THE CHEMICAL COMPOSITION AND THE OSMOTIC PRESSURE OF THE AQUEOUS HUMOR AND PLASMA OF THE RABBIT*

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(Received for publication, July 29, 1950)

The primary purpose of this investigation was to obtain information on the relative concentration of those substances in the aqueous humor and plasma which contribute significantly to the osmotic pressure of these fluids. A secondary purpose was to compare the osmotic pressure of aqueous humor from the anterior chamber of the eye with that from the posterior chamber, and with plasma.

While many previous studies of the various constituents of aqueous humor and plasma have been made, there have been none in which all the major constituents have been determined on the same set of samples of aqueous humor and plasma withdrawn essentially simultaneously from unanesthetized animals. Thus the usefulness of the analytical data obtained heretofore as a basis for inferring the mechanism of formation of aqueous humor has been limited. Furthermore, while recent investigations all show that the aqueous humor of cats (Benham, Duke-Elder, and Hodgson, 1938 (1)) and dogs and man (Hodgson, 1938 (2); Roepke and Hetherington, 1940 (3)) is slightly hypertonic to plasma, there have been only four measurements of the osmotic pressure of aqueous humor in rabbits (anesthetized) (Roepke and Hetherington, 1940 (3)) and, to the author's knowledge, none of the relative osmotic pressure of the aqueous humor of the posterior and anterior chambers.

Incidentally, in none of the above experiments has consideration been given to the possibility that the partial pressure of carbon dioxide in the aqueous humor may be different from that in the plasma, and therefore different from the 5 per cent carbon dioxide–oxygen mixtures commonly employed in determining the osmotic pressure. Failure to take into account the actual carbon dioxide tension can give rise to a small systematic error in determining the osmotic pressure.

The general plan of procedure in this investigation was to determine the chief constituents of pooled samples of aqueous humor and plasma taken at approximately the same time. The extent to which the total dissolved substances

* Supported in part by a grant from the Snyder Ophthalmic Foundation, Inc., New York City.

in these fluids was accounted for by chemical analysis was checked by measurement of the total osmotic pressure.

Methods

Albino rabbits, approximately 9 months old, were used in all the experiments. They were fed Purina rabbit chow and given access to water the night before the experiment. To avoid acute changes in normal fluid balance as a result of exercise, animals were removed from the cages, placed gently in baskets, and carried to the laboratory where they were kept as quiet as possible. They were not given water for the hour previous to withdrawal of samples of blood and aqueous humor. No anesthetic was used for taking blood samples; a topical anesthetic (1 per cent pontocaine) was employed for withdrawing aqueous humor. Blood was removed before aqueous humor and if the animals struggled during this operation they were temporarily discarded. To facilitate withdrawal of aqueous humor the rabbits were wrapped in a towel. Only eyes which appeared normal were used.

In experiments in which the osmotic pressure of aqueous humor from single eyes was to be determined, this fluid was removed from the anterior chamber with special micropipettes (4) which could be capped to prevent evaporation during the time required to make the determination. In experiments in which aqueous humor from a number of rabbits was pooled, a larger micropipette (5) was employed to remove samples. Immediately upon withdrawal the aqueous humor was introduced directly into a glass-stoppered weighing bottle. To minimize loss of carbon dioxide, alveolar air was blown into the bottle each time it was opened.

Samples of aqueous humor from the posterior chamber were withdrawn by inserting the needle of the special micropipette through the sclera just posterior to the ciliary body.

Arterial blood was obtained from the rabbits by heart puncture. It was withdrawn into a syringe containing a solution of heparin in sodium chloride which was approximately isotonic with plasma. The volume of heparin solution was less than 1 per cent of the volume of blood. The blood was introduced beneath paraffin oil into centrifuge tubes which were then stoppered and plasma was obtained by centrifuging for 10 minutes at about 2000 R.P.M. Plasma from different animals was pooled in quantities proportional to the amount of aqueous humor used from the same animal.

Osmotic pressure was determined by the thermoelectric method of Baldes (6), using apparatus as modified by Kinsey (7). The determinations were performed in an atmosphere of 5 per cent carbon dioxide in air at 28°C.

Osmotic pressure was expressed in terms of millimols equivalent of sodium chloride and absolute values in these terms were obtained by using standard solutions of sodium chloride. The osmotic activity of other ions and some non-electrolytes, relative to sodium chloride, was determined by measurement against standard solutions of sodium chloride. These relative osmotic activities were determined at approximately the same concentration of sodium chloride as is found in plasma.

The carbon dioxide tension in aqueous humor was shown in separate experiments to be less than that of plasma (corresponding to 3.6 instead of 5 per cent carbon dioxide in air), hence determination of the osmotic pressure in an atmos-

phere containing 5 per cent carbon dioxide will lead to a systematic error because of an increase in the total bicarbonate concentration in the aqueous humor. The magnitude of error introduced in this manner can be readily calculated from the Henderson-Hasselbalch equation and the relative osmotic activity of bicarbonate to sodium chloride. For rabbits with an average bicarbonate concentration of about 34 mM/liter the error would amount to 0.6 mM/liter bicarbonate (0.2 milliosmol per liter equivalent of sodium chloride on the basis of a relative osmotic activity of 0.9). The reported values have been reduced appropriately to correct for this effect.

The conversion of millimols per kilogram of water to milliequivalents per kilogram of water for each ion was performed by multiplying by the appropriate valence, except for phosphate and calcium. For phosphate, a factor of 1.8 was used for the conversion because at the normal pH of plasma and aqueous humor, 20 per cent of the concentration of this radical carries one equivalent of base and 80 per cent carries two equivalents of base. For calcium, the non-diffusible fraction in plasma (45 per cent) was included. The base equivalent of protein as milliequivalents per liter was obtained by multiplying the grams of protein per 100 ml. by the Van Slyke factor of 2.43 (8). The concentration of protein was estimated from the total solids.

Millimols were converted to milliosmols by multiplying by the osmotic activity of each substance relative to sodium chloride.

The conversion of non-protein nitrogen to millimols per kilogram of water was made on the assumption that there are, on the average, two atoms of nitrogen in each molecule of the non-protein nitrogen fraction of aqueous humor and plasma. From present knowledge of the relative concentration of nitrogenous components of the aqueous humor and plasma this assumption is approximately correct.

All the analyses except those for calcium and potassium were performed in duplicate or triplicate.

The following methods were employed for performing the chemical analyses:—

Chloride—titration with mercuric nitrate without removal of protein in the case of plasma (9). All plasma chloride concentrations increased 2 mM per kilogram of water to compensate (10).

Total bicarbonate—Van Slyke gasometric procedure (11).

Ascorbic acid—titration with 2,6-dichlorophenolindophenol in 4 per cent metaphosphoric acid filtrate (12).

Lactate—oxidation to acetaldehyde and colorimetric determination of the product formed with parahydroxybiphenyl (13).

Phosphate—colorimetric method of Fiske and SubbaRow (14).

Sodium—gravimetric method of Butler and Tuthill (15).

Potassium—titrimetric method of Fiske and Litarczek (16).

Magnesium—colorimetric method of Gillam (17).

Calcium—titrimetric method of Fiske and Logan (18).

Non-protein nitrogen—Nessler method described by Folin (19).
Urea—microdiffusion method of Kinsey and Robison (5).
Glucose—colorimetric procedure described by Folin (20).

RESULTS

Duplicate values for the osmotic pressure of the left and right eyes of nine rabbits in comparison with arterial plasma are shown in Table I, part A. The differences in osmotic pressure in the aqueous humor from the two eyes are not significant. The average osmotic pressure of the aqueous humor for the eighteen eyes was 2.5 mM/liter equivalent of sodium chloride higher than that of plasma.

The tonicity of aqueous humor in comparison with plasma of thirteen additional animals is shown in Table I, part B. In five instances (indicated by double daggers) the osmotic pressure of plasma and aqueous humor was measured separately against sodium chloride standards. In three of these animals, and in eight others, the difference in osmotic pressure was determined directly by placing plasma on one thermoelement of the osmometer and aqueous humor on the other element. The osmotic pressure of aqueous humor in these experiments was 3.7 mM/liter equivalent of sodium chloride higher than that of plasma. The average osmotic pressure of aqueous humor in thirty-one eyes (Table I, parts A and B) was 3.0 mM/liter equivalent of sodium chloride higher than that of plasma. This value is approximately twice that reported by Roepke and Hetherington (3) for anesthetized rabbits (1.9 mM/liter equivalent of sodium chloride, uncorrected for carbon dioxide effect).

The results of determining the difference in osmotic pressure between aqueous humor obtained from the anterior and posterior chambers are shown in Table II. The average value for osmotic pressure in the posterior chamber was 0.5 mM/liter equivalent of sodium chloride higher than that in the anterior chamber, and the median was 1.7 mM/liter equivalent of sodium chloride lower than that in the anterior chamber. Considering the variability of the separate values these results indicate that there is no difference in the osmotic pressure of aqueous humor in the anterior and posterior chambers.[1]

[1] In a previous paper (4) the author reported that the concentration of ascorbic acid in the posterior chamber was approximately 1.4 times that of the anterior chamber. In view of the observation that the osmotic pressure in the posterior and anterior chambers appeared to be the same, it seemed desirable to support this finding with some direct analytical evidence. Since chloride could be determined in quantities of aqueous humor as small as 5 to 10 microliters with an accuracy of better than 10 per cent, analyses were made on the concentration of chloride in the anterior and posterior chambers of twenty-two animals. The concentration in the posterior chamber in this series of experiments averaged 3 milliosmols per liter higher than in the anterior chamber. In view of the variation found in the individual eyes this difference in concentration is of questionable significance. It is concluded, therefore, that little, if any, difference in concentration of chloride exists in these two chambers.

TABLE I

Osmotic Pressure of Plasma and Aqueous Humor

Part A

Plasma (arterial)	Difference between aqueous humor left eye and plasma			Difference between aqueous humor right eye and plasma			Difference between left and right eyes
mM/l. equiv. NaCl	*mM/l. equiv. NaCl*		Mean	*mM/l. equiv. NaCl*		Mean	
157.0	+4.4,*	+5.5*	+5.0*	+10.0,*	+9.3*	+9.7*	−4.7
153.3	−3.7,	−2.7	−3.2	+1.5,	+0.4	+0.9	−4.1
155.9	−1.1,	0	−0.8	+2.7,	+4.6	+3.7	−4.5
152.3	−0.7,	+0.7	0	−1.2,	−1.4	−1.3	+1.3
156.4	+2.7,	+3.3	+3.0	+0.9,	+1.7	+1.3	+1.7
159.8	+6.5,	+5.2	+5.9	+6.2,	+5.6	+5.9	0
161.3	+2.4,	+2.3	+2.4	+0.2,	+0.8	+0.5	+1.9
161.8	+3.9,	+4.0	+4.0	+3.3,	+3.1	+3.2	+0.8
155.4	+1.9,	+2.1	+2.0	+2.7,	+2.7	+2.7	−0.7
Average..157.0			+2.0			+2.9	−0.9

Part B

Plasma (arterial)	Aqueous humor	Difference
mM/l. equiv. NaCl	*mM/l. equiv. NaCl*	*mM/l. equiv. NaCl*
154.2	156.8	+2.6*,‡
157.0	161.2	+4.2‡
156.8	158.2	+1.4‡
—	—	+1.2
154.3	155.5	+1.2‡
—	—	+3.0
156.8	158.2	+1.4‡
—	—	+1.2
—	—	+3.3; 2.7; 2.8; 11.1; 5.0; 6.9; 8.7; 3.1

Average..... +3.7 (13 animals)
Median..... +3.1

*The + and − signs indicate aqueous humor hypertonic and hypotonic, respectively, to plasma.

‡ The osmotic pressure of aqueous humor was determined directly against sodium chloride standard. All others measured against plasma.

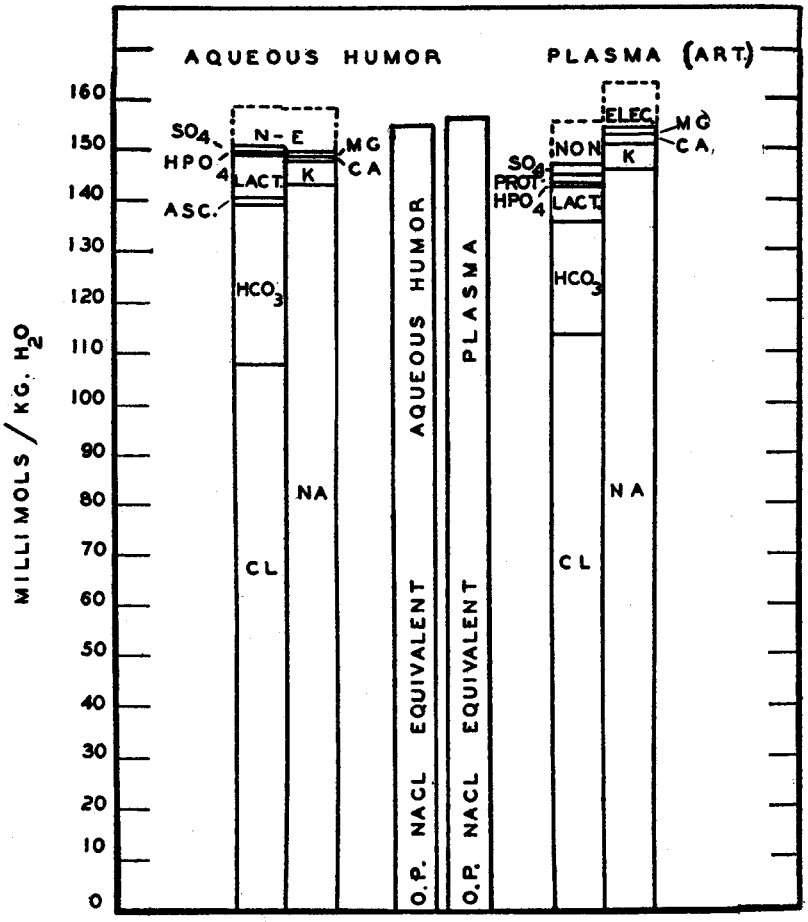

FIG. 1. Histogram of the distribution of major components in the aqueous humor and plasma of rabbits in comparison with the osmotic pressure in terms of millimols equivalent of sodium chloride.

TABLE II

Osmotic Pressure of Aqueous Humor from the Posterior and Anterior Chambers

Difference between osmotic pressure posterior and anterior chambers	Average
mM/l. equiv. NaCl	*mM/l. equiv. NaCl*
+10.1, +9.5	+9.8
−2.2, −1.2	−1.7
+6.7, +6.7	+6.7
+2.6, +2.8	+2.7
−4.2, −1.2	−2.8
−4.6, −3.6, −3.6	−3.9
+4.1, +4.6	+4.4
+4.4, +4.4	+4.4
+0.3, −0.5	−0.1
−4.0	−4.0
−4.3	−4.3
+10.7, +10.1	+10.4
−5.9	−5.9
−4.0	−4.0
−4.3	−4.3
Average	+0.5
Median	−1.7

(+ indicates aqueous humor of posterior chamber hypertonic to that of anterior chamber.)

TABLE III

Composition of the Aqueous Humor and Plasma of the Rabbit

Dissolved substances	Aqueous humor				Plasma (arterial)				Average ratio Aq/Pl
	Experiment No.				Experiment No.				
	11	12	13	Average (72 eyes)	11	12	13	Average (36 animals)	
	millimols/kg. H_2O				*millimols/kg. H_2O*				
Anions:									
Chloride	106.2	108.0	109.5	**107.9**	111.0	113.0	117.5	**113.8**	0.95
Bicarbonate*	33.0	32.4	30.3	**31.9**	21.8	20.7	23.2	**21.9**	1.45
Ascorbate	1.2	1.2	1.2	**1.2**	0.1	0.1	0.1	**0.1**	12
Lactate	9.3	9.8	7.2	**8.8**	7.9	7.2	4.7	**6.6**	1.3
Phosphate	0.6	0.75	0.75	**0.7**	1.0	1.5	1.2	**1.2**	
Sulfate†	0.6	0.6	0.6	**0.6**	1.2	1.2	1.2	**1.2**	
Protein	<0.05	<0.05	<0.05	**<0.05**	2.0 (approx.)	2.0 (approx.)	2.0 (approx.)	**2.0** (approx.)	
Total	150.9	153.0	149.6	**151.1**	145.0	145.7	149.9	**146.8**	1.04 (diff. ions only)
Cations:									
Sodium	142.4	141.7	145.0	**143.0**	143.0	146.0	149.0	**146.0**	0.98
Potassium	6.3 est.	3.2	4.5	**4.7**	6.5 est.	3.2	4.7	**4.8**	
Calcium (diffusible)	1.4	1.2	1.6	**1.4**	1.8	1.8	1.75	**1.8**	
Magnesium§	0.5	0.5	0.5	**0.5**	0.9	0.9	0.9	**0.9**	
Total	150.6	146.6	151.6	**149.6**	152.2	151.9	156.3	**153.5**	0.973
Non-electrolytes:									
Carbonic acid	1.0 est.	1.0	0.9	**1.0**	1.2 est.	1.1	1.2	**1.2**	
Non-protein nitrogen	11.3	12.0	10.5	**11.1**	11.3	13.1	9.5	**11.3**	
Glucose	1.3	7.9	7.6	**5.6**	2.5	8.6	8.8	**6.6**	
Total	13.6	20.9	19.0	**17.7**	15.0	22.8	19.5	**19.1**	
Grand total	315.1	320.5	320.2	**318.4**	312.2	320.4	325.7	**319.4**	
Osmotic pressure, *mM/l. equiv. NaCl*	154.3	154.5	156.0	**155.0**	156.3	154.5	158.2	**156.4**	

Total Solids in Plasma

Experiment No.	Per cent	Conversion factor mM/l. to mM/kg. H_2O
11	7.6	1.055
12	6.5	1.045
13	7.4	1.052

* Average bicarbonate content determined separately in aqueous humor from 20 other eyes and 11 plasma samples was **34.1** and **25.8** mM/kg. water, respectively.

† Data from Heubner and Meyer-Bish (24).

§ Based on determinations performed on samples obtained from 13 other rabbits.

The concentrations of various constituents in aqueous humor and plasma, expressed in millimols per kilogram of water, and some ratios of concentrations in these fluids, are given in Table III. The same data are shown in a conventional histogram in Fig. 1 in comparison with the total osmotic pressure ex-

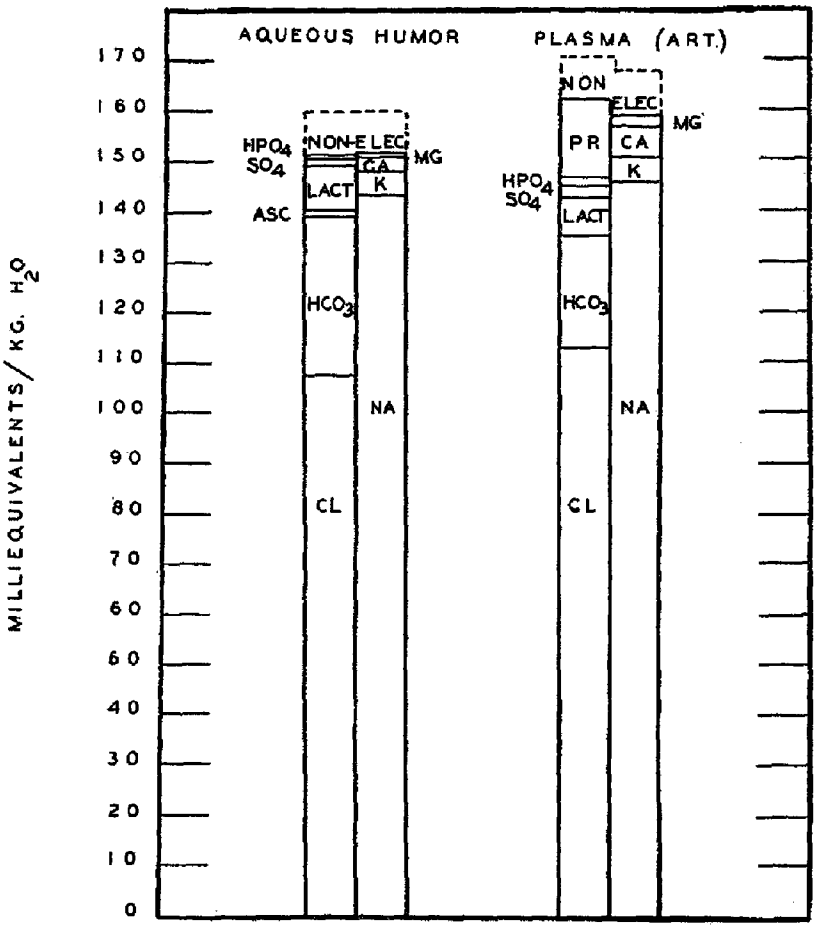

FIG. 2. Histogram of the distribution of charges carried by the major components of the aqueous humor and plasma of rabbits.

pressed as millimols equivalent of sodium chloride. To indicate the variability between the results obtained on different groups of animals, separate values for each group of twelve animals (twenty-four eyes) are presented as well as the average values for thirty-six animals (seventy-two eyes).

The distribution of electric charges and the concentrations of dissolved sub-

stances in the aqueous humor and plasma, expressed in milliequivalents per kilogram of water, are shown in Fig. 2. The total positive and negative charges in aqueous humor are essentially equal. In plasma, the total negative charges appear to be slightly in excess of the total positive charges; possible reasons for this discrepancy will be taken up in the discussion.

DISCUSSION

The validity of comparing measurements of osmotic pressure of relatively simple salt solutions with those obtained with solutions as complex as plasma has been questioned. Roepke (21) has suggested that values for the osmotic pressure of viscous solutions may be incorrect because of the presence of surface films, difference in non-solvent volume, and various other factors associated with such solutions. These possibilities have been investigated by Roepke and Baldes (22) and by Roepke (21) who found that the error incurred with viscous mixtures of hemolyzed blood cells containing 35 per cent dry solids, or egg yolk containing 55 per cent dry material, was less than 2 per cent of the difference in vapor pressure. While these results seem to indicate the adequacy of the thermoelectric method for determining the osmotic pressure of plasma, the availability of crystalline human albumin which had been recently electrodialyzed and shown to be salt-free by conductivity measurements, made it possible to check the adequacy of the method in a viscous solution more nearly resembling plasma.

A solution containing albumin was placed on one thermocouple of the osmometer and distilled water on the other. Duplicate determinations of the osmotic pressure indicated that the albumin solution was +0.4 mM/liter equivalent of sodium chloride in excess of the water. Within the limits of experimental error this corresponds well with the osmotic pressure produced by the albumin, thus indicating that the rate of evaporation of water was not influenced appreciably by the presence of protein in approximately the same concentration as that present in plasma, and therefore that the comparative measurements of osmotic pressure of aqueous humor and plasma are valid.

The indication that the osmotic pressure of the right and left eyes is equal confirms the earlier work by Bárány (23).

The similarity in osmotic pressure, and in chloride concentration, between aqueous humor in the posterior and anterior chambers suggests that water coming from plasma rapidly equilibrates with aqueous humor in the posterior chamber. These observations are in accord with previous determinations of the relatively high degree of permeability for water in the barriers separating aqueous humor and blood.

Some comment is in order concerning the relative composition of the aqueous humor and plasma (Table III). The bicarbonate concentration for pooled samples of aqueous humor and plasma is slightly lower than that obtained in

a series of separate determinations made on other rabbits (Table III, asterisk). The values for the concentration of bicarbonate as given in the footnote probably more nearly represent the concentration existing *in vivo* since some loss of carbon dioxide undoubtedly occurs in making up the pools.

The carbonic acid concentration (1.0 mM/kilo H_2O) was calculated from a determination of the pH of the aqueous humor and plasma, the significance of which will be discussed in another paper.[2]

Because of time limitations in preparing pooled samples, the concentrations for magnesium and sulfate were obtained from fluids taken from animals at another time. Since the contribution of these ions to the total quantity of ions present was less than one-third of 1 per cent, the magnitude of error which might be introduced by using these data is insignificant. In Experiment 11 an estimate for the amount of calcium and non-protein nitrogen was included. This was based on results obtained in experiments 12 and 13.

In Experiment 11, the seemingly low values observed for glucose, both in aqueous humor and plasma, were checked but no source of error was found. The explanation for these low values is not apparent.

The ratio of concentration of substances in the aqueous humor compared with plasma (Table III) indicates that some individual ions in the aqueous humor, for example, bicarbonate, are far from being in equilibrium with those in the plasma. These results indicate clearly that the aqueous humor cannot be static. The ratios for the distribution of total positive and negative diffusible ions were 0.973 and 1.04, respectively. This kind of distribution results from the difference in protein content of the two fluids, and, although approximately that required by the Gibbs-Donnan theory, has no especial significance with respect to the mode of formation of aqueous humor.

As stated in the introduction, the estimation of the total concentration of dissolved substances from the osmotic pressure makes it possible to estimate the completeness with which the analytical data account for all the solutes in aqueous humor and plasma. In making such an estimate it is necessary to determine whether each millimol of substance present contributes 1 milliosmol equivalent of sodium chloride to the osmotic pressure.

Measurements were made for sodium bicarbonate, sodium lactate, and glucose by adding the same quantity of these substances, as found in the plasma, to solutions of sodium chloride of such strength that the total would be equal to 150 mM/liter, and measuring the osmotic pressure of these solutions against a 150 mM/liter solution of sodium chloride. In this manner the total ionic strength was made approximately equal to that of plasma.

The relative osmotic activity of lactate and glucose did not differ sufficiently from that of sodium chloride to introduce a significant error. The apparent osmotic pressure of sodium bicarbonate solutions, however, was only 0.9 that of the same concentration of sodium chloride. The same value for the relative

[2] Kinsey, V. E., A unified concept of aqueous humor dynamics and the maintenance of intraocular pressure; an elaboration of the secretion-diffusion theory, *Arch. Ophth.*, 1950, **44**, 215.

osmotic activity of sodium bicarbonate was observed by determining the osmotic pressure of plasma to which additional bicarbonate had been added. Thus the contribution of sodium bicarbonate to the osmotic pressure of the aqueous humor and plasma was only 0.9 that of an equivalent concentration of sodium chloride. Since the osmotic pressure was measured in terms of sodium chloride equivalent, it was necessary to correct for this effect in comparing the total osmotic pressure of dissolved substances as determined analytically with that obtained by osmometry. This correction was made assuming the 0.9 factor to apply equally to bicarbonate and to an equivalent amount of sodium. The results are shown in Table IV in comparison with the total osmotic pres-

TABLE IV

Summary of Data on the Composition of Aqueous Humor and Plasma Corrected for Osmotic Activity Relative to Sodium Chloride

Dissolved substances	Aqueous humor	Plasma (arterial)
	milliosmols/kg. H_2O	*milliosmols/kg. H_2O*
Anions	147.7	144.8
Cations	146.4	151.5
Non-electrolytes	17.9	19.1
Total (by chemical analysis)	312.0	315.4
Total* (by osmotic pressure determination)	310.0	312.7
Percentage of dissolved substances present accounted for by chemical analysis	100.6	100.8

* Expressed as milliosmol equivalent of sodium or chloride.

sure as determined by direct measurement. All the results are expressed as milliosmols per kilogram of water. The agreement between the totals to within better than 1 per cent is well within the experimental errors. Thus the chemical analyses apparently have accounted for all the dissolved substances which contribute appreciably to the osmotic pressure of the aqueous humor and plasma. It seems probable that the total concentration of substances other than those for which analyses were made is less than 2 mM/liter.

A comparison of the measured osmotic pressure of the aqueous humor and plasma (Table III) indicates that for each experiment the fluids were essentially isosmotic. This result does not agree with the average measurements obtained on individual rabbits (Table I). In all but four of the cases the osmotic pressure of the aqueous humor exceeded that of the plasma by an average of approximately 3 mM/liter equivalent of sodium chloride (6 milliosmols per liter). That errors in the determination of osmotic pressure are not responsible for the difference in results is suggested by the agreement (within 2 mM/liter

equivalent of sodium chloride) between duplicate determinations, and the similarity of total concentration of dissolved substances in the aqueous humor and plasma, as found by chemical analysis.

The explanation for this discrepancy presumably lies in the difference in procedures used in making the osmotic pressure measurements on individual rabbits and those in the experiments in which pooled samples were employed, but the particular factor responsible is not apparent.

Neither evaporation nor loss of carbon dioxide coincident with pooling the aqueous humor or plasma could account for the discrepancy, since any evaporation which might occur would contribute equally toward increasing the osmotic pressure of both the aqueous humor and plasma. Loss of carbon dioxide, while affecting the analytical results, would not influence the relative osmotic pressure of the aqueous humor and plasma because, as stated above, the determinations were made in an atmosphere containing 5 per cent carbon dioxide which approximately reestablishes the original physiologic balance between carbon dioxide and bicarbonate.

Two possible explanations for the observed differences occur to the author. In a series of separate experiments the osmotic pressure of plasma was found to increase approximately 4 milliosmols after standing for 3 hours at room temperature. This change was not observed with aqueous humor. In the pooled experiments approximately 3 hours elapsed between obtaining the first plasma sample and performing the osmotic pressure measurements, thus a similar increase in osmotic pressure may have occurred.

Second, it seems possible that while the animals were handled gently to avoid exercise which is known to increase the osmotic pressure of the blood, excitement coincident with being brought in groups into the laboratory may have affected the animals in such a manner as to increase slightly the osmotic pressure of the plasma.

Whatever the explanation, this difference in results in the relative osmotic pressure of aqueous humor and plasma in the experiments in which pooled samples were used, and those in which individual samples were employed, did not lessen the usefulness of osmotic pressure determinations as an indication of the extent to which all the major constituents of the aqueous humor and plasma could be accounted for by analytical means.

The distribution of charges in aqueous humor (Fig. 2) was equal. In plasma, however, a slight excess of negative ions appeared to be present. Clearly this cannot be the case. The values reported may result simply from accumulation of experimental errors, or more likely, in the author's opinion, from the necessity of estimating the charge carried by protein from data on horse serum obtained by Van Slyke (8). It is apparent that a slight error in estimating this charge could give rise to a relatively large error in the total charge carried by this anion.

SUMMARY

Measurements were made of the osmotic pressure of plasma, and of aqueous humor taken from the anterior chamber of the right and left eyes and from the posterior chamber of unanesthetized rabbits. Aqueous humor from the anterior chamber was found to be hypertonic to the plasma by approximately 3 mM/liter equivalent of sodium chloride. The aqueous humor from the anterior and posterior chambers of the right and left eyes was isotonic. The concentration of chloride in the anterior and posterior chambers was the same.

The concentration of all the major components of the aqueous humor and plasma has been determined by chemical analysis on fluid samples obtained from unanesthetized rabbits at approximately the same time. The calculated osmotic pressure of the total of these substances in terms of sodium chloride equivalent agrees to within better than 1 per cent of the total osmotic pressure as measured experimentally.

The distribution of some individual anions and cations of the aqueous humor and plasma was determined. This distribution is widely different from that which would obtain at a state of equilibrium. The positive and negative charges carried by the ions in the aqueous humor were approximately equal. Sources of error in the experiments are discussed.

The author wishes to thank Mrs. June Pelham, Miss Elaine Pekarski, and Miss June Twomey for technical assistance, and Dr. Frederic Merriam for performing the lactic acid analyses.

BIBLIOGRAPHY

1. Benham, G. H., Duke-Elder, W. S., and Hodgson, T. H., The osmotic pressure of the aqueous humour in the normal and glaucomatous eye, *J. Physiol.*, 1938, **92,** 355.
2. Hodgson, T. H., Studies on the aqueous humour in normal and glaucomatous eyes, *Tr. Ophth. Soc. U. Kingdom*, 1938, **58,** 87.
3. Roepke, R. R., and Hetherington, W. A., Osmotic relation between aqueous humor and blood plasma, *Am. J. Physiol.*, 1940, **130,** 340.
4. Kinsey, V. E., Dehydroascorbic acid—ascorbic acid in the aqueous humor of rabbits, *Am. J. Ophth.*, St. Louis, 1950, **33,** 257.
5. Kinsey, V. E., and Robison, P., Micromethod for the determination of urea, *J. Biol. Chem.*, 1946, **162,** 325.
6. Baldes, E. J., A micromethod of measuring osmotic pressure, *J. Scient. Instr.*, 1934, **11,** 223.
7. Kinsey, V. E., Modification of the apparatus for the Baldes thermoelectric method of measuring osmotic pressure, *J. Scient. Instr.*, 1950, **21,** 767.
8. Van Slyke, D. D., Hastings, A. B., Miller, A., and Sendroy, J., Jr., Studies of gas and electrolyte equilibria in blood. XIV. The amounts of alkali bound by serum albumin and globulin, *J. Biol. Chem.*, 1928, **79,** 769.

9. Schales, O., and Schales, S. S., A simple and accurate method for the determination of chloride in biological fluids, *J. Biol. Chem.*, 1941, **140,** 879.
10. Kinsey, V. E., Aqueous humor/plasma chloride ratios in rabbits, dogs, and human beings, *J. Gen. Physiol.*, 1949, **32,** 329.
11. Peters, J. P., and Van Slyke, D. D., Quantitative Clinical Chemistry. Volume 2. Methods, Baltimore, The Williams and Wilkins Co., 1932.
12. Mindlin, R. L., and Butler, A. M., Determination of ascorbic acid in plasma; macromethod and micromethod, *J. Biol. Chem.*, 1938, **122,** 673.
13. Barker, S. B., and Summerson, W. H., The colorimetric determination of lactic acid in biological material, *J. Biol. Chem.*, 1941, **138,** 535.
14. Fiske, C. H., and SubbaRow, Y., The colorimetric determination of phosphorus, *J. Biol. Chem.*, 1925, **66,** 375.
15. Butler, A. M., and Tuthill, E., An application of the uranyl zinc acetate method for determination of sodium in biological material, *J. Biol. Chem.*, 1931, **93,** 171.
16. Folin, O., Laboratory Manual of Biological Chemistry, New York, D. Appleton-Century Co., Inc., 5th edition, 1934, 353.
17. Gillam, W. S., A photometric method for the determination of magnesium, *Ind. and Eng. Chem., Anal. Ed.*, 1941, **13,** 499.
18. Fiske, C. H., and Logan, M. A., The determination of calcium by alkali-metric titration. II. The precipitation of calcium in the presence of magnesium, phosphate, and sulfate, with applications to the analysis of urine, *J. Biol. Chem.*, 1931, **93,** 211.
19. Folin, O., Laboratory Manual of Biological Chemistry, New York, D. Appleton-Century Co., Inc., 5th edition, 1934, 265.
20. Folin, O., The micro method for the determination of blood sugar, *New England J. Med.*, 1932, **206,** 727.
21. Roepke, R. R., Thesis, A study of the osmotic properties of blood by the thermoelectric method, University of Minnesota, 1938.
22. Roepke, R. R., and Baldes, E. J., A critical study of the thermoelectric method of measuring vapor pressure, *J. Biol. Chem.*, 1938, **126,** 349.
23. Bárány, E., The relative importance of ultrafiltration and secretion in the formation of aqueous humour as revealed by the influence of arterial blood pressure on the osmotic pressure of the aqueous, *Acta physiol. scand.*, 1947, **13,** 81.
24. Heubner, W., and Meyer-Bish, R., Über den Sulfatgehalt im Blutserum und Kammerwasser, *Biochem. Z.*, 1926, **126,** 184.