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THE LENS AS AN OSMOMETER AND THE EFFECTS OF MEDIUM OSMOLARITY ON WATER TRANSPORT, ^{86}Rb EFFLUX AND ^{86}Rb TRANSPORT BY THE LENS

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SUMMARY

The transport of water into or out of the lens was regulated by the osmotic gradients between lens and incubation media. Rabbit lenses incubated in hyposmotic Tyrode's media gained water while water losses were found in lenses incubated in hyperosmotic Tyrode's media. Concomitant decreases or increases of intralenticular osmolarities to equilibrate the osmolarity of the bathing fluid were found in lenses incubated in the anisotonic media. The water gains or losses from lenses, induced by osmolarity changes, were reversible. Within the 238–368-mosM range of media osmolarities, the lens behaved as a "perfect osmometer". Minimal lens swelling resulted in hydropic changes in lens epithelium cells and in lens fibers. Additional overhydration led to fluid collection in the interfibrillar space. The rate of ^{86}Rb efflux from the lens increased in swollen lenses proportionally to the degree of hydration. The net transport of ^{86}Rb into the lens was increased (+8, +18%) in minimally swollen lenses and markedly decreased (—50%) in lenses incubated in media —260 mosM hypotonic to the lens. The water permeability coefficient (k) of lens was 0.4 $\mu \cdot min^{-1} \cdot atm^{-1}$, a value smaller than the k of erythrocytes, of similar magnitude to the k of leukocytes and slightly higher than the k of marine invertebrate eggs.

INTRODUCTION

In terms of water transport the lens can be compared to a unicellular structure as it is enveloped by a capsule, and changes in its water volume follow alterations of the environmental osmolarity[1]. The swelling or dehydration of the lens is responsible for refractive errors, and scattering of light entering the eye[2]. Recent studies indicate the accumulation of impermeable sugar alcohols by lenses incubated in high glucose or high galactose media may result in increased lens osmolarity and movement of water into the lens[3]. Measurements of intralenticular osmolarity indicate the lens, aqueous humor, and vitreous humor have identical osmolarities. NORDMANN[4,5] found the freezing point depression (Δ) of bovine lens varied from —0.51 to —0.63°, and averages for rabbit and human lens to be —0.53 and —0.58°, respectively. The latter values correspond to osmolarities of 288 and 312 mosM/kg lens water. To quantitate

the effects of media osmolarity on the lens, we have incubated rabbit lenses in control Tyrode's media and in various anisotonic media, and measured the transport of water and the intralenticular osmolarities. The majority of these studies were conducted in media slightly hypertonic (+33, +66 mosM) or hypotonic (−32, −64 mosM) to the lens. In extremely hypotonic media (−260 mosM) marked lens hydrations resulted from osmolarity changes. In addition, the effects of medium osmolarity on the efflux and uptake of ^{86}Rb by the lens were studied. It is known Rb^+ behave as K^+, and that they are actively transported against the concentration gradient[6] and efflux rapidly from the lens[7].

MATERIAL AND METHODS

Rabbit lenses and Tyrode's media of various osmolarities

Albino rabbits (Haskins Rabbitry, Creve Coeur, Mo.), 2–3 kg weight, were sacrificed painlessly by intracardiac air injection, and the eyes enucleated immediately. The lenses were obtained by opening the eyes at the posterior pole, and gently separating the vitreous humor to the side, and sectioning the zonules with curved blunt scissors.

TABLE I

COMPOSITION OF TYRODE'S MEDIA OF VARIOUS OSMOLARITIES

Media	*NaCl (mM)*	*Sucrose (mM)*	*Calc. osmolarity (mosM)*
Control Tyrode's	137.0	—	302
−32 mosM hyposmotic Tyrode's	120.4	—	270
Na^+-substituted isosmotic Tyrode's	120.4	30.45	299
−64 mosM hyposmotic Tyrode's	102.8	—	238
Na^+-substituted isosmotic Tyrode's	102.8	60.8	300
−260 mosM hyposmotic Tyrode's	0.0	—	42
Na^+-substituted isosmotic Tyrode's	0.0	231.8	302
+32 mosM hyperosmotic Tyrode's	137.0	30.4	334
+64 mosM hyperosmotic Tyrode's	137.0	60.8	366

The composition of complete Tyrode's media was similar to that used in previous studies on lens transport[6,8]. Hyposmolar Tyrode's medium was prepared by omission of various concentrations of NaCl and hyperosmolar Tyrode's medium by addition of various sucrose concentrations as shown in Table I. Na^+-substituted isosmotic Tyrode's medium was prepared by replacing equal molarities of NaCl by sucrose. The rationale for including the Na^+-substituted experiments was that the absence of Na^+ from the media affected the lens efflux or uptake of ^{86}Rb in addition to osmolarity. Consequently, all osmolarity experiments on ^{86}Rb uptake or efflux had to be corrected for such Na^+ effects. Osmolarities were expressed in mosM (mosM/kg water). Hyposmotic solutions were referred to as the difference between isotonicity

and the calculated value for osmolarity with the negative sign (−). In hyperosmotic solutions the difference between isotonicity and the calculated osmolarity were indicated with the positive sign (+).

Measurements of lens water transport

Two methods were used for lens water determinations: (a) intermittent weighing during incubations, (b) water content by differential weighing before and after drying at 100°.

(a) *Intermittent weighing.* Determinations with this method carried the assumption that the dry weight of the lens (33.3% of the original weight) remained constant during the incubations, and that any changes in total lens weight reflected variations in lens water. Immediately after removal from the eyes, the lenses were weighed in a Roller-Smith precision balance. After initial weighing, the lenses were carefully transferred to quadrant-styled petri dishes containing 10 ml of media of various osmolarity in each quadrant, and incubated at 37° in a rocking water bath moving at a speed of 18 times/min. At intervals of 30 min, 1 h, 2 h, 3 h, and 4 h, the lenses were temporarily removed from the media, and weighed. Extreme care was exercised in the transfer maneuvers to avoid any trauma to the lens capsule. The results indicated the water and total weight content of the lens remained constant after 5 h of incubation in media isotonic to the lens, and validated the method of repeated weighing.

(b) *Water content by differential weighing.* At the end of experiments on either water transport by the lens or on ^{86}Rb uptake and efflux, the lenses were weighed, dried for 24 h at 100°, and weighed again. The differential weight between the fresh and dry lenses represented lens water.

Intralenticular osmolarities

The lens osmolarities were measured by adapting methods previously used in red blood cells and brain[9–11]. Basically, it consisted of homogenizing the tissue in a solution of a known osmolarity. Total mosM in the homogenate = total mosM in the tissue (lens) + total mosM in the diluting solution. The differential value between the osmolarity of the tissue homogenate and its solvent represented the tissue osmolarity. Calculations were based on the formula[9,10]:

$$\pi_s W_s + 2/3 W_l \pi_l = \pi_h (W_s + 2/3 W_l) \quad (1)$$

π_s = observed osmolarity of the solvent Tyrode's solution; W_s = weight (volume) of solvent Tyrode's solution; W_l = weight of the lens (or lenses), 2/3 of this value represents the tissue water, average value of 66.6% for rabbit lens; π_l = osmolarity of lens (unknown); π_h = observed osmolarity of the homogenate; from where

$$2/3 W_l \pi_l = \pi_h (W_s + 2/3 W_l) - \pi_s W_s \quad (2)$$

$$\pi_l = \frac{\pi_h (W_s + 2/3 W_l) - \pi_s W_s}{2/3 W_l} \quad (3)$$

In practice two lenses were homogenized in a conical pyrex tube containing 1.8 ml of isotonic Tyrode's solution (302 mosM). The lens to solvent ratio was approx. 1:4. Osmolarities of the solutions and lens homogenates were obtained in a freeze point

depression Advanced Osmometer (Advanced Instruments, Inc., Newton Highlands, Mass.). Osmolarities in lens fluid were expressed in mosM/kg lens water.

Na^+ and K^+ determinations in lenses

Following tissue water determination, the dried lenses were digested in HNO_3 as previously described. 10 ml of distilled water were added, the digests were centrifuged at 2500 rev./min and 2 ml of supernatant used for Na^+ and K^+ determinations by flame photometry[8]. Tissue cation content was expressed in mequiv/kg dry lens weight.

^{86}Rb efflux from rabbit lenses into media of various osmolarities

Rabbit lenses were transferred to Kjeldahl round-bottom flasks containing 10 ml of K^+-free Tyrode's medium and 0.4 μC/ml ^{86}Rb (Abbott Laboratories, Abbott Park, Ill.) (specific activity, 0.82 mC/mg) and incubated as previously described for 2 h (ref. 6). At the end of the incubations, the lenses were transferred into 2 ml of Tyrode's medium of various osmolarities. In a separate set of experiments to prevent the re-entry of ^{86}Rb and obtain estimates of efflux independent of the (Na^+-K^+)-ATPase "pump", ouabain, in concentrations of 1 mM, was added to the media of some flasks. Aliquots of 20 μl were obtained from the efflux media at 15, 30, 60 min, and 2, 3, and 4 h, and plated in planchets. At the end of the incubation period, the lenses were removed from the media, weighed, and transferred to conical homogenizers containing 2 ml of 15%(w/v) trichloroacetic acid, and ground. The homogenates were centrifuged at 2500 rev./min for 20 min, and 20-μl aliquots of the supernatants plated in planchets. The plated samples of efflux media and supernatants were counted in a Nuclear Chicago gas-flow counter at infinite thickness. The results were calculated in ^{86}Rb counts/min per ml of efflux media and ^{86}Rb counts/min per ml of lens after the corresponding background subtractions. The percentage of ^{86}Rb effluxed from the total ^{86}Rb present in the lens at various timed intervals was:

$$\text{Eff lens} = \frac{\text{Eff media}}{\text{Acc lens} + \text{Eff media}} \times 100 \qquad (4)$$

Eff lens = efflux from lens or efflux at any time interval as % of total initial ^{86}Rb in lens; Eff media = total ^{86}Rb efflux from the lens into the media at any time interval = counts/min per ml final media × final medium volume; Acc lens = total ^{86}Rb accumulated by the lens at any time interval = ^{86}Rb counts/min per ml lens water × volume lens water.

^{86}Rb transport into lenses in Tyrode's media of various osmolarities

After removal, the lenses were transferred into 2 ml of (1) control Tyrode's media, (2) Na^+-substituted isotonic Tyrode's media, and in (3) hypo- or hyperosmotic Tyrode's media with 0.4 μC/ml of ^{86}Rb in each media. The lenses were incubated in Kjeldahl flasks processed as in previous experiments[5,7], and the rates of accumulation after 4 h calculated in tissue to medium ratios of the ^{86}Rb counts/min per ml of lens water over the ^{86}Rb counts/min per ml of final media. The total ^{86}Rb (in counts/min per lens) transported into lens (transp. lens) in 4-h incubations was determined by adding the total ^{86}Rb accumulated by the lens (counts/min per lens) at 4 h to the total ^{86}Rb effluxed from the lens during 4 h experiments (counts/min per lens). The later

values were obtained from the efflux experiments, using the nomenclature in (5).

$$\text{Transp. lens} = \text{Acc lens} + \text{Eff lens} \tag{5}$$

Glucose transport and utilization by lenses incubated in Tyrode's media of various osmolarities

In 20-μl samples of the initial and final incubation media glucose was determined by glucose oxidase with Worthington Glucostat (Worthington Biochemical Corporation, Freehold, N.J.). The lenses had been incubated in complete Tyrode's media, and in Tyrode's media of various osmolarities as in other experiments on this series. The difference between initial and final glucose content of the media represented total glucose transported and utilized by the lens, and was expressed in mmoles/kg wet lens weight per h.

Histology of incubated lenses

After incubations for 4 h in complete Tyrode's media and in Tyrode's media of various osmolarities, the lenses were fixed in 2.1% formaldehyde. 6-μ sections of the lenses were stained with hematoxylin–eosin.

Fitness of the lens to a "perfect osmometer"

Changes in the extracellular osmotic pressure brought about changes in volume by transfer of water to or from the lens. If an infinitely large volume of extracellular fluid was present, the application of the modified van 't Hoff osmotic pressure equation[12] (PONDER's[13] equation) that follows, determined the fitness of the lens to a "perfect osmometer".

$$V = W_0 \left(\frac{\Delta_0^t}{\Delta_1^t} - 1 \right) + 100 \tag{6}$$

where V = cell volume (which is arbitrarily set at 100 at isomolarity); W_0 = fraction of cell volume occupied by H_2O at isosmolarity (the lens = 66%); Δ_0^t = freezing point depression of intracellular (lens) solvent phase at isosmolarity; Δ_1^t = freezing point depression of extracellular (Tyrode's) fluid.

The V value was the theoretically expected value for change in lens volume (lens water content) with changes in tonicity of the media (Δ_1^t/Δ_0^t). With the tonicity of the media equal to 1, the cell volume remained unchanged at 100%. The parameters Δ_1^t, Δ_0^t, and W_0 were measured experimentally to determine whether the found value V fitted the theoretically expected value for the corresponding change in osmolarity.

Permeability coefficient (k) of water influx into lens

The volume of water crossing per unit area of lens membrane per unit time in response to a difference of one atmosphere represented the osmotic permeability of the lens. The permeability coefficient k was expressed in μ^3 of water per μ^2 of lens surface per min per atm (μ^3 water$\cdot\mu^2$ lens surface $^{-1}\cdot$atm $^{-1} = \mu\cdot$min$^{-1}\cdot$atm^{-1}). If the water diffusion through the lens membranes was a process of simple diffusion, and the resistance of the membrane remained constant during the osmotic volume change then[14,18]:

$$\frac{dV^e \text{ lens}}{dt} = k \cdot A(\pi_0 - \pi_e) \tag{7}$$

where V^e lens = volume of lens at osmotic pressure π_e; t = time of measurement; A = surface area of the lens (lens capsule area); π_0 = internal osmotic pressure at $t = 0$ (in mosM/kg lens water); π_e = external osmotic pressure (in mosM/l of media); and from the basic relationships between osmotic pressure and volume

$$\pi_e V^e \text{ lens} = \pi_0 V^0 \text{ lens} \tag{8}$$

where V^0 lens = volume of lens at osmotic pressure π_0. If π = internal osmotic pressure of the lens in equilibrium with π_e at any time interval t and substituting in (7) and (8)

$$-\frac{\pi_0 V^0 \text{ lens}}{\pi^2}\frac{d\pi}{dt} = kA(\pi - \pi_e) \tag{9}$$

and integrating

$$kAt = \frac{\pi_0 V^0 \text{ lens}}{\pi_e^2} \ln \frac{\pi(\pi_0 - \pi_e)}{\pi_0(\pi - \pi_e)} + \frac{\pi_0 V^0 \text{ lens}}{\pi_e} + \left(\frac{1}{\pi_0} - \frac{1}{\pi}\right) \tag{10}$$

By solving (10) the water permeability coeffiecient k was found. The volume of the lens was calculated form the lens weight and specific gravity (s.g. 1.12). Assuming the lens is an oblate spheroid, the surface area (A) was found as

$$A = 2\pi a^2 + \frac{b^2}{e} \log_e \frac{1+e}{1-e} \tag{11}$$

where a and b are the major and minor semi-axes, respectively, and e the eccentricity. The average value used for A was 240 mm^2.

RESULTS

Effect of medium osmolarities on water transport into lens

Fig. 1 shows the percentage of change in water content of rabbit lenses incubated in media of various osmolarities for a period of 4 h. The water content of those lenses incubated in isotonic media remained unchanged at the end of 4 h. Those lenses incubated in hypotonic media of —32, —64, and —260 mosM, showed gains of 5%, 14%, and 64.5% of the original water content, respectively. Lenses incubated in hypertonic media of +33 and +66 mosM lost 5% and 11% of total water content, respectively. Absolute values in mg/lens are shown in Fig. 2. Some of the changes in water content were reversible as demonstrated in Fig. 3. In those experiments, the lenses were incubated in various hypotonic solutions for 3 h, and then transferred to a +100 mosM hypertonic medium. The results showed gradual reverse of the previous lens water gains, and even a loss of part of their original water content. Only 30% of the total water gains of lenses incubated in —260 mosM hypotonic media effluxed when transferred to a +100 mosM hypertonic medium (Fig. 4) for 4 h.

Effect of medium osmolarities on intralenticular osmolarities

The change in water content of lenses incubated in media of various osmolarities was accompanied by alterations in intralenticular osmolarities. Table III shows the intralenticular osmolarities of rabbit lenses incubated in various experimental conditions after 4 h and 22 h. At 4 h, lenses in both hypotonic (—32 and —64 mosM),

and hypertonic Tyrode's medium (+33 to +66 mosM) had attained complete osmotic equilibrium with the media, and the intralenticular osmolarity values approached osmolarity values very close to those of the media. Of interest, is the decrease of lens osmolarity of 61 mosM when incubated in a medium hypotonic by —67 mosM as compared to an increase of lens osmolarity of 38 mosM when incubated in a medium

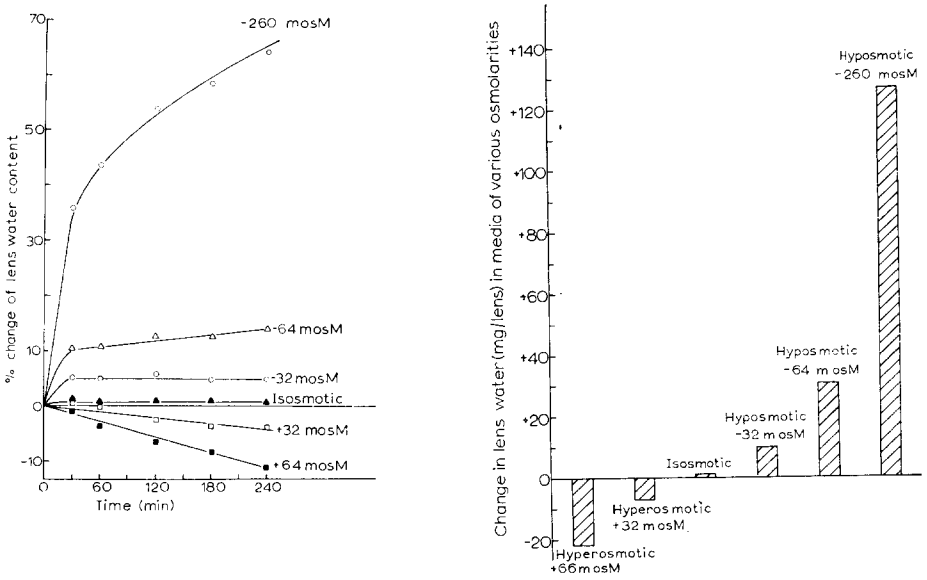

Fig. 1. Change in water content of lenses incubated in isosmotic, hyposmotic, and hyperosmotic Tyrode's media as the percentage of total water in the lenses at the beginning of the experiments. Each curve represents the average of an experimental set of 10 or more lenses.

Fig. 2. Change in water content of lenses incubated in isosmotic and anisosmotic Tyrode's media in mg H_2O per lens, compared to the initial water content at the beginning of the experiments. Each block represents the average of an experimental set of 10 or more lenses.

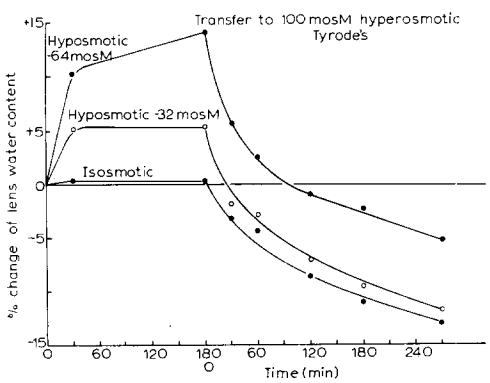

Fig. 3. Change in water content of lenses incubated in isosmotic and hyposmotic Tyrode's media made *plus* 100 mosM hyperosmotic by addition of sucrose, expressed as the percentage of the total water in the lenses at the beginning of the experiments. Each experimental set includes 4 or more lenses.

hypertonic by +64 mosM. These values parallel the water gains or losses shown in Table II.

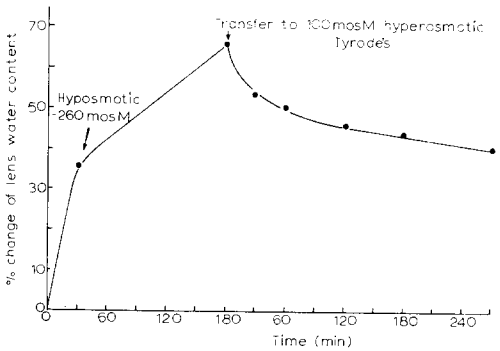

Fig. 4. Change in water content of lenses incubated in —260 mosM hyposmotic Tyrode's media and transferred at 2 h to +100 mosM hyperosmotic media, in percentage of the water present at the beginning of the experiment. The individual dots are the average of 4 lenses.

TABLE II

EFFECT OF OSMOLARITY ON LENS WATER CONTENT

Average of 24 lenses in each group incubated as described in METHODS.

Change of calc. osmolarity (mosM)	*Δ of lens volume at end of 4 h (% of control)*	*Δ of calc. volume to fit a "perfect osmometer" (% of control)*	*Change of lens water (mg/lens) at 4 h*
+ 66	− 11	− 12	− 22
+ 33	− 5	− 6	− 10
Isosmotic	+ 0.5	0	0.6
− 32	+ 5	+ 8	+ 10
− 64	+14	+ 18	+ 31
−260	+64.5*	+432	+127

* +102 % of control at 22 h.

Fitness of the lens to a "perfect osmometer"

From the measurements of intralenticular osmolarities (Table III), it was evident lenses incubated in hypo- or hyperosmolar media (—64 to +66 mosM) were in equilibrium with the environmental fluid at 4 h as no substantial changes in osmolarities occurred at 22 h of incubation. However, in a —260 mosM hyposmotic Tyrode's medium further decreases in intralenticular osmolarities and water gains took place in the interval between 4 and 22 h of incubation, indicating the lens was not equilibrated with the media.

The results in Table II and Fig. 5 show no major discrepancies between experimental and expected values for ΔV (Δ lens volume; Δ lens water) of lenses subjected to osmolarities of —64 to +66 mosM from isotonicity. This would indicate the lens fits a "perfect osmometer" within that experimental range but not when the medium is highly hypotonic.

Efflux of ^{86}Rb from rabbit lenses incubated in media of various osmolarities

The ^{86}Rb effluxes from lenses incubated in media hypotonic to the lens by —32, —64, and —260 mosM, as compared to those incubated in isotonic Tyrode's solution with and without Na^+ substitutions, are shown in Figs. 6–8. The increased ^{86}Rb efflux from lenses in hypotonic media as compared to those lenses incubated in

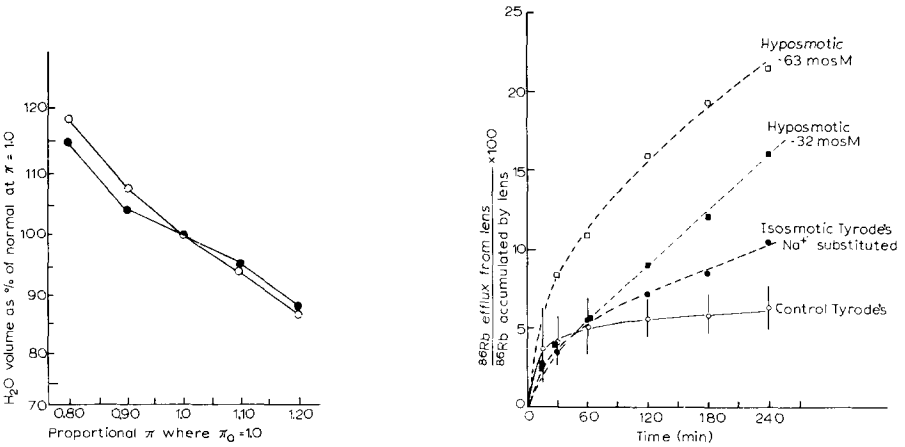

Fig. 5. Experimental (●—●) and expected (○—○) water volume of lenses incubated in media of various tonicities π, where π_0 or isotonicity to the lens = 1.0.

Fig. 6. The efflux of ^{86}Rb from rabbit lenses transferred to isosmotic control Tyrode's medium and to isosmotic Na^+-substituted and —32 and —64 mosM hyposmotic media. Each experimental set represents an average of 4 lenses. Bars represent S.D. of controls.

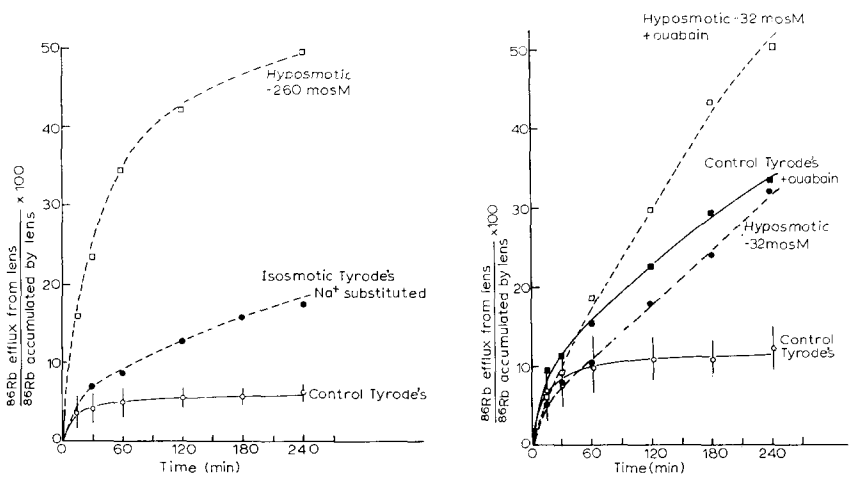

Fig. 7. The efflux of ^{86}Rb from rabbit lenses transferred to isosmotic control Tyrode's medium and to isosmotic Na^+-substituted and —260 mosM hyposmotic media. Each experimental set represent average of 4 lenses. Bars represent S.D. of controls.

Fig. 8. The efflux of ^{86}Rb from rabbit lenses transferred to isosmotic control Tyrode's medium to isosmotic control Tyrode's medium *plus* 0.1 mM ouabain ((Na^+-K^+)-ATPase), to —32 mosM hyposmotic Tyrode's and to —32 mosM hyposmotic Tyrode's *plus* 0.1 mM ouabain ((Na^+-K^+)-ATPase). Each experimental set represents averages of 4 lenses. Bars are S.D. of controls.

TABLE III

EFFECT OF MEDIUM OSMOLARITY ON INTRALENTICULAR OSMOLARITY

Each value average of 2 sets of experiments as described in the text.

Compositions of Tyrode's media	137 mM NaCl + 60.8 mM sucrose	137 mM NaCl + 30.4 mM sucrose	137 mM NaCl	120.4 mM NaCl	102.75 mM NaCl	0 mM NaCl
	Hyperosmotic ←		*Isosmotic*		→ *Hyposmotic*	
Osmolarity of media (measured)	366	333	302	266	235	39
Osmolarity of lens in various media after 4-h incubation	340	322	302	269	239	106
Osmolarity of lens in various media at 24-h incubation	357	325	298	267	234	45

TABLE IV

EFFECT OF MEDIUM OSMOLARITY ON RABBIT LENS Na^+ AND K^+ CONCENTRATION

Each value average of 3 lenses incubated for 1 h and processed as described in the text.

Tyrode's medium osmolarity (mosM)	*ΔH_2O (mg/lens) mean*	*Na^+ in lens (mequiv/kg dry wt.) mean*	*K^+ in lens (mequiv/kg dry wt.) mean*	*Total $Na^+ + K^+$ in lens (mequiv/kg dry wt.) mean*
302	+ 0.5	34.4	247	281.4
237	+11.7	40.0	234	274.0
165.9	+41.2	50.1	220	270.1
102.1	+71.9	59.8	202	261.8

TABLE V

EFFECT OF OSMOLARITY AND Na^+ ON THE ^{86}Rb EFFLUX FROM THE LENS (4 h)

Each value average of 6–8 lenses incubated as described in the text.

Media	*^{86}Rb effluxed into media (% of total ^{86}Rb in lens)*	*Experimental control (total) (%)*	*Experimental control (Na^+ deficit) (%)*	*Experimental control (↓ isomolarity) (%)*
Control Tyrode's (137 mM Na^+)	6.2			
−32 mosM Tyrode's (120.4 mM Na^+)	15.9	9.7	5.0	4.7
−64 mosM Tyrode's (102.75 mM Na^+)	27.4	15.2	4.0	11.2
−260 mosM Tyrode's (0 mM Na^+)	49.0	42.8	11.2	31.6

control Tyrode's medium was due to (1) osmolarity factors, (2) the decreased Na^+ concentration in the media. The contributions of these various fractions to the increased ^{86}Rb effluxes from lens are separated, and analyzed in Table V. In the 4-h experiments, osmolarity accounted for ^{86}Rb efflux increases of 4.7%, 11.2%, and 31.6%, in media hypotonic to the lens by —32, —64, and —260 mosM, respectively.

There was, however, an active ^{86}Rb lens "pump" that could counteract the increased ^{86}Rb effluxes into the media when the osmolarities were varied. To establish whether the osmolarity effects were independent from or associated with a stimulation or deficit of the Na^+–K^+ "pump" in the following series of experiments, the Na^+–K^+ "pump" was suppressed with ouabain. The results in Fig. 8 are indicative of

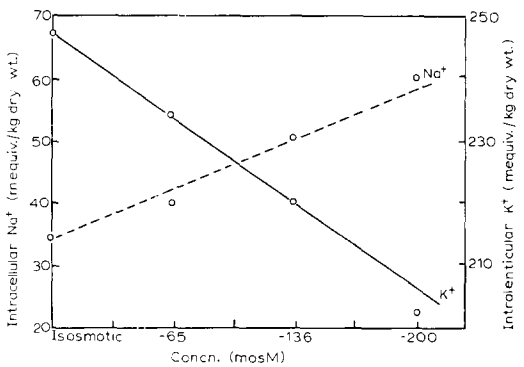

Fig. 9. The Na^+ and K^+ concentrations of lenses (in mequiv/kg dry wt.) incubated for 1 h in Tyrode's media isotonic and hypotonic to the lens.

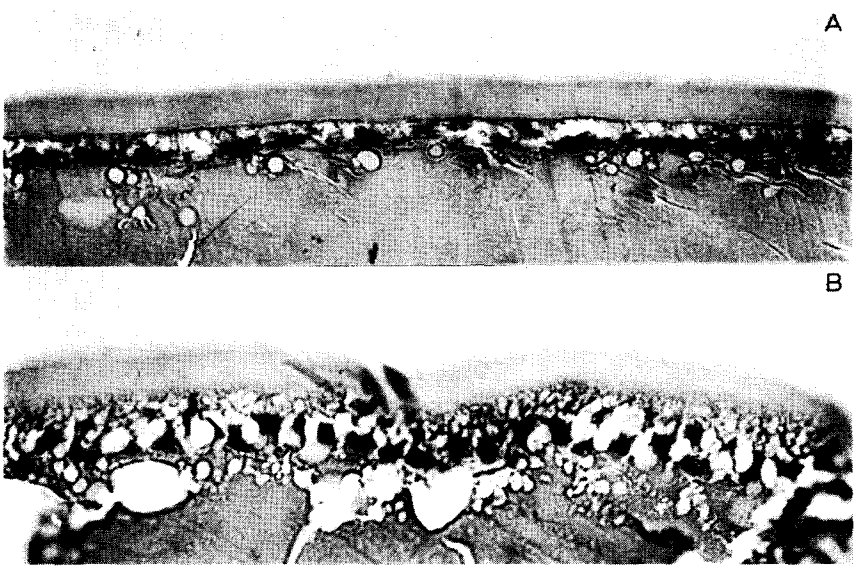

Fig. 10. Section through capsule and lens epithelium of lenses incubated —32 mosM hyposmotic Tyrode's medium (A) and —64 mosM hyposmotic Tyrode's medium (B) for 1 h. In the photograph above (A) notice hydration of the cytoplasm of epithelial cells, flattening of the nuclei and hydropic subepithelial vacuoles. In photograph below (B) marked epithelial and subepithelial vacuolation. (Hematoxylin–eosin staining, magnification 400 ×.)

the independent effects of osmolarity on the ^{86}Rb effluxes from lenses with a suppressed (Na^+-K^+)-ATPase "pump". The percentage of increased effluxes from lenses hypertonic to the media with and without an active (Na^+-K^+)-ATPase are shown in Table VI. At the low medium osmolarities (−260 mosM) there was no additive effect. The disruption of the lens epithelium and fibers evident by histology in these overhydrated lenses provided an explanation for the latter results.

Transport of ^{86}Rb into lens incubated in Tyrode's media of various osmolarities

Tissue to medium accumulation ratios represent approximate estimates of active transport into tissues incubated *in vitro*, when (a) short-term experiments are carried out, and (b) the efflux is only a small and constant fraction of the total transported into the tissue. Due to the high ^{86}Rb efflux from the lenses, the above requirements were not met by the experiments of lenses incubated in media of various osmolarities. To determine the exact net fluxes of ^{86}Rb into lens, the ^{86}Rb losses to efflux were added to that ^{86}Rb accumulated by the tissue. The total ^{86}Rb transported by lenses

TABLE VI

EFFECT OF OSMOLARITY AND (Na^+-K^+)-ATPASE ON ^{86}Rb EFFLUX FROM THE LENS (4 h)

Each value average of 6–8 lenses incubated as described in the text.

Media	*^{86}Rb effluxed into media (% of total ^{86}Rb in lens) − ouabain*	*^{86}Rb effluxed into media (% of total ^{86}Rb in lens) + 0.1 mM ouabain*
Control Tyrode's (137 mM Na^+)	6.2	16.6
−32 mosM (120.4 mM Na^+)	15.9	24.9
−64 mosM (102.75 mM Na^+)	21.4	34.5
−260 mosM (0 mM Na^+)	49.0	50.0

TABLE VII

EFFECT OF OSMOLARITY ON THE TRANSPORT AND UTILIZATION OF GLUCOSE BY THE LENS

Each value average of 4 lenses incubated as described in the text.

Media	*NaCl (mequiv/l)*	*Tris–HCl (mequiv/l)*	*Osmolarity (mosM)*	*% of control*
Isosmotic Tyrode's	137	-	298	100
Hyposmotic Tyrode's	68.5	- -	166	87.5
Na^+-substituted Tyrode's	68.5	68.5		112.0
Hyposmotic Tyrode's	34.25	—	99	63.5
Na^+-substituted Tyrode's	34.25	102.7		122.0
Hyposmotic Tyrode's	0	-	38	12.7
Na^+-substituted Tyrode's	0	137.0		94.6

TABLE VIII

^{86}Rb TRANSPORTED INTO LENS INCUBATED IN TYRODE'S MEDIA OF VARIOUS OSMOLARITIES

Each value average of 6–8 lenses incubated as described in the text.

Media	*Osmolarity (Δ from isotonic)*	*^{86}Rb in lens water / ^{86}Rb in final media at 4 h*	*% of control*	*Accumulated (counts/min per lens)*	*Effluxed (counts/min per lens)*	*Total transported (counts/min per lens)*
Complete Isotonic Tyrode's		30.1	100	274 000	17 300	291 300
Hyposmotic Tyrode's	− 32 mosM	25.2	83	265 000	50 000	315 000
Isotonic Tyrode's Na^+-substituted		31.2	103	277 000	34 300	311 300
Hyposmotic Tyrode's	− 64 mosM	21.8	72	266 000	75 000	341 000
Isotonic Tyrode's Na^+-substituted		32.2	107	278 000	34 300	312 300
Hyposmotic Tyrode's	−260 mosM	1.5	5	74 800	70 300	145 100
Isotonic Tyrode's Na^+-substituted		13.9	46	204 000	45 000	249 000
Hyperosmotic Tyrode's	+ 33 mosM	30.4	101	273 000	14 600	287 600
Hyperosmotic Tyrode's	+ 66 mosM	37.3	124	288 000	14 300	302 300

in various osmolarities in 4-h experiments are shown in Table VIII. Medium hypotonicity of −32 or −64 mosM resulted in 8% and 18% increases in ^{86}Rb transported into lens as compared to lenses in isotonic Tyrode's medium. Thus, a slight increase in the active transport of ^{86}Rb resulted from the gains in water, probably a compensatory mechanism for the increased effluxes of the ion. At much lower medium tonicities (−260 mosM), a decrease in 50% of ^{86}Rb transport into lens, as compared to control lenses in isotonic Tyrode's medium was found. The large gains in lens water of these lenses markedly affected the active cation "pump".

Effect of medium osmolarities on lens K^+ and Na^+ content

The entrance of water into the lens following incubation in hyposmotic media resulted in shifts of the Na^+ and K^+ concentrations of the lens (Fig. 11, Table IV). Lowering medium osmolarity to 102 mosM resulted in 20% losses and 80% increases of the lens K^+ and Na^+ concentrations, respectively, in 1 h. The K^+ effluxes from the lens were a further confirmation of the above experiments on increased ^{86}Rb effluxes in overhydrated lenses. The increased Na^+ concentrations either followed the movement of water as a "solvent drag" or represented the inhibition of the extrusion 'pump' for Na^+.

Histology of lenses incubated in Tyrode's medium of various osmolarities

The fixation of lenses for routine histology resulted in disruption of the hydration of lens fibers and epithelium. The osmolarity of 10% formalin routinely used for tissue fixation was 1430 mosM. It was possible to maintain the tonicity of the fixation

Fig. 11. Section through the equatorial lens fibers demonstrates intrafibrillar vacuoles and interfibrillar accumulation of water. The lens had been incubated in −64 mosM hyposmotic Tyrode's medium for 2 h and fixed in 2.1% formaldehyde (Hematoxylin–eosin staining, magnification 200 ×.)

solution at isotonicity with the normal lenses by using 2.1% formalin. Lenses incubated in +33 and +66 mosM hypertonic Tyrode's media had lost 5% and 14% of the total water content, respectively. However, no histologic evidence of dehydration was found. The lens epithelium, lens fibers and capsule of these lenses were

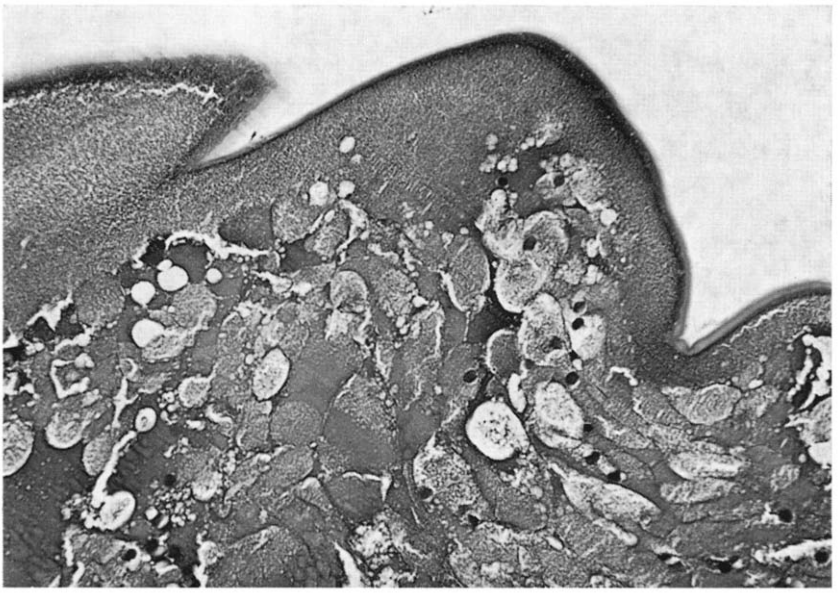

Fig. 12. Section through the equatorial region of a lens incubated —260 mosM hyposmotic Tyrode's medium for 4 h and fixed in 2.1% formaldehyde. The shrinking of the equatorial region induced by fixation is evident in the folds of the lens capsule. Many overhydrated lens fibers at the disrupted lens bow. Swollen fibers with displaced nuclei at the lens box resemble 'balloon-cells' seen in human and experimental cataracts. (Hematoxylin–eosin staining, magnification 400 ×.)

normal. Lenses incubated in —32 mosM and —64 mosM hypotonic solutions had hydropic vacuoles in the cytoplasm of epithelial cells (Figs. 10A and 10B) and lens fibers (Fig. 11), in addition to water-induced separation of lens fibers at the equatorial areas. Otherwise, the capsules were normal, and the lens bow intact. The most striking histologic changes were found in lenses incubated in very hyposmotic (—260 mosM) Tyrode's solutions. In those lenses the cells of the lens epithelium were over-hydrated to the point that many cell membranes were broken. In addition, the lens bow was disrupted (Fig. 12), and nuclei of equatorial cells migrated posteriorly. The lens fibers were separated by large vacuoles and clefts. Many areas showed broken and disrupted lens fibers.

Effect of medium osmolarity on the transport and utilization of glucose, by the lens

The transport and utilization of glucose by lenses incubated in media hypotonic to the lenses by —100 mosM or less were found within normal limits (Table VII). This pointed to the relative insensitivity of glucose transport and utilization mechanisms to the osmolarity changes. At medium osmolarities of 166, 99, and 38 mosM, the glucose transport and utilization by the lenses were 87.5, 63.5, and 12.7% of the corresponding controls in 4-h experiments. Control lenses utilized glucose at a rate of 3–4 mmoles/kg lens per h (mean $\pm$ S.D., 3.58 $\pm$ 0.23).

DISCUSSION

From the above experiments, it was evident that the transport of water into and out of the lens was largely regulated by the osmolarity gradient between the lens and the surrounding media. The increased or decreased water content of the lens were proportional to these osmolarity gradients. As the volume of media was large (2 or 10 ml) compared to the volume of lens, the changes in osmolarity to equalize both sides of the membrane, were evident only in the measurements of intralenticular osmolarities. The lens behaved as a "perfect osmometer" in the range of osmolarities from 238 to 368 mosM. A perfect biological osmometer would be a cell that could hydrate or dehydrate as an artificial physical model. Most cells, however, resist changes in area brought about by changes in water content. This is due to physical limitations of thin cell membranes or the pressure of the adjoining cells. Red blood cells gained or lost sufficient water to remain in equilibrium with the surroundings in the range from 210 to 520 mosM[15]. Red blood cells and lens probably represent the best examples of mammalian cells whose osmotic response approaches that of an artificial osmometer.

The half-time, $t_{1/2}$, for water diffusion towards attainment of a new steady state between lens and media was dependent on the osmolarity gradients. The lens $t_{1/2}$ to equilibrate to —32 mosM hypotonic Tyrode's medium was 5.5 min; to equilibrate to —64 mosM hypotonic was 11 min, and to equilibrate to —260 mosM hypotonic was 160 min. When compared to red blood cells the transport of water into the lens, to equalize osmolarity gradients, was slow. Under an osmotic gradient, the hemolysis of red blood cells suspended in distilled water occurred in 2.4 sec (ref. 15).

Measurements of permeability coefficient (k) of lens were also rather small ($k = 0.36$–0.45 $\mu \cdot min^{-1} \cdot atm^{-1}$) when compared to human erythrocyte ($k = 5.7$ $\mu \cdot min^{-1}$–atm^{-1})[16] or ox erythrocyte ($k = 2.5$ $\mu \cdot min^{-1} \cdot atm^{-1}$). The k values for lens were equal to mouse or rat lymphocytes ($k = 0.41$–0.56 $\mu \cdot min^{-1} \cdot atm^{-1}$), and higher than marine invertebrates eggs ($k = 0.05$–0.30 $\mu \cdot min^{-1} \cdot atm^{-1}$), or rabbit leukocyte ($k = 0.26$ $\mu \cdot min^{-1} \cdot atm^{-1}$)[17]. Our k measurements for the lens were obtained from the changes in volume following minimal (—32 mosM) alterations in osmolarity, and carried the assumption that the capsule was the only barrier to water diffusion into the lens. This point could be debated as the lens epithelium and superficial cortical lens fibers as cell membranes probably offered resistance to the intracellular water transport. On the other hand, as the intralenticular membranes are probably a limiting factor to water transport, the half-time required for osmotic water movement through the lens capsule alone may be considerably less.

A water "pump" independent of the "cation pump" does not operate in the lens. The mean of measurements of intralenticular osmolarities (302 mosM) is superimposable to the mean of rabbit aqueous humor osmolarities (302 mosM) (B. BECKER, personal communication). As the lens is isotonic to aqueous humor it would not require energy to maintain an osmolarity gradient.

The increased ^{86}Rb or K^+ efflux from overhydrated lenses were probably the consequence of the physical stretching of the cell membrane with the opening of additional "pores", changes in the K_m for efflux, or activation of efflux "carriers". That the increased effluxes of ^{86}Rb were not caused by an inhibition of the $(Na^+$-$K^+)$-ATPase is shown by the additional ^{86}Rb efflux in the presence of ouabain. These

increased ^{86}Rb effluxes caused by lens swelling can, however, simulate the increased ^{86}Rb effluxes from inhibition of the (Na^+-K^+)-ATPase. Although minimal swelling results in a stimulation of the ^{86}Rb "pump", the more marked hydrations eventually inhibit active transport by 50%.

The lens electrolytes probably contribute the major fraction of the 302 mosM inside the lens. Although values for K^+, Cl^- and PO_4H^- are available for the rabbit lens, there are no comparable adequate values for CO_3H^-. Furthermore, it is difficult to assess the contribution of free amino acids, inositol, glutathione, ascorbic acid and anionic proteins. In intact red blood cells where hemoglobin concentrations are 34 g/100 ml, its contribution to a total of 285 mosM is only 18.7 mosM[15]. Osmolarity contribution of the lens proteins in concentrations of 330 g/kg wet tissue may not be significantly higher than that of erythrocytes.

Electrolyte inbalances can bring about osmolarity changes that result in water gains by the lens. For instance, when the cation "pump" that regulates the active transport of K^+ and extrusion of Na^+ by the lens is completely abolished by iodoacetate (1 mM), the concentrations of lens K^+ decrease from 125 to 16.4 mequiv/l and the concentration of Na^+ increase from 24 to 148.3 mequiv/l (ref. 19). With an increase in the total concentration of Na^+ *plus* K^+ in the lens, there is a net increase in anion (Cl^- and/or CO_3H^-) and osmolarity to the point that the lens imbibes water (30% increase in total water content)[19]. On the other hand, inhibitors such as ouabain (0.1 mM) with an effect on the lens (Na^+-K^+)-ATPase, do not suppress the "pump" completely and increase in Na^+ concentrations to 71.6 mequiv/l and decrease in K^+ concentrations to 75.2 mequiv/l (unpublished results) result but in 25% increases in Cl^- transport (as measured with ^{36}Cl)[20].

Although this report deals with "freely-transported" water, bound water or water as a part of protein molecules may be significant in the lens. It is accepted that the physicochemistry of protein hydration is complex but that the H_2O dipoles are oriented around the exposed charged groups of the protein molecules. The hydration of protein might be similar to that of electrolytes in solution, may involve an ice-like lattice around the apolar side chain of protein molecules, or may immobilize water between the filaments of intertwining protein molecules[21,22]. Water inside the protein macromolecules could furnish the crystalline state needed for the preservation of lens transparency.

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