J Forensic Sci, Jul. 1972, Vol. 17, No. 3

W. Q. Sturner,[1] *M.D., A. B. C. Dowdey,*[1] *M.D.,*
R. S. Putnam, [1]*M.D., and J. L. Dempsey,*[1] *B.S.*

Osmolality and Other Chemical Determinations in Postmortem Human Vitreous Humor

The measurement of osmotic pressure in blood and urine has been used extensively in clinical investigation and is beginning to be available as a diagnostic test in the hospital laboratory [*1–4*]. It is dependent on the number of solute particles in solution, rather than their shape, weight, or charge. The determination of the freezing point depression of a solution gives a good estimate of the number of particles and is the basis for the calculation of the osmolality. Serum osmolality has a normal range of 275 to 295 mOsm /kg, with electrolytes, particularly sodium, contributing over 90 percent of this value. Glucose, nonprotein nitrogen, and protein substances make up a large portion of the remainder. The average osmolality of urine ranges from 300 to 1090 mOsm /kg, varies with dietary intake, and is generally higher in males [*3*]. The mean value for cerebrospinal fluid osmolality in fifty living patients was found to be 281 mOsm /kg, and the range of values extended from 269 to 304 mOsm /kg [*5*]. Postmortem spinal fluid studies to date have not included osmolality determinations.

Specimens of vitreous humor from the eyes of human subjects are easily withdrawn following death and have been submitted to a number of chemical [*6,7*] and more recently, toxicologic determinations [*8,9,10*]. The concentrations of vitreous humor electrolytes (with the exception of potassium) and other substances such as urea nitrogen, reflect terminal serum concentrations and are maintained several days following death in the absence of severe putrefaction [*6*]. It was believed that osmolalities in vitreous humor, to our knowledge not previously recorded, should be obtained and compared to other measurable factors in this medium. Since determination of osmolality is essentially a nondestructive testing technique, its performance does not compete for sample with other desired studies.

Materials and Methods

Forty-five specimens were obtained by inserting a no. 20 needle attached to a 12-ml disposable plastic hypodermic syringe into the lateral sclera of each eye, with approximately 4 ml of vitreous humor being collected from each subject. The specimens were frozen in rubber-stoppered, chemically clean test tubes and analyzed at a later date. The subjects involved died from various traumatic and natural disease processes. Each was a case of sudden death at the scene or was dead on arrival at a local hospital. No parenteral fluid or medication had been terminally administered.

Received for publication 22 Dec. 1971; accepted for publication 26 Jan. 1972.

[1] Department of Pathology, University of Texas Southwestern Medical School of Dallas and the Southwestern Institute of Forensic Sciences, Dallas, Tex.

TABLE 1—*Osmolality and other chemical studies in vitreous humor.*

Case Number	Age	Sex	Cause of Death	Post-mortem Interval, h	Blood Alcohol, (g/100 ml)	Osmo-lality, mOsm/kg	Na	K	CO_2	Ca	Glucose	VUN	Uric Acid	SGOT
1	31	M	Craniocerebral trauma	15	0.14	332	145	7.3	12	6.0	45	10	1.3	61
2	70	M	Arteriosclerotic heart disease	1	neg.	300	144	6.0	18	6.0	70	17	1.5	21
3	24	M	Multiple injuries	4	neg.	294	143	6.1	15	4.8	58	14	1.0	128
4	26	M	Intravenous narcotism	7	0.166	352	147	7.0	12	6.0	45	12	1.4	30
5	49	M	Multiple injuries	12	0.33	385	143	8.0	11	6.5	29	7	3.0	21
6	27	M	Craniocerebral trauma	11	0.01	306	147	7.2	14	5.5	40	9	0.7	85
7	51	F	Craniocerebral trauma	18	neg.	317	151	9.0	11	6.8	21	8	2.1	40
8	47	M	Myocardial infarction	15	neg.	321	151	7.7	7	6.5	90	10	1.3	30
9	70	M	Stabwounds	4	neg.	299	139	5.0	12	6.2	280	10	1.4	37
10	55	M	Multiple injuries	15	neg.	310	147	7.6	12	5.4	45	16	1.6	40
11	34	M	Tuberculosis	3	neg.	301	143	6.8	13	6.1	33	3	1.5	53
12	14	F	Myocardial infarction (congenital heart disease)	5	...	248	144	6.9	11	5.3	49	4	0.8	36
13	63	F	Multiple injuries	2	neg.	303	146	5.3	13	6.0	95	15	1.0	35
14	2	F	Darvon poisoning	2	neg.	299	143	6.6	16	5.9	51	14	0.7	55
15	19	M	Rupture of bowel; peritonitis	3	neg.	307	146	5.4	13	6.4	125	17	1.3	25
16	52	F	Multiple injuries	5	0.13	340	145	6.5	14	5.8	55	23	1.3	65
17	40	F	Craniocerebral trauma	5	0.08	333	146	7.5	11	6.0	50	10	0.8	95
18	39	M	Intravenous narcotism	6	0.005	342	159	9.1	13	6.7	40	15	1.8	41
19	22	M	Craniocerebral trauma	9	0.26	379	148	7.2	11	6.3	49	10	1.8	60

20	54	M	Gunshot wound	18	neg.	306	144	8.0	11	6.1	30	16	1.3	33
21	54	F	Gunshot wound	17	neg.	320	153	8.3	6	5.0	45	16	1.4	36
22	48	M	Arteriosclerotic heart disease	5	neg.	296	145	6.1	14	5.0	39	10	1.5	5
23	50	F	Drowning	3	neg.	298	145	5.0	17	5.5	75	5	0.9	69
24	49	F	Multiple injuries	14	0.03	321	147	7.7	7	6.2	50	9	1.3	47
25	49	M	Arteriosclerotic heart disease	22	neg.	307	138	+10.0	7	5.4	9	14	1.9	13
26	40	M	Gunshot wound	17	0.13	321	147	8.5	9	3.7	20	14	1.5	16
27	35	M	Fatty cirrhosis of liver	4	neg.	299	141	6.4	11	6.1	40	4	1.5	5
28	14	M	Craniocerebral trauma	17	neg.	302	145	9.2	10	3.5	15	8	1.2	10
29	35	F	Rupture of aortic aneurysm	16	neg.	311	149	7.5	6	4.4	40	13	0.9	25
30	46	M	Gunshot wound	16	0.03	314	147	7.8	7	4.3	30	8	1.5	15
31	59	M	Coronary thrombosis	23	neg.	310	150	8.1	7	5.2	40	8	1.9	30
32	2	M	Asthmatic bronchitis	12	neg.	315	137	+10.0	6	3.7	15	14	2.1	197
33	1	M	Craniocerebral trauma	24	neg.	305	142	+10.0	8	4.5	26	17	0.8	15
34	50	M	Alcohol and barbiturate poisoning	22	0.20	397	145	+10.0	2	7.3	20	7	4.0	12
35	46	M	Arteriosclerotic heart disease	2	neg.	300	144	8.4	10	5.0	20	13	1.5	7
36	19	M	Gunshot wound	13	0.198	340	147	7.3	12	5.1	40	14	0.9	19
37	40	M	Hanging	18	...	295	143	8.1	8	3.2	22	10	1.5	14
38	54	M	Rupture of liver	14	0.06	299	135	7.4	9	3.9	25	9	1.7	15
39	18	M	Multiple injuries	4	neg.	298	142	8.7	12	2.6	45	11	1.2	41
40	18	M	Gunshot wound	3	neg.	300	145	6.9	13	5.2	40	8	1.2	11
41	49	M	Multiple injuries	5	0.24	369	151	8.8	12	5.4	20	5	1.5	5
42	40	M	Cerebral hemorrhage	17	neg.	308	146	9.1	4	5.0	35	16	2.3	10
43	18	M	Gunshot wound	2	neg.	295	145	6.7	11	2.7	40	9	1.0	10
44	2	F	Microcephaly	4	neg.	332	158	7.5	6	5.5	75	27	2.0	30
45	18	M	Multiple injuries	18	neg.	304	148	9.2	12	3.8	20	12	1.5	10

The osmometer employed was the Cryomatic, Model 31CM[2] which requires a 2-ml sample for a single determination. The results obtained with this instrument were checked by using the manual, *Advanced Wide Range Osmometer*, manufactured by the same company. Twenty independent replicate determinations were performed on samples of vitreous humor using each instrument and a comparison of the results obtained revealed a correlation coefficient of 0.99428, using the method of least squares [*11*]. The regression equation is Osmolality (manual) = $-3.8344 + 1.0047 \times$ Osmolality (automated). Precisions of the two methods may be expressed by their standard deviations. They are 0.8257 and 0.9293 for the manual and automated procedures respectively, based on the differences between 22 sets of duplicate assays on each instrument. The F value for the variances (S.D.^{2}) is 1.2666. From these data, there is probably no significant difference in the values obtained from the two instruments.

Biochemical profiles were obtained of the original specimens using the Model SMA-12 Auto Analyzer.[3] Ethyl alcohol was determined in the blood using standard gas chromatographic methods. Specimens of pooled vitreous humor were prepared by combining the contents of 22 random samples and refreezing 2-ml aliquots in B-D Vacutainer test tubes. At periodic intervals over twenty months, a single aliquot was submitted for osmometry using the Cryomatic, Model 31CM, and other chemical analyses were performed using routine microchemical methods.

Results

The values obtained in the 45 cases with pertinent information concerning age, sex, cause of death, blood alcohol concentration, and postmortem interval are listed in Table 1. In four instances the remaining sample was insufficient to reanalyze for a high potassium concentration and these results are indicated with the plus (+) sign indicating greater than. The blood alcohol concentration was not determined in two instances. The results of the analyses of the pooled samples are shown in Table 2.

Discussion

A wide range of osmolalities were found in the specimens of vitreous humor. Using a percent cumulative frequency distribution curve plotted on probability paper, we estimated the range (98 percent) for osmolality in all cases to be from 280 to 350 mOsm/kg. The standard deviation was not calculated for the total number of cases because of the abnormal distribution of the values. The numerous factors inherent in natural disease, immediate response to injury, and autolytic processes following death undoubtedly contributed to this variation. No relationship between osmolality and the postmortem interval or manner of death was evident.

However, the osmolality was noted to be increased proportionally to the concentration of ethyl alcohol present in the blood. This supports the findings of Redetski et al and others in studies done on serum from living patients [*12,13*]. Lactic acid produced within the subject, as well as the congener content of the ingested beverages have been shown to alter the expected osmolality. In those cases in our series which were negative for alcohol (28) the ± 2 standard deviation normal range is 288 to 323 mOsm/kg, with a mean value of 305.7 mOsm/kg [*11*].

The concentrations of other chemical substances obtained in this group revealed occasional alterations. In one patient inflicted with multiple stab wounds, the glucose

[2] Advanced Instruments, Inc., Newton Highlands, Mass.

[3] Technicon Instruments Corp., Ardsley, N.Y.

TABLE 2—*Vitreous humor—pooled specimens.*

Date	11/21/69	12/22/69	1/19/70	2/10/70	3/10/70	4/10/70	5/18/70	6/17/70	7/28/70	8/28/70	10/22/70	11/23/70	2/1/71	3/4/71	4/26/71	7/1/71	8/4/71
Osmolality	305	305	306	308	312	298	308	308	307	298	315	304	312	303	303	302	308
Sodium	138	139	139	139	139	142	140	144	133	138	140	140	145	141	140	141	141
Potassium	8.4	8.0	8.0	8.4	8.2	8.0	8.5	8.8	8.0	7.8	8.4	7.7	8.5	7.8	8.4	9.5	8.4
Chloride	119	122	121	122	121	121	121	121	119	121	122	121	125	123	124	124	122
Uric Acid	2.3	2.5	2.5	2.4	2.4	1.2	2.5	2.0	2.0	2.2	1.6	1.5	2.1	1.6	1.6	1.6	1.4
Total Protein	20	40	40	70	23	50	40	50	30	42	14	35	80	34	50	59	46
Urea Nitrogen	14	13	14	16	12	14	13	17	14	13	14	12	16	15	13	17	14
Glucose	34	37	18	18	29	35	QNS	34	35	39	35	42	30	36	34	38	36

Osmolality values given in mOsm/kg.
Electrolyte values given in mEq/l.
Uric Acid, total protein, urea nitrogen and glucose values given in mg/100 ml.

value of the vitreous humor was 280 mg% without a corresponding increase in osmolality. The pancreas was free from disease and injury and there was no history of diabetes mellitus. We have been unable to explain this observation. Another finding of interest was a depression of the calcium concentration in several traumatic cases. The concentration of calcium in the vitreous humor approximates half that of the normal serum range of 8.8 to 10.5 mg% [*14*]. A value of less than 4.0 mg% may indicate a hypocalcemic state, but we have observed only a limited number (6) of such cases and these have not included relevant clinical data. Furthermore, Coe [*6*] has suggested that abnormal calcium concentrations, as indicated by antemortem serum findings, may not be reflected as such in postmortem vitreous humor analyses. We have been unable to document a decrease of vitreous humor calcium in a group of infants dying from a variety of causes, including the crib death syndrome [*15*]. As expected, those instances in which sodium was elevated there was a proportional increase in osmolality, but the small number of such cases prohibited separate statistical analysis.

The frozen pooled specimens of vitreous humor which were tested during regular intervals for twenty months showed no significant alterations from their original values. Two specimens revealed decreased glucose and may have been contaminated with microorganisms, but bacteriologic studies were not performed. The concentrations of potassium and total protein were somewhat variable and uric acid showed a mild depression, but the other substances remained fairly constant over the period of study.

Summary

The osmolalities of forty-five human postmortem vitreous humor specimens were determined. The normal range was estimated to be from 280 to 350 mOsm/kg. No correlation with time and manner of death was observed, but the higher values corresponded to the amount of ethyl alcohol present. In those instances in which ethyl alcohol in the blood was not detected, the standard deviation range was 288 to 323 mOsm/kg. Measurements of other chemical substances were recorded and calcium depression was noted in some traumatic cases. Pooled samples of frozen vitreous humor showed only minor changes during periodic analyses over an interval of twenty months.

References

[*1*] Abele, J. E., "The Physical Background to Freezing Point Osmometry and its Medical-Biological Applications," *American Journal of Medical Electronics*, Jan.-March, 1963, pp. 32–41.

[*2*] Holmes, J. H., "Measurement of Osmolality in Serum, Urine and Other Biologic Fluids by the Freezing Point Determination," preworkshop Manual of the Workshop of Urinalysis and Renal Function Studies, Advanced Instruments, Inc., Newton Highlands, Mass.

[*3*] Lobdell, D. H., A Practical Guide to Clinical Osmometry, *St. Vincent's Hospital Medical Bulletin*, Vol. 8, No. 2, 1969, pp. 7–15.

[*4*] Warhol, R. M., Eichenholz, A., and Mulhausen, R. O., "Osmolality," *Archives of Internal Medicine*, AIMDA, Vol. 116, 1965, pp. 743–749.

[*5*] Goldberg, N. B., Sawinski, V. J., and Goldberg, A. F., "Human Cerobrospinal Fluid Osmolality at 37 C," *Anesthesiology*, ATSYA, Vol. 26, 1965, p. 829.

[*6*] Coe, J. I., "Postmortem Chemistries on Human Vitreous Humor," *American Journal of Clinical Pathology*, JCPAA, Vol. 51, 1969, pp. 741–749.

[*7*] Sturner, W. Q. and Gantner, G. E., Jr., "The Postmortem Interval—A Study of Potassium in the Vitreous Humor," *American Journal of Clinical Pathology*, JCPAA, Vol. 42, 1964, pp. 137–144.

[*8*] Coe, J. I., "Comparative Study of Postmortem Vitreous Humor and Blood Alcohol," *Journal of Forensic Sciences*, JFSCA, Vol. 15, 1970, pp. 185–190.

[*9*] Felby, S. and Olsen, J., "Comparative Studies of Postmortem Barbiturate and Meprobamate in Vitreous Humor, Blood and Liver," *Journal of Forensic Sciences*, JFSCA, Vol. 14, 1969, pp. 507–514.

[*10*] Sturner, W. Q. and Coumbis, R. J., "The Quantitation of Ethyl Alcohol in Vitreous Humor and Blood by Gas Chromatography," *American Journal of Clinical Pathology*, JCPAA, Vol. 46, 1966, pp. 349–351.

[11] Youden, W. J., *Statistical Methods for Chemists*, John Wiley & Sons, New York, 1951, pp. 17, 29, 40.
[12] Redetski, H. M., Koerner, T. A., and Hughes, J. R., "The Use of Osmometry for the Evaluation of Alcohol Intoxication," *Clinical Toxicology Bulletin*, CTOXA, Vol. 1, No. 5, 1970. p. 5.
[13] Robinson, A. G. and Loeb, J. N., "Ethanol Ingestion—Commonest Cause of Elevated Plasma Osmolality?," *New England Journal of Medicine*, NEJMA, Vol. 284, 1971, pp. 1253–1255.
[14] Davson, H., *Physiology of the Ocular and Cerebrospinal Fluids*, J. and A. Churchill Ltd., London, England, 1956, pp. 245–247.
[15] Sturner, W. Q. and Dempsey, J. L., "Sudden and Unexpected Infant Death: Postmortem Chemistry Studies of the Vitreous Humor," in preparation.

Department of Pathology
The University of Texas Southwestern Medical School at Dallas
5323 Harry Hines Boulevard
Dallas, Texas 75235