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Postmortem Chemistries on Blood with Particular Reference to Urea Nitrogen, Electrolytes, and Bilirubin

A great deal of work has been done on postmortem chemistry of blood, cerebrospinal fluid, and vitreous humor with the hope that biochemical abnormalities which exist during life can be demonstrated from examination of postmortem material. The author began investigation into this field in 1967 by performing tests on both serum and vitreous humor taken from cadavers at varying postmortem intervals. The results of the investigations on vitreous humor have already been published [*1*,*2*] as well as some of the studies on postmortem serum [*3*,*4*]. The following material represents part of the original investigation and has been presented orally both formally [5] and informally, but has never been available for review by other people interested in the subject.

Procedure

Blood was obtained from patients who died in Hennepin County General Hospital and from bodies brought to the morgue as Medical Examiner's cases. In appropriately selected cadavers, blood was aspirated from the heart using a parasternal approach immediately after admission of the body to the morgue. At the same time, all of the vitreous humor was gently extracted from one eye. A second sample of blood was later aspirated from the heart at the time an autopsy was performed or when the body was to be removed by a mortician. The vitreous humor was taken from the second eye at this time.

All samples were immediately taken to the hospital laboratory where the blood was centrifuged and the serum removed. If the specimens were obtained during the working day they were analyzed immediately. Otherwise, they were refrigerated in stoppered tubes until the following morning. Analyses were performed on the SMA 12/30 Autoanalyzer (Technicon Instruments Corp., Ardsley, N.Y.) that gave values for sodium, potassium, chloride, carbon dioxide content, total protein, albumin, calcium, alkaline phosphatase, bilirubin, urea nitrogen, and glucose.

Hospital patients were selected primarily on the basis of having had an SMA 12/30 battery of tests performed shortly before death, that could be used as comparison with postmortem results. A wide variety of pathological conditions showing numerous biochemical abnormalities were available for evaluation. In contrast the cases selected for study from the Medical Examiner's material were quite limited and consisted primarily

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of individuals showing no antemortem evidence of disease, who died rapidly from traumatic injuries where the exact time of death was known.

Results

Blood Urea Nitrogen

There were 108 cases in which there was no antecedent history or autopsy evidence of renal problems and where death occurred rapidly as a result of accident or natural disease. The causes of death in these individuals included suicidal gunshot wounds to the head, traumatic injuries from automobile accidents, sudden death from cardiac arrhythmia, massive intracerebral or subarachnoid hemorrhage, dissecting aneurysms of the aorta, ruptured arteriosclerotic aneurysms, "café" coronaries, etc. In all of them the interval between the traumatic injury or onset of terminal illness and death was less than an hour. For this group the postmortem blood urea nitrogen was found to vary from 6–24 mg/dl with an average of 13.8 mg/dl.

Blood obtained from 58 hospital patients with terminal nitrogen retention are listed in Table 1, along with postmortem values from the same individuals showing that values obtained after death do accurately reflect antemortem levels.

The stability of urea nitrogen in the cadaver is portrayed in Fig. 1, where data are plotted from 170 cases who had serum drawn at two postmortem intervals. Each point on the

TABLE 1—*Comparison of antemortem and postmortem blood urea nitrogen in individuals with nitrogen retention.*

Blood Urea Nitrogen			
Antemortem[a]	Postmortem	Antemortem[a]	Postmortem
54	54	100	120
55	52	104	129
55	68	108	105
56	62	110	121
57	62	110	119
58	70	110	118
60	64	113	135
64	63	114	124
64	74	114	144
64	79	117	105
68	74	119	138
68	70	119	141
69	75	121	145
70	77	124	144
74	84	125	127
76	78	125	135
78	97	129	135
80	98	130	152
83	101	136	155
86	92	140	147
87	124	140	143
89	100	146	141
89	98	147	155
90	95	151	155
90	96	160	172
93	93	160	165
96	107	195	205
96	99	210	230
96	120	224	241

[a] All antemortem values were obtained within 24 h of death.

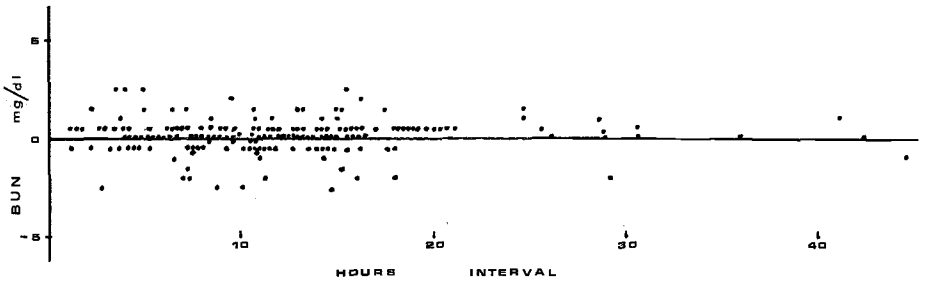

FIG. 1—*Each point represents the difference between two urea nitrogen values from postmortem specimens drawn at varying intervals from the same cadaver. The value of the second specimen is subtracted from the first so that a positive result means an increase while a negative value means a decrease in BUN with increasing time after death.*

graph represents the difference between the values for the first and second specimens drawn. Thus, a positive value represents an increase in urea nitrogen between the first and second specimen, while a negative value represents a decrease. Over 90 percent of the cases varied less than 3 mg/dl between specimens taken as much as 44 h apart. An additional four cases were available with paired results that could not be fitted on the graph because of postmortem intervals extending from 60–122 h. In all four of these additional cases there was less than 3 mg/dl difference between the first and second postmortem specimens.

Electrolytes

There were 155 hospital patients on whom SMA 12/30 determinations had been performed less than 8 h prior to death. The great majority of these were individuals who had normal electrolytes, although occasional patients with elevated or lowered sodium and/or chloride values were present. Two postmortem specimens were obtained from most of these individuals. The results of the analyses are shown in Figs. 2 and 3, where each point represents the difference between antemortem and postmortem values for the postmortem intervals indicated. Thus, a positive value represents an increase in the postmortem levels of sodium or chloride, while a negative value represents a decrease in the value after death. Several mathematical models to express these data were tried. While improvement for long postmortem intervals was found by using the square root of time or an exponential equation, the simple least squares regression analysis based on the formula $d = \beta t$ (where $\beta = -0.9$ for sodium and -0.97 for chloride, d = difference, and t = time), gave nearly as satisfactory a fit for the early hours after death and is expressed by the dotted line in both graphs.

The Autoanalyzer procedure for carbon dioxide determines total carbon dioxide content including carbonate ions, bicarbonate ions, and dissolved carbon dioxide gas in the serum. In clinical usage the loss of carbon dioxide from serum samples in the Auto-

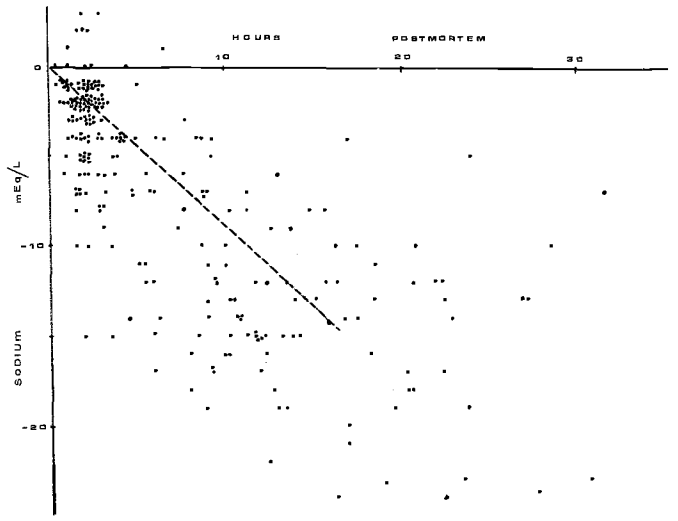

FIG. 2—*Each point represents the difference* (d) *between an antemortem and a postmortem sodium value on the same individual. Thus a negative value indicates a fall in the sodium level after death. The dotted line represents least squares regression analysis using the equation* $d = \beta t$ *where* $\beta = -0.9$.

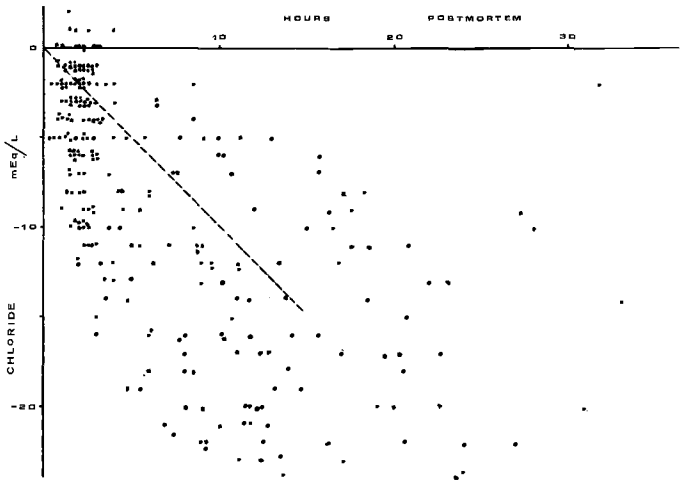

FIG. 3—*Each point represents the difference* (d) *between an antemortem and a postmortem chloride value on the same individual. Thus a negative value indicates a fall in the cloride level after death. The dotted line represents least squares regression analysis using the equation* $d = \beta t$ *where* $\beta = -0.97$.

analyzer tray does not appear to be significant if the tray is covered. The results of this analysis on 155 individuals are shown in Fig. 4, where each point represents the difference between antemortem and postmortem values for each individual at varying postmortem times. Several mathematical models were tried and the curve that best fit the data is least squares regression analysis using the exponential equation $d = \beta (1 - e^{-\alpha t})$ where $\beta = -17.3$ and $\alpha = 0.32$.

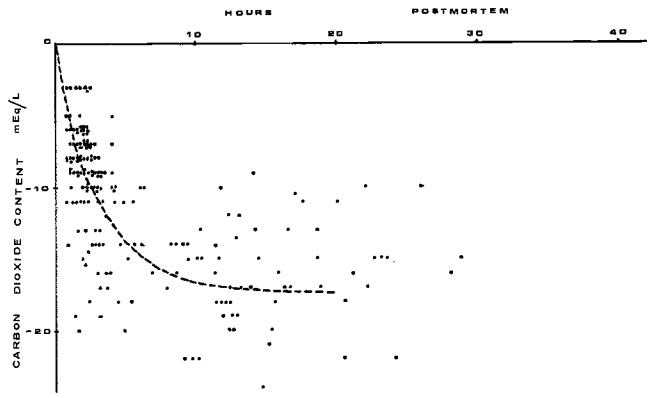

FIG. 4—*Each point represents the apparent fall in value of postmortem serum carbon dioxide content expressed as the difference* (d) *between the antemortem and postmortem level as determined by Autoanalyzer. The dotted line represents least squares regression analysis using the exponential equation* $d = \beta(1 - e^{-\alpha t})$ *where* $\beta = -17.3$ *and* $\alpha = 0.32$. *The apparent decline is felt to represent a procedural artifact (see discussion).*

Bilirubin and Alkaline Phosphatase

There were 94 hospital patients who had terminal bilirubin values between 0.3 and 1.9 mg/dl and normal alkaline phosphatase values, upon whom 160 postmortem observations were made. These are shown in Table 2. There was found to be a slight, but definite, rise in total bilirubin as determined by the Jendrassik and Grof procedure used in the Autoanalyzer.

TABLE 2—*Antemortem bilirubin and alkaline phosphatase values for 94 normal individuals with 160 postmortem observations at varying intervals on the same individuals.*

	Normal Individuals		
No. of Cases	Avg. Time, h (range)	Avg. Bilirubin, mg/dl (range)	Avg. Alk. P'tase KA units (range)
94	Antemortem	0.82 (0.3–1.9)	12.7 (7–20)
76	1.8 (0–3)	0.98 (0.4–3.2)	15.2 (6–43)
52	7.6 (3 ¼–12)	1.24 (0.4–3.5)	22.7 (11–50)
32	18.8 (12 ¼–44)	1.52 (0.5–4.8)	30.0 (12–60)

The mild elevation of bilirubin that apparently occurs after death does not interfere with evaluation of clinical jaundice as demonstrated in Table 3, where early and late postmoretm serum bilirubin values are compared with terminal antemortem levels from 20 jaundiced individuals.

Alkaline phosphatase is determined by a modification of the King-Armstrong procedure on the Autoanalyzer. The values from 94 normal individuals at varying postmortem

TABLE 3—*Antemortem and postmortem bilirubin values from 20 jaundiced individuals.*

Bilirubin (mg/dl)—Jaundiced Individuals				
Antemortem Bilirubin	1st Postmortem Interval, h	Bilirubin	2nd Postmortem Interval, h	Bilirubin
5.0	2 1/2	5.7	3 3/4	6.0
5.4	2	6.2	6	6.1
5.6	3	5.1	23	4.3
6.1	1 1/4	5.5	6 1/4	6.0
6.6	1 3/4	6.6	8 1/2	6.5
6.6	1 3/4	6.0	8 3/4	6.5
6.7	1 3/4	7.2	13 1/2	6.9
6.8	2 3/4	7.4	15 1/4	5.4
6.8	2	6.8	28	6.9
6.8	2 1/2	7.4	5 1/2	7.3
7.0	3 1/4	5.9	...	...
7.4	2	7.7	...	...
8.4	1 1/2	6.6	17	6.0
10.1	1 1/4	8.3	...	...
11.7	2 1/2	14.0	...	...
15.5	4 1/2	15.0	33	15.0
16.2	1/2	16.8	13 1/4	17.4
20.4	1	18.9	...	...
21.0	1	20.0	19 1/2	24.0
25.5	3	25.0	14	23.0

intervals are given in Table 2. From this it is apparent that alkaline phosphatase increases with increasing time after death. The values nearly doubled in 8 h and essentially tripled in 20 h postmortem.

Calcium

Determination of calcium in the Autoanalyzer is by use of cresolphthalein complexone. This dye also reacts with magnesium and the Autoanalyzer includes several steps to minimize interference from this cation. As a consequence, the technique is satisfactory for determining clinical serum calcium levels when magnesium levels are consistently in a a narrow range. However, examination of the data obtained from postmortem blood revealed an apparent increase between the first and second postmortem specimens drawn from each cadaver. Experiments conducted by adding small amounts of magnesium to postmortem serum gave similar increases. Consequently, it seems impossible to accurately determine postmortem serum calcium with the present method incorporated in the Autoanalyzer.

Protein

Determination of total protein in the Autoanalyzer is by the standard biuret method while albumin used 2-(4-hydroxybenzeneazo) benzoic acid. It was found that postmortem levels of both total protein and albumin closely approximated known antemortem values. The data on this and comparative antemortem and postmortem electrophoretic patterns are given in a separate publication [*4*].

Other Substances

Since much of the blood was obtained by blind cardiac puncture during the early collection of data for this paper, it was frequently unknown whether the sample was obtained from the left or right side of the heart. As a consequence no evaluation of glucose

is possible. Frequently levels were markedly elevated over those known to have existed immediately prior to death and, just as frequently, postmortem values were found to be much reduced. The problem of interpretation posed by glycolysis and glycogenolysis has been well discussed in the literature and will not be considered here.

Consistent with the findings of Jetter [*6*] and other investigators [*7*] postmortem serum potassium values were found to rise so rapidly that any attempt to evaluate potassium metabolism was considered impossible.

Glutamic oxalic transaminase begins to rise very shortly after death and rapidly reaches very high levels as determined by the Autoanalyzer. It was felt that interpretation of postmortem values was not possible and no analyses of the data on this procedure were attempted.

Discussion

Nearly 50 years ago Paul [*8*] proved the stability of urea in the body by drawing blood samples from the same cadaver at varying postmortem intervals. Values remained constant between specimens drawn as early as ½ h after death to others taken over two days later. This was true whether the urea nitrogen was in the normal range or markedly elevated. However, subsequent work by Pucher and Burd [*9*], Polayes et al [*10*], and Naumann [*11*] on hospital patients all indicated there was a postmortem increase in urea nitrogen. It was not until the 60's that work by Levonen et al [*12*], Jensen [*13*], and Fekete and Kerenyi [*14*] reconfirmed Paul's observations. They established that urea nitrogen in the postmortem serum closely approximated values found in the terminal antemortem blood. Fekete and Kerenyi found postmortem urea nitrogen values from individuals who died suddenly to average 15.5 mg/dl, in contrast to an average of 47.4 mg/dl in hospital patients who died without evidence of kidney disease. Levonen et al demonstrated the stability of urea in the body after death, showing little variation in values of samples drawn from the same cadaver daily over a four-day period. The present work substantiates the findings of Levonen et al and Fekete and Kerenyi. Postmortem urea nitrogens from individuals who died suddenly are all within the normal range, and the average for this group is identical to that of the normal living population. The stability of urea nitrogen is demonstrated by a variation of less than 3 mg/dl in over 90 percent of the cases on specimens drawn up to 120 h apart from the same cadaver.

The comparison of terminal antemortem and postmortem urea nitrogen values in many hospital patients demonstrates that elevated postmortem values do reflect evidence of antemortem nitrogen retention. Whether nitrogen retention represents prerenal factors or kidney disease with clinical uremia, must be determined by evaluation of clinical history and autopsy findings in each individual case.

Previous work on serum electrolytes has established there is a progressive decline in the serum chloride values with increasing time after death. Jetter [*6*] stated this was caused by a shift of chlorides to the erythrocytes causing the average plasma chloride values to be in the range of 80–90 mEq/l by one day postmortem. Schleyer [*15*] and other European workers substantiated the plasma chloride decline and reported the rate of decrease to be approximately ¼–1 mEq/l per hour. The present work shows that the rate of fall is quite individual, but the average rate of fall (0.97 mEq/l per hour) was at the upper limits of the rate stated by previous investigators.

In contrast to chloride there is only a single direct reference to sodium in the English literature. This is a statement by Jetter [*6*] that the serum sodium remains constant during the first 12 h postmortem, following which a somewhat inconclusive decrease begins.

Jetter's statement was made on analysis of only 20 cases. Several recent articles [*16,17*] concerned with electrolyte changes found in drowning have indirectly shown that a decline in serum sodium begins much earlier than 12 h. The present investigation demonstrated that sodium begins to decline immediately after death. While there is a great deal of individual variation, the average rate of fall in this series was approximately 0.9 mEq/l per hour.

The marked individuality and rather rapid rate of fall in both sodium and chloride makes evaluation of hyponatremia or hypochloremia very difficult from the examination of serum alone. However, unusually marked depression of these substances, in conjunction with a short postmortem interval, may support a diagnosis based on lower vitreous humor values. The use of such serum electrolyte studies for corroborative evidence of lower salt levels in the vitreous is illustrated by the author [*18*] in a paper concerned with electrolyte imbalance found in alcoholic patients.

In contrast to the difficulty of interpreting low serum levels, elevated serum levels of sodium and/or chloride are strongly indicative of antemortem hypernatremia and hyperchloremia. They are conclusive when combined with elevated levels for the same substances in vitreous humor.

The work on bilirubin in this study establishes that the antemortem degree of clinical jaundice can be quite accurately determined by postmortem examination of total serum bilirubin. However, in a nonjaundiced individual with questionable liver disease, where it would be desirable to determine evidence of antemortem "chemical jaundice," the present work would indicate this to be difficult. Naumann [*19*] in earlier studies felt that postmortem bilirubin values accurately reflected antemortem serum levels. However, he gives an average of only twelve cases, and the value for total bilirubin was 0.1 mg/dl higher than the upper limit of his normal antemortem range. These data are consistent with the present study, where a small but definite rise in average bilirubin values is demonstrated with increasing postmortem time. As a consequence, total bilirubin levels consistent with chemical jaundice were found several times in individuals established as having had normal antemortem values.

Jetter [*6*] and Naumann [*20*] report that postmortem serum calcium levels accurately reflect antemortem values. However, the method of calcium determination was not given in either Jetter's article or Naumann's personal communication. Presumably it was the Clark and Collip or some other procedure specific for calcium. There is an apparent increase in serum calcium after death when determined by the Autoanalyzer but this is thought to be an artifact caused by increasing amounts of serum magnesium in the cadaver. The demonstration that serum calcium shows an apparent increase so soon after death can be interpreted as evidence that magnesium begins to leave the cells long before significant intravascular hemolysis occurs, and in greater amounts than Jetter felt occurred at the time of his publication.

Jetter [*6*] stated in his study that the carbon dioxide combining power begins to decrease in the serum rapidly after death, but that the carbon dioxide content remains constant. Initially, this seems at variance with the present data where presumably the carbon dioxide content is being determined by the Autoanalyzer procedure. However, the postmortem values undoubtedly do not reflect terminal antemortem carbon dioxide content due to procedural artefacts. While the loss of carbon dioxide in the serum samples from living patients may not be significant, it is felt that in the postmortem specimen this probably accounts for the apparent striking reduction in carbon dioxide content. After death with decreasing blood pH, there is undoubtedly a fall in CO_2 combining power with a resultant increased amount of dissolved carbon dioxide in the serum. Running the

specimens now in open cups through the Autoanalyzer exposes the serum to the air, affording ample opportunity for the dissolved carbon dioxide to escape. The marked postmortem reduction in total carbon dioxide content probably reflects the fall in the carbon dioxide combining power.

Work by a number of authors has demonstrated the postmortem determination of serum protein and their fractions by chemical, electrophoretic, and immunological techniques and accurately portrays any antemortem abnormality which might have existed. The present investigation supports this contention as published in a separate communique [*4*].

Both Enticknap [*21*] and Naumann [*11*] have previously shown that alkaline phosphatase values tend to rise after death. This is supported by the present investigation. Enticknap also demonstrated a progressive rise in postmortem glutamic oxalic transaminase. Averages from a large number of patients revealed an essentially linear increase with postmortem time, so that a rough estimate of the postmortem interval could be calculated. The present work substantiates the early and quite rapid rise in glutamic oxalic transaminase after death, but this is very erratic for individual cases and no attempt was made to analyze the data on a statistical basis in the present series.

Summary

Postmortem serum evaluations of sodium, potassium, chloride, CO_2 content, total protein and albumin, urea nitrogen, glucose, alkaline phosphatase, and calcium are presented. It is found that urea nitrogen levels accurately reflect terminal antemortem nitrogen retention. Postmortem serum bilirubin is an accurate measure of antemortem jaundice, but slight postmortem increases of total bilirubin with increase in postmortem time cause difficulty in interpreting borderline abnormal bilirubin values in the non-jaundiced individual.

The present study demonstrates that serum sodium begins to fall immediately after death and that the rate of fall on the average is similar to the decrease in chloride ion.

The investigation also establishes that the present procedure used in the Autoanalyzer is unsatisfactory for postmortem calcium determination. This is thought to be the result of interference from magnesium. Postmortem evaluation of serum protein is found to be reliable with excellent correlation by all present techniques between antemortem and postmortem specimens. Finally, the previously reported postmortem rise in both alkaline phosphatase and glutamic oxalic transaminase has been substantiated.

Acknowledgment

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