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Variations in Vitreous Humor Chemical Values As A Result of Instrumentation

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ABSTRACT: Urea nitrogen, glucose, sodium, potassium, and chloride were measured in common vitreous humor samples using a variety of instruments. There was found to be variation in values obtained by the different procedures for each of these constituents. The variation in electrolyte values between the different procedures can pose real problems in attempting to determine the presence of an antemortem dehydration or low salt condition. Possible reasons for these variations are discussed, and the normal range of values of both sodium and chloride for the different instrumentalities is provided. However, variations in values for both urea nitrogen and glucose would not pose any problems of interpretation for forensic science evaluations.

KEYWORDS: pathology and biology, vitreous humor, comparative analysis

Interpretation of laboratory values from living patients depends in part on establishment of a "normal" value for any particular chemical substance. It has been well established in the clinical laboratory that a normal range for any compound will depend upon the particular laboratory procedure used to measure the substance. The same holds true in processing fluid from a dead body whether it be blood, cerebrospinal fluid, or vitreous humor. This was first pointed out in 1949 by Tonge and Wannan [*1*] who demonstrated that postmortem blood contained considerable quantities of nonfermentable reducing substances so that by the routine laboratory methods of that time it was rare to find a zero value for glucose, although no true glucose was present. Coe [*2*] in 1973 reported a signficant variation in postmortem vitreous glucose values when the laboratory changed procedures, and Daae et al [*3*] reported striking differences in glucose values when five different analytical methods were compared on common vitreous samples. Similar discrepancies have been demonstrated for other substances such as serum calcium [*4*] and vitreous bilirubin [*5*].

Recently the clinical laboratory at the Hennepin County Medical Center investigated various instruments for the determination of serum sodium, potassium, chloride, urea nitrogen, and glucose. As these constituents are most commonly determined in vitreous humor, a series of vitreous specimens was analyzed for each of these constituents by a variety of different procedures. The results of these tests form the basis of this report.

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Methods

Vitreous humor was aspirated in a standard manner by inserting a 20-gage needle through the outer canthus of the eye into the center of each globe and gently aspirating the contents with a 10-cm^3 syringe. Crystal clear fluid without particular matter was routinely obtained. This was then centrifuged and the supernatent portion used for testing. Thirty-five specimens were analyzed using four different procedures for glucose, sodium, and potassium, and three different procedures for urea nitrogen and chloride.

Sodium and potassium were measured by direct potentiometry with a Kodak Ektachem 400, by indirect potentiometry with a Beckman Astra, and by flame photometry with a Corning Flame Photometer 450 and a Technicon SMA 6/60. Glucose was measured by the following four methods: A Beckman glucose analyzer and the Beckman Astra using glucose oxidase and an oxygen sensor that measures the rate of change in oxygen concentration; a Kodak Ektachem 400 using an adaptation of the glucose oxidase-peroxidase-chromgen-coupled system, with reflectance readings taken at 540 nm; and a Technicon SMA 6/60 utilizing an automated glucose-oxidase procedure with a peroxidase indicator reaction measured at 600 nm. Chloride was measured by the Beckman Astra by means of coulometric titration, by the Technicon SMA 6/60 utilizing a colormetric end point technique, and on a Kodak Ektachem 400 using an ion-selective electrode for potentiometric measurements. Urea nitrogen was measured by the Kodak Ektachem 400 based on a urease-catalyzed hydrolysis of urea to amonia and carbon dioxide, by the Beckman Astra using an enzymatic conductivity rate method, and by the Technicon SMA 6/60 utilizing a two-step colorimetric reaction measured at 460 nm.

All instruments were standardized and run according to the specifications of the manufacturer. The specificity, sensitivity, and dynamic range of each instrument, as described by the manufacturer, were verified in our laboratory and were well within acceptable limits. No known interferences were encountered. Vitreous samples were obtained for routine analysis from the Medical Examiner's Office and performed in random order. Commercial sera controls and a pooled vitreous fluid control were analyzed on each instrument for each amalyte. Within-run precisions ($n = 18$) and between run precisions ($n = 26$) were well within acceptable ranges for both low and high controls (all coefficients of variations (CVs) were less than or equal to 4.3%), and no significant differences were noted between instruments. Therefore, analytical bias cannot be attributed to the method used. Note that all guidelines given in this report are based on reference ranges established in our laboratory.

Results

The results of the different analyses are given in Tables 1 through 5 with the same sample occupying an identical position in each table. The method used for determining each substance in the laboratory at the Hennepin County Medical Center through 1983 is presented in the first column of each chart, and values based on these procedures have been the ones presented in publications from this office to date.

Examination of the tables reveals that some variation from the normal range is noted in each of the constituents that have established normal values. Five specimens (1101, 1313, 1355, 1603, and 3468) give evidence of nitrogen retention of varying degree. Eight specimens (1101, 1354, 1355, 1606, 1959, 2176, 3091, and 3092) reveal elevated glucose values indicating an antemortem hyperglycemia. Three specimens (1101, 3019, and 3091) have sodium values by flame photometry exceeding the normal range of 130 to 155 meq/L. With chloride, there are two specimens (1355 and 3091) showing elevated values and one specimen (1610) showing a depressed value compared to the normal range of 105–135 meq/L that was established with the Technicon SMA 6/60 using a colorimetric end point.

Potassium has no normal range after death even in the early postmortem period because of an essentially linear elevation in value with increase in postmortem time until putrefaction sets in.

TABLE 1—*Urea nitrogen.*

Specimen Number	Technicon SMA 6/60, mg/dL	Ektachem 400, mg/dL	Beckman Astra, mg/dL
685	13	15	12
686	21	23	11
726	16	17	13
727	15	17	6
730	13	13	2
976	8	7	7
979	18	19	15
980	12	13	9
1101	97	104	99
1102	14	15	13
1103	15	14	14
1312	12	12	11
1313	79	99	79
1354	19	20	16
1355	77	87	78
1603	53	57	54
1606	13	13	10
1608	12	13	3
1610	25	24	25
1959	31	34	31
1968	19	19	19
1969	9	8	9
2176	44	50	46
2177	16	16	15
2468	11	11	9
2609	26	27	24
2966	29	17	18
3018	22	21	18
3019	11	11	10
3021	11	12	12
3023	15	13	14
3091	13	13	11
3092	13	13	8
3231	12	14	13
3468	38	45	38
Mean	24.3	25.9	22.1

Discussion

The whole purpose of performing postmortem chemistries is to enable the forensic pathologist to better determine the antemortem physiological condition of the dead individual. Postmortem variations prevent using very narrow ranges as "normal" for most substances but do enable the pathologist to establish many gross biochemical abnormalities that existed prior to death. Because of the limited interpretation imposed on postmortem studies, mild variations in value may cause no problem for the forensic scientist. Thus the variation in urea nitrogen values between the various procedures is of no importance. Individuals having values in the normal range (less than 30 mg/dL) were easily identified by any of the procedures, and individuals having nitrogen retention showed approximately equivalent levels by all tests.

Similarly, interpretation of glucose values is not difficult. The four different methods examined do show the variations reported by previous investigators, and these could conceivably cause problems in the hospital laboratory where incipient diabetes or hypoglycemia are important. However, because of the routine but irregular fall of vitreous glucose with increasing

TABLE 2—*Glucose.*

Sample Number	Beckman Glucose Analyzer, mg/dL	Ektachem 400, mg/dL	Beckman Astra, mg/dL	Technicon SMA 6/60, mg/dL
685	56	49	57	30
686	30	34	36	5
726	71	59	93	46
727	60	56	69	36
730	60	45	57	25
976	17	27	21	15
979	74	59	79	18
980	47	43	58	12
1101	126	132	125	125
1102	56	45	52	25
1103	48	35	44	5
1312	55	53	60	35
1313	22	31	29	10
1354	107	123	101	105
1355	230	240	226	218
1603	48	41	49	17
1606	231	188	237	200
1608	17	20	21	5
1610	41	41	41	35
1959	383	325	349	325
1968	58	43	57	20
1969	83	58	79	40
2176	148	142	157	120
2177	38	37	41	6
2468	59	65	64	50
2609	27	30	34	5
2966	38	33	38	10
3018	29	33	41	25
3019	63	52	76	30
3021	11	12	23	0
3023	17	22	23	0
3091	176	147	170	145
3092	139	145	140	128
3231	22	20	24	0
3468	14	15	18	0
Mean	77.2	71.4	79.7	53.5

time between death and drawing of a specimen [*6*], it is not possible to determine antemortem hypoglycemia from postmortem specimens. Also, it is apparent that the low glucose values show the greatest percentage difference between the various procedures. At the high end of the range, there is no need to determine the borderline or incipient diabetic in a dead individual. Only definite evidence of antemortem hyperglycemia is of significance to the forensic pathologist. All four procedures demonstrated essentially equivalent values in those individuals with elevated vitreous glucose.

By contrast, the variations in sodium and chloride values found between the different procedures would cause definite problems in determining antemortem electrolyte abnormalities. Coe [*6*], Swift et al [*7*], and Forman et al [*8*] have shown that vitreous sodium levels either above or below a normal range reflect a corresponding antemortem serum abnormality. Coe in extensive studies determined the normal range of vitreous sodium to be from 130 to 155 meq/L when determined by flame photometry. This has been published in several articles discussing hyper- and hypo-natremia [*9, 10*] and used by investigators as a basis for demonstrating water intoxication [*11*], dehydration [*12*], low salt levels from diuretics [*10*], and so on. Like-

TABLE 3—*Sodium.*

Sample Number	Corning Flame, meq/L	Ektachem 400, meq/L	Beckman Astra, meq/L	Technicon SMA 6/60, meq/L
685	141	150	145	142
686	142	152	144	141
726	146	156	146	146
727	143	158	145	144
730	144	156	145	144
976	143	152	144	143
979	154	162	152	149
980	144	154	142	142
1101	158	158	158	156
1102	139	151	144	138
1103	144	154	145	141
1312	148	157	156	147
1313	145	150	148	143
1354	136	145	143	136
1355	154	164	162	155
1603	137	144	134	135
1606	133	140	135	131
1608	140	150	142	139
1610	140	145	141	138
1959	136	142	137	132
1968	142	147	147	142
1969	145	153	149	143
2176	143	151	147	142
2177	143	151	149	143
2468	147	156	153	145
2609	147	156	148	143
2966	146	156	146	143
3018	154	152	147	144
3019	157	154	150	145
3021	149	151	146	143
3023	149	161	156	148
3091	167	176	169	160
3092	141	149	148	139
3231	139	151	144	142
3468	147	156	149	148
Mean	145.2	153.1	147.3	143.2

wise extensive studies have established a normal range of vitreous chloride to be from 105 to 135 meq/L when determined by the Technicon SMA 6/60. These data have likewise been published and used by others in determining hyper- or hypo-chloremia [*13*].

The present study shows that these published "normal" ranges are valid only when certain analytical procedures are used. With the introduction of the Kodak Ektachem 400 into the laboratory at the Hennepin County Medical Center, a new range of normal values for electrolytes became necessary. While only three cases show sodium values exceeding 155 meq/L by flame photometry, there are twelve cases with sodium values over 155 meq/L when the Ektachem 400 is used.

The variation in chloride values is even more striking. With the Ektachem 400, three cases show values below 105 meq/L indicating antemortem hypochloremia, while there are none when the Technicon SMA 6/60 was used. In contrast, there are nine cases with chloride values in the hyperchloremic range (over 135 meq/L) when the Beckman Astra is used, compared to only two such cases with the Technicon SMA 6/60.

The reason for the striking differences between the various instruments for both sodium and chloride is not altogether clear. With serum, the values for these constituents by the

TABLE 4—*Chloride.*

Sample Number	Technicon SMA 6/60, meq/L	Ektachem 400, meq/L	Beckman Astra, meq/L
685	127	114	136
686	120	117	136
726	129	118	126
727	132	120	128
730	130	120	133
976	128	120	125
979	132	123	132
980	128	118	127
1101	121	110	122
1102	125	115	139
1103	120	119	130
1312	133	124	142
1313	125	116	133
1354	115	106	119
1355	138	128	152
1603	121	107	121
1606	112	102	120
1608	129	113	133
1610	105	104	106
1959	110	104	120
1968	124	113	133
1969	129	118	135
2176	124	114	130
2177	124	116	128
2468	130	121	134
2609	128	118	131
2966	129	118	128
3018	128	118	126
3019	129	119	134
3021	126	118	131
3023	129	120	136
3091	>140	141	>150
3092	126	115	145
3231	123	114	132
3468	130	121	132
Mean[a]	125.3	115.9	130.4

[a]Mean excludes Sample 3091.

various instruments tested were identical within the limits of laboratory error. The vitreous variations thus represent some significant differences between this fluid and serum which alter the perceived values of the electrolytes.

The sodium concentration discrepancy between the flame photometer and direct potentiometry in vitreous fluid, as contrasted to no discrepancy in sera, might be explained by the differences in the water content of serum (93%) and vitreous fluid (99%). The greater sodium concentration by direct potentiometry would be in agreement with studies in sera by Apple and coworkers [*14*]. They showed that water content clearly influenced sodium values, with direct potentiometry values appearing more accurate. However, this mechanism does not explain the discrepancy for chloride values which is almost of equal magnitude but in the opposite direction. Presently, our laboratory is investigating the possibility of chloride binding substances in vitreous fluid that would give falsely low readings by direct potentiometry. A system to study this phenomenon based on pH alterations of vitreous fluid is being developed.

Whatever the reason for variation in vitreous values of both sodium and chloride, problems occur in interpretation of these ions. The previously established normal ranges using flame

TABLE 5—*Potassium.*

Sample Number	Technicon SMA 6/60, meq/L	Ektachem 400, meq/L	Beckman Astra, meq/L	Corning Flame, meq/L
685	5.3	5.6	5.3	5.2
686	5.7	6.3	5.9	5.8
726	5.7	6.1	5.4	5.6
727	5.1	5.7	5.3	5.2
730	5.3	6.0	5.4	5.5
976	8.4	9.0	8.8	8.3
979	5.4	6.0	5.5	5.5
980	5.6	6.2	5.6	5.6
1101	4.8	5.0	4.8	4.9
1102	5.8	6.5	5.9	5.8
1103	4.7	5.5	5.0	5.0
1312	6.4	6.9	6.8	6.5
1313	6.2	6.5	6.5	6.3
1354	5.0	5.3	5.0	5.0
1355	5.4	5.9	5.8	5.4
1603	4.3	4.6	4.4	4.3
1606	5.2	5.7	5.5	5.3
1608	7.8	8.3	8.4	7.9
1610	4.8	5.2	5.0	4.9
1959	8.2	8.9	8.8	8.5
1968	4.9	5.1	5.1	4.8
1969	6.1	6.6	6.1	6.0
2176	5.7	6.0	6.0	5.8
2177	5.7	6.0	6.0	5.7
2468	5.5	6.0	5.5	5.6
2609	4.9	5.4	5.1	5.0
2966	4.7	5.5	4.9	4.8
3018	6.0	6.3	6.2	6.4
3019	5.8	6.0	6.0	6.2
3021	>10.0	13.9	>10.0	13.9
3023	6.5	7.3	6.9	6.5
3091	8.2	9.6	9.2	8.4
3092	4.7	5.4	5.2	4.8
3231	7.7	8.3	7.7	7.6
3468	9.2	9.3	9.5	9.0
Mean[a]	5.9	6.4	6.1	5.8

[a]Mean excludes Sample 3021.

photometry were based on comparison of postmortem vitreous values against antemortem serum values, and their validity has been supported not only by research of the senior author but by other investigators as well [7,8].[3] With the different values being obtained with the new instruments, no direct comparisons between antemortem and postmortem values has been possible to date. However, the present study shows consistent differences that enable us to estimate what the normal range of values for sodium and chloride will be by the different methods tested. These are given in Table 6. In this table, as explained in earlier publications, a moderate elevation of urea nitrogen supports the diagnosis of hypertonic dehydration. A low potassium level in the low salt syndrome is necessary to eliminate postmortem deterioration as the cause of hyponatremia or hypochloremia or both. When potassium values exceed 20 meq/L, it must be assumed that putrefaction has begun which will invalidate low vitreous electrolyte values as being the result of antemortem disease.

There is insufficient data in this study to determine whether procedural variations in potas-

[3]Ronald Wright, Medical Examiner's Office, Fort Lauderdale, FL, personal communication, 1976.

TABLE 6—*Range values of sodium and chloride by different tested methods.*

Antemortem Abnormality	Vitreous Humor Values		
	Flame photometry or SMA 6/60	Ektachem 400	Beckman Astra
Dehydration			
Sodium, meq/L	>155	>165	>155
Chloride, meq/L	>135	>125	>140
Urea nitrogen, mg/dL	40-100	40-100	40-100
Low salt condition			
Sodium, meq/L	<130	<135	<130
Chloride, meq/L	<105	<95	<110
Potassium, mg/dL	<15	<15	<15

sium values will seriously affect the graphs and formulae that have been developed to estimate the postmortem interval from vitreous potassium.

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