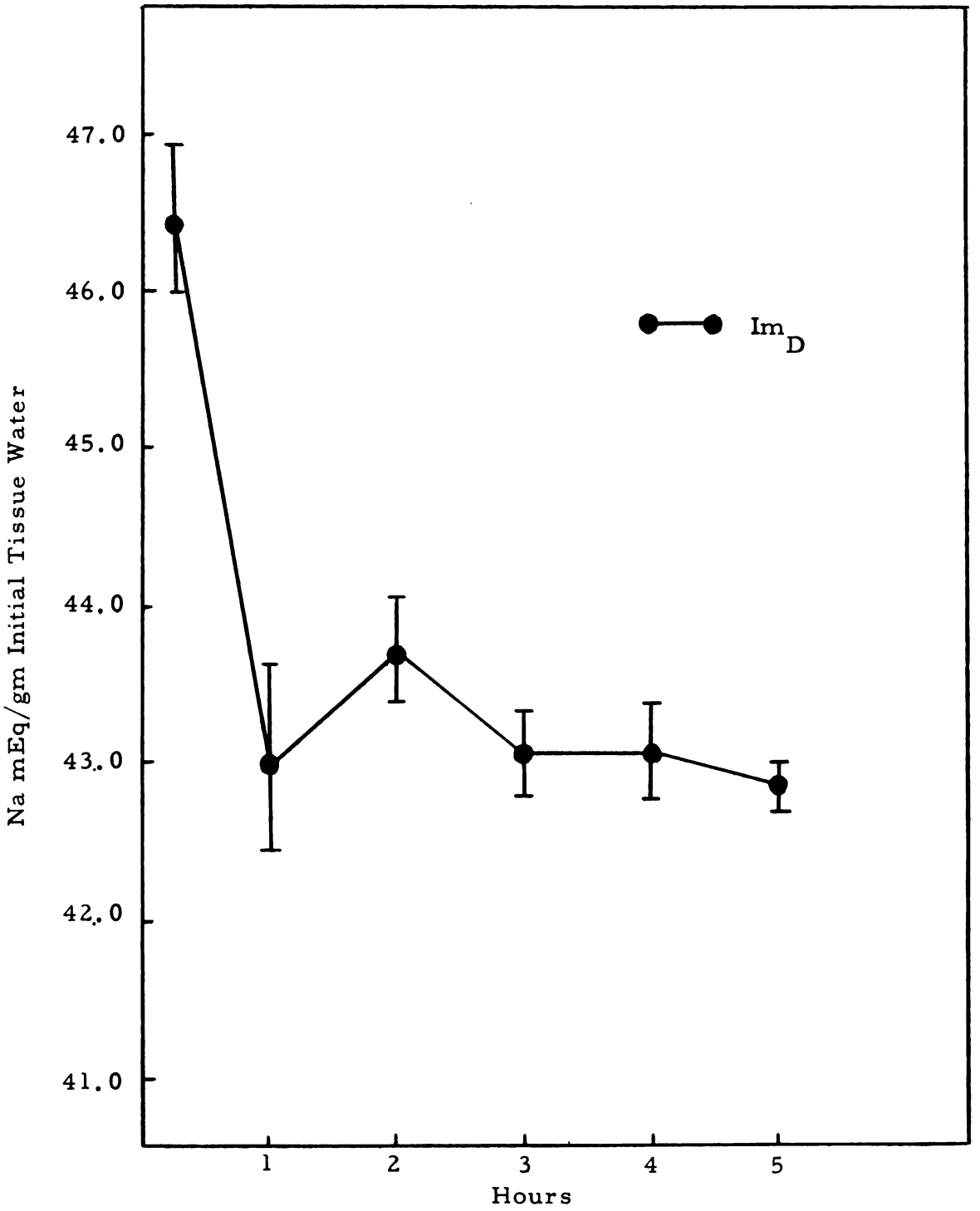


Figure J. Media Cl; experiment: O_D/Im_D.

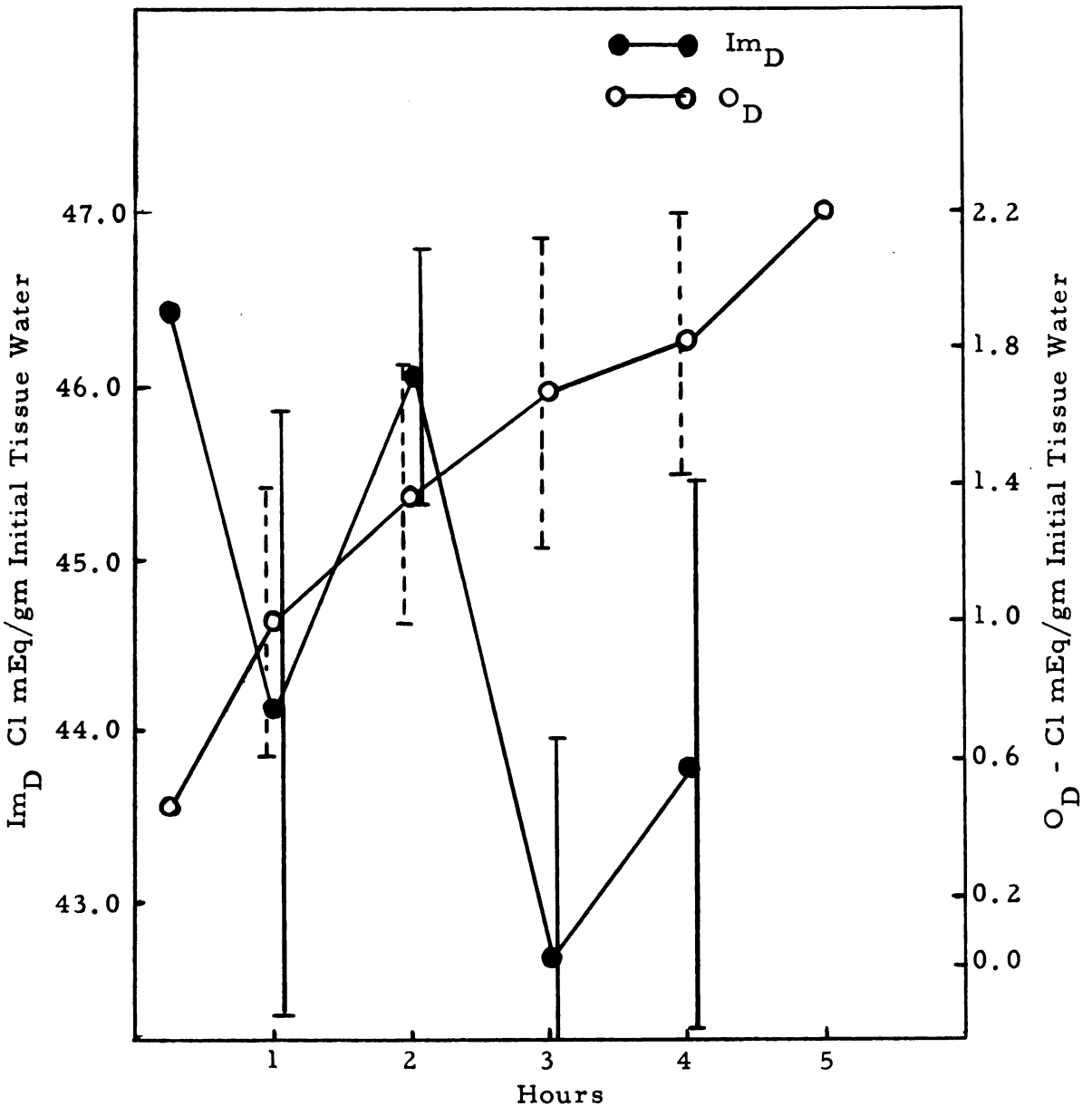


Figure K. Media K; experiments: O_D/Im_D, O_S/Im_S.

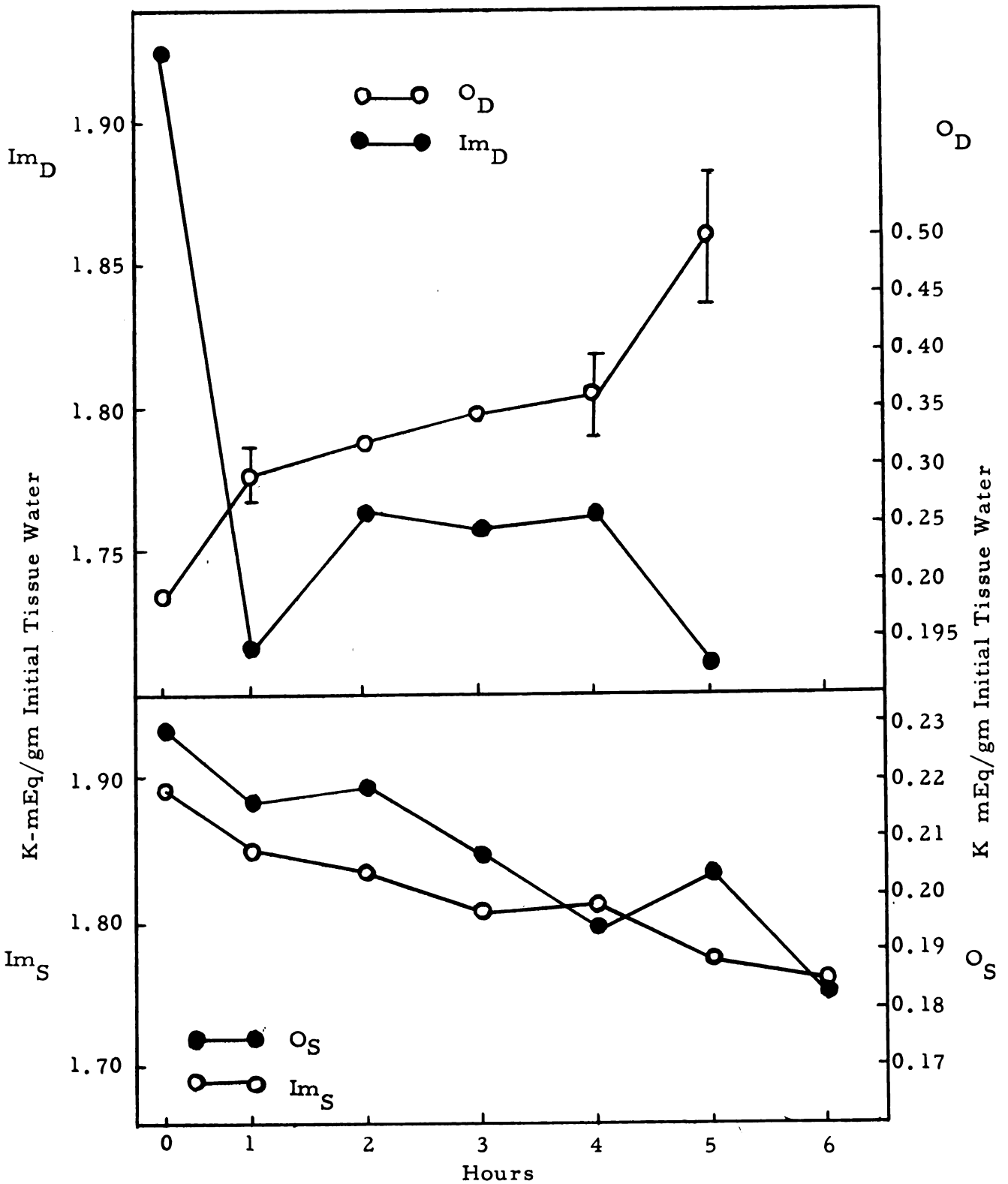


Figure L. Media Na; experiment: O_S/Im_S.

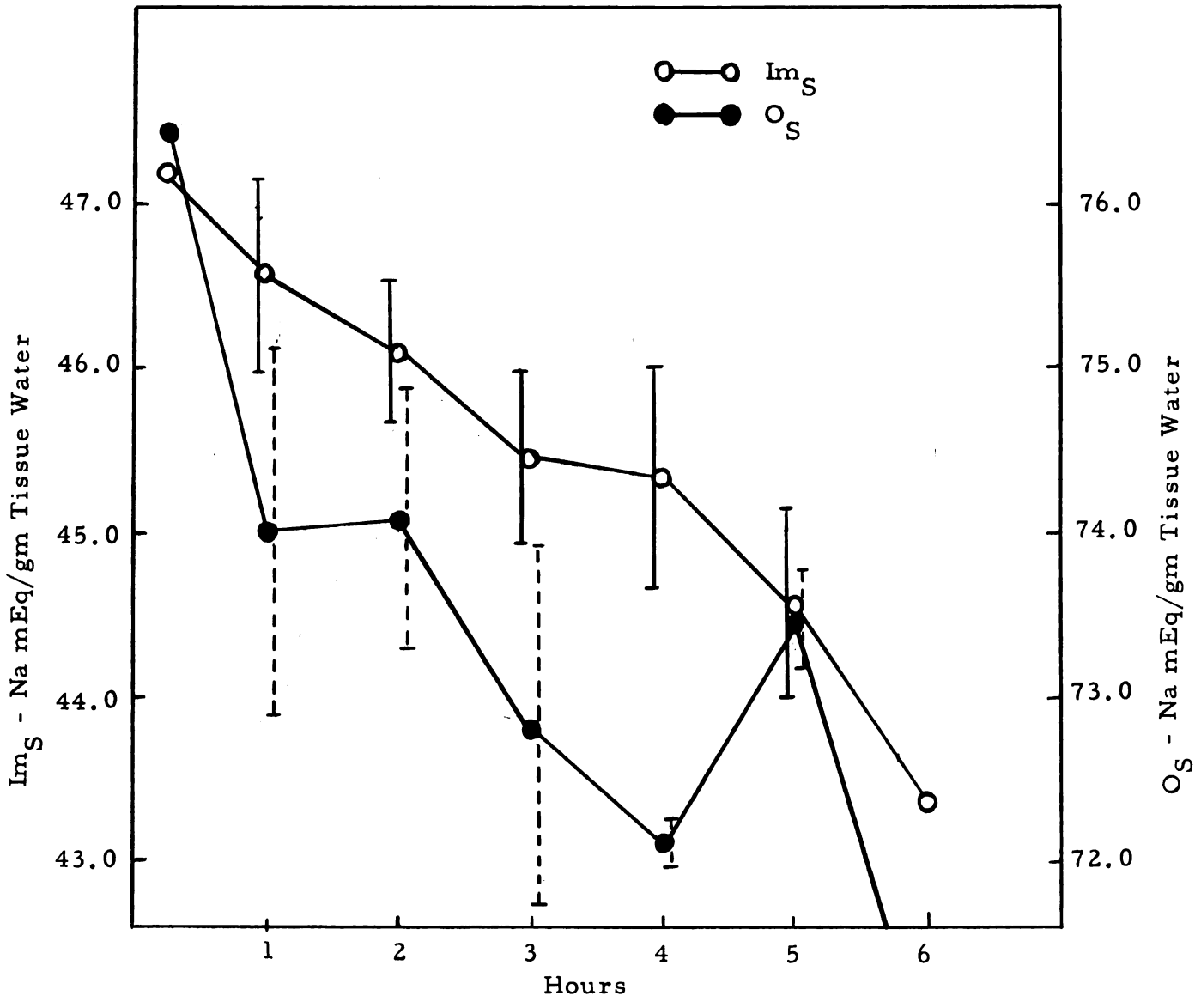
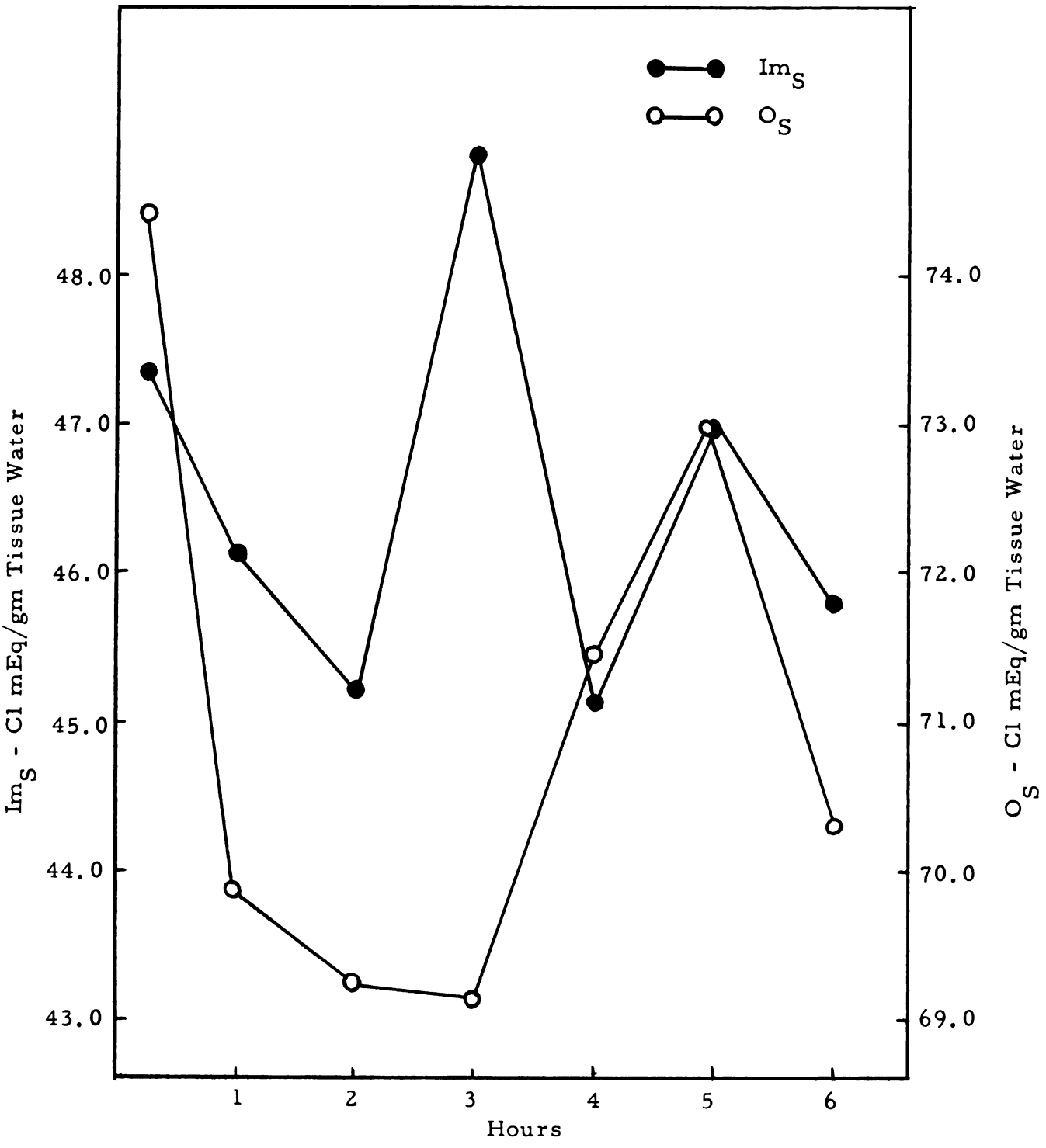


Figure M. Media Cl; experiment: O_S/Im_S.



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