Osmotic Relations Between the Fluids of the Rabbit Eye (37495)

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Kinsey and Reddy (1) have reviewed the published data on the osmotic pressure of the rabbit aqueous humor and reports that it is 2.0–6.9 mOsmolal hypertonic to serum obtained from large vessels or from the heart. Kinsey (2) also agrees with Levene (3) that there is no significant difference in osmolality between posterior and anterior chamber aqueous humors. However, Kass and Green (4) reported that aqueous humor from the anterior chamber was significantly hyperosmotic to posterior chamber aqueous and both were hyperosmotic to serum or plasma from the same rabbit. Bleeker, Van Haeringen and Glasius (5) reported an osmotic swelling of the vitreous body of rabbits several hours after the intravenous administration of hyperosmotic amounts of urea. Duncan, Ellis and Paterson (6) reported data on the osmotic relation between anterior chamber aqueous humor and vitreous humor obtained from the same eye and of the relation between the osmolalities of serum and vitreous humor. They also found that, although intravenous osmotic agents did not enter the vitreous body in significant amounts during 1 hr experiments, the vitreous humor osmolality paralleled that of serum.

The report that the crystalline lens incubated in silicone oil secretes a fluid from its anterior surface (7) raises the question of the osmolality of that fluid compared to aqueous and vitreous humors. The lens is bathed with aqueous humor on one side and is covered with the vitreous body on its other side. The osmolality of the vitreous and aqueous humors and of the fluid secreted by the lens all measured by the same osmometer is therefore reported here.

Materials and Methods. Albino rabbits of either sex weighting 2.5–3 kg were used. They were killed by intravenous air embolism. Aqueous humor samples were removed immediately postmortem using a 1 ml disposable syringe with No. 27 needle inserted through the cornea. After the removal of the sclera and retina from the posterior half of the eyeball, vitreous humor was removed directly into needleless 2 ml disposable plastic syringes. All syringes used in this work had been washed three times with triple distilled water and then air dried.

The osmolality of the various fluids obtained from the eye or from the excised lens was determined using a Mechrolab Model 301A vapor pressure osmometer with aqueous solvent probes. The osmometer was operated with the probes and standard solutions maintained at 37 ± 0.002°. A 200 mOsmolal standard NaCl solution was used in the standard vapor cup and on the reference probe. The osmometer was standardized daily using 340 and 280 mOsmolal NaCl solutions. These standard solutions of NaCl were prepared according to the table (8) in the manual supplied with the osmometer which includes corrections for the thermodynamic activity of NaCl in water. Thus the osmolalities are reported in units of millimoles equivalent of osmotically active substance per kilogram of water, abbreviated, mOsmolal. Samples for which sufficient fluid was available were measured two or more times in succession and sample means were used to calculate the means of sets of samples.

The osmolality of fluid secreted by the lens in silicone oil was determined by a modification of the micromethod recommended by the manufacturer of the instrument (9, p. 13). The modification consisted of adding 4 to 10 μl of fluid to a probe that had been freshly washed with 4 or 5 drops of 280 mOsmolal standard solution. Any visible excess fluid on the probe was sucked back

into the syringe containing the wash solution before delivering the sample to the probe.

The fluid secreted by the lens in silicone oil was collected by low speed centrifugal separation from the lens held on a 60 mesh stainless steel screen welded to a stainless steel ring. The screen had a radius of curvature of 5.3 mm and supported the lens with its anterior surface next to the screen under silicone oil in Lucite plastic cups which had a V-shaped depression in the center bottom to collect the centrifugate, Fig. 1. The fluid under silicone oil was removed from the cup directly into the micro-syringes supplied for use with the osmometer.

Between uses the Lucite cups, lens support screens, and syringes were carefully washed then rinsed several times with ordinary distilled water and twice with triple distilled water. They were air dried while protected from dust and laboratory fumes. The screens were handled with forceps washed and dried as described above in order to prevent contamination with osmotically active substances.

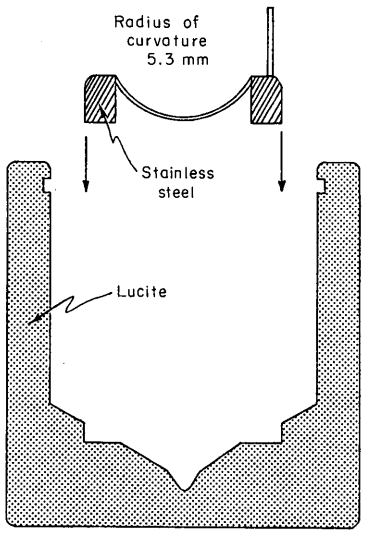

FIG. 1. Sectional drawing of the polymethacrylate centrifuge cup designed to support the stainless steel ring with attached 60 mesh stainless steel lens support screen during low speed centrifugation. For ease of placement or removal the ring has a handle and the cup has a groove machined into the inside surface near the top.

The silicone oil used, Dow Corning 360 Medical fluid, was washed 5–10 times with triple distilled water. It was filtered through a Millipore Mitex filter with 5 μm pores in a 25 mm micro-syringe filter holder. The solubility of water in silicone oil is unmeasurably low[1] as is the case of all chemicals except CO_2 and O_2 known to be present in the humors of the eye.

Results and Discussion. The mean osmolalities ($\pm$ the standard error of the means, SE) of serum, vitreous humor, anterior chamber aqueous humor (obtained immediately postmortem), and the fluid secreted by the lens incubated at 37° in dimethyl siloxane are shown in Table I. The number of animals or eyes used are also shown. In addition to analyzing the data obtained as reported in Table I, the means ($\pm$SE) of the individual differences between the osmolalities of fluids obtained from the same animals or eyes were calculated. The mean difference, osmolality of aqueous humor minus osmolality of vitreous humor, was 10.6 $\pm$ 8.8 mOsmolal (for 18 eyes) which appears to be statistically significant ($p < 0.001$). This level of significance is confirmed by the fact that in 16 of the 18 eyes the difference was positive. The range of values of the difference was from —2 to 28 mOsmolal.

A comparison of the differences in osmolality between serum, and the humors obtained from the same 6 rabbits (9 eyes) was made. The difference (vitreous humor minus serum) was 1.78 $\pm$ 6.9 mOsmolal and the difference (anterior chamber aqueous humor minus serum) was 18.0 $\pm$ 5.9 mOsmolal. Thus from all the data, it appears that anterior chamber aqueous humor removed immediately postmortem is hyperosmotic to serum although the vitreous humor is isosmotic to

[1] Water containing 10^6 cpm/ml of tritium label was shaken for 1 hr with 2 vol of silicone oil. After separation of phases and filtration of the silicone oil through Millipore Mitex filter 1 ml of the oil was mixed with 10 ml of a dioxane based scintillation mixture then counted for a sufficient number of times to have detected an increase >5 in the count rate of the predetermined background of that sample of scintillator in that vial. Only a slight decrease in count rate was obtained.

TABLE I. Osmolality of Fluids from Rabbit Eyes.

	No.	Osmolality (mOsmolal)	SE[a]
Serum	10 animals	302.8	5.9
Anterior chamber aqueous humor	19 eyes	314.1[b]	9.3
Vitreous humor	70 eyes	302.9	7.2
Fluid secreted by the lens in silicone oil	24 lenses	262.4[c]	16.5

[a] SEM.

[b] Aqueous humor is significantly hyperosmotic to each of the other fluids ($p < 0.01$). The fluid secreted by the lens is significantly hypotonic to each of the other fluids ($p < 0.005$).

[c] Corrected for apparent bias in the determination (see text).

serum.

The fluid secreted by the lens in silicone oil is an additional intraocular fluid if the lens secretes a fluid *in vivo* as is highly probable. The osmolality of this fluid was determined by the microdrop method detailed above. Data which justified the method and determines a correction which must be included are given in Table II. The data from the 300 mOsmolal NaCl standard solution indicate that the microdrop method gave slightly high but satisfactory results. However, when 20 μl samples of 295 mOsmolal Tyrode's solution or 300 mOsmolal NaCl standard solution were added under silicone oil to the supporting screens in the Lucite cups, centrifuged, collected, and the osmolality determined using the microdrop method above it was found that the mean osmolalities from 10 determinations on the Tyrode's solution and from 10 determinations on the NaCl standard solutions were each significantly higher than the osmolalities before submission of these solutions to the fluid collection procedure. Thus it is evident that fluid, which contains the major salts of aqueous humor put through the same collection procedure as for fluid secreted by the lens, has an apparent osmolality which is 39.5 mOsmolal high by this microdrop method.

It is well known that at the interfaces of simple salt solutions the concentration of monovalent cations and anions are lower than in the main body of such solutions (10). It was observed that about half of the fluid added to the screen was collected into the cup after centrifugation. Thus a partial separation of water and solutes was achieved because the fluid which remained attached to the stainless steel screen of the lens support contained less solute than the centrifugate whether NaCl solution or the aqueous like solution, modified Tyrode's, was tested. Since the approximately 10 μl of fluid which remained attached to the support screen was spread over at least 2 cm^2 and had two interfaces the enrichment of solutes in the centrifugate is about theoretically correct. It is almost certain that the major osmotically active components of the secreted lens fluid are sodium and potassium salts. All of the secreted fluid was not collected by the centrifugation procedure. Thus a negative correction was correctly applied to determine the

TABLE II. Comparison of Macro- and Microdrop Methods for Determination of Osmolalities and the Effect of Fluid Collection Procedure.

Solution used	Method (drop)	No. of determinations	Osmolality	
			Mean	± SE
300 mOsmolal NaCl standard	Macro	22	299.8	4.8
300 mOsmolal NaCl standard	Micro	36	301.3	8.6
295 mOsmolal Tyrode's	Micro[a]	10	337.3	15.3
300 mOsmolal NaCl standard	Micro[a]	10	338.0	14.7

[a] Samples (20 μl) subjected to the same collection procedure as for fluid secreted by the lens.

osmolality of the fluid secreted by the lens. It is important to emphasize that the centrifugal force used was not sufficient to separate fluid from lenses which had not secreted fluid onto their anterior surfaces. One must conclude from these data that the fluid secreted from the lens in silicone oil is probably hypotonic to vitreous body fluid by approximately 40 mOsmolal and to anterior chamber aqueous humor by as much as 52 mOsmolal.

In none of the reports in the literature has this same type of vapor pressure osmometer been utilized for determination of osmolality of eye fluids nor has a temperature as high as 37° been used previously. One might expect to find small discrepancies in the absolute values for the osmolality of a particular fluid when reported from different laboratories which used different animals, methods, and equipment for the determination. One should not expect to find large discrepancies in the values reported from different laboratories for the *difference* in osmolality between serum and aqueous humor of the rabbit eye, regardless of the methods or equipment used in the measurement, if similar breeds are used as the experimental animal. A rather large discrepancy exists between the results for anterior chamber aqueous reported herein and also by Kass and Green (4) compared to the results reported by other investigators cited above. It should be noted that values for osmolality of rabbit serum cited above and the values reported herein and also by Kass and Green (4) are very close to each other which means that the reported discrepancy is not likely to be due to the experimental procedure used in measuring the osmolality. That the discrepancy is not due to the temperature used for the measurement of tonicity or to the particular animals used, or to changes which took place in aqueous humor as the animals died by air embolism is evident from the agreement between data herein and the data of Kass and Green (4).

The suggestion from the data reported herein that the lens secretes a fluid which is hyposmotic to serum and vitreous humor is consistent with the observation (4) that posterior chamber aqueous is significantly hyposmotic to anterior chamber aqueous. A hyposmotic posterior chamber aqueous humor would be expected if the lens secretes fluid into the posterior chamber whether a fluid slightly hyperosmotic or isosmotic to serum were secreted by the corona ciliaris.

Summary. The osmotic activity of serum and the fluids and humors of the eye was measured (37°) using a vapor pressure osmometer. Vitreous humor was found to be isosmotic to serum. Aqueous humor obtained immediately postmortem was approximately 11 mOsmolal hyperosmotic to serum. On comparison of anterior chamber aqueous humor and vitreous humor obtained from the same eyes, the mean difference was 10.6 ± 8.8 mOsmolal ($p < 0.001$) aqueous hyperosmotic.

The fluid secreted from the anterior surface of rabbit lenses incubated in silicone oil was collected by low speed centrifugal separation. The osmolality of this fluid from 24 lenses was 262.4 ± 16.5 mOsmolal. The fluid secreted by the lens is significantly hyposmotic to each of the other fluids ($p < 0.005$).

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