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Estimation of Postmortem Interval Based on the Third Component of Complement (C3) Cleavage

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ABSTRACT: To estimate postmortem interval (PMI), the spontaneous conversion of the native third component of complement (C3) to its derived fragments in whole blood was studied by crossed immunoelectrophoresis. C3 cleavages in vitro at different temperatures showed that the incubation of whole blood at a higher temperature led to a faster conversion of beta 1C (native C3) to beta 1A (C3c). In cadaveric blood, we found a significant positive correlation between percentage of C3 cleavage and PMI. From these results, it is possible to estimate PMI from the ratios of C3 cleavage.

KEYWORDS: pathology and biology, postmortem interval, blood, complement (biology)

Various objective methods for determining postmortem interval (PMI) have been used over the past 50 years [*1*]. Almost every body fluid has been tested for the level of inorganic and organic constituents and for enzyme activities, such as sugar, nitrogenous compounds, and immunoglobulins in postmortem blood [*2-4*]. In practice, however, these methods are not commonly used except for determining vitreous potassium level for assessing PMI. The major reason for this is the presence of individual variations in antemortem values. Therefore, only substances, which are not influenced by individual antemortem values, are suitable for the estimation of PMI. One possible substance that can be used to estimate PMI is the third component of complement (C3). It is known that C3 is spontaneously cleaved into several fragments in nonactivated plasma, which cannot be detected in circulation; thus the ratio of C3-derived fragments among individuals remains constant.

C3 is composed of two disulfide-linked polypeptide chains, an alpha chain of molecular weight (MW) 120 000 and a beta chain of MW 75 000 [*5*]. Previously, immunoelectrophoretic analysis showed only one precipitin line in fresh serum, namely beta 1C (native C3), and two additional major precipitin lines in aged normal serum [*6*], such as beta 1A and alpha 2D, which are considered to be C3-derived fragments. Using agarose gel immunofixation electrophoresis, Alper and Johnson demonstrated that beta 1A increased with longer storage of serum [*7*]. We report here a spontaneous conversion of native C3 to its fragments in whole blood using crossed immunoelectrophoresis (CIE) and the relationship between postmortem interval (PMI) and the ratio of C3 conversion in cadaveric blood.

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Materials and Methods

Blood

The ratio of C3 cleavage was measured in vitro and in cadaveric blood. Blood samples collected were incubated at 37, 23, and 14°C. Sera from each sample were removed at various time intervals: every 12 h at 37°C until 72 h, every day at 23°C until five days, every two days at 14°C until ten days. These were immediately stored at −80°C, thawed within two weeks, and analyzed using crossed immunoelectrophoresis (CIE).

Cadaveric blood was obtained from autopsy cases for which the time of death had been determined from eyewitness accounts, including bystanders and attending physicians. In addition, the rectal temperature was simultaneously recorded. Blood samples were drawn from the hearts, stored at 0°C within 3 h, centrifuged at 3000 *g* for 30 min to remove sera, and stored at −80°C. The blood sample storage at −80°C and the freeze-thaw do not have any significant effect on C3 cleavage.

Antiserum to Beta 1A

The antiserum to beta 1A which precipitated beta 1C as well as beta 1A was kindly provided by Professor N. Tamura of the Department of Immunology, Institute of Basic Medical Sciences, University of Tsukuba.

Crossed Immunoelectrophoresis (CIE)

The method described by Weeke [*8*] was used to examine beta 1A and beta 1C qualitatively and quantitatively, and modified by using 50- by 50-mm gelbond film for agarose gels (LKB, USA). First, electrophoresis was carried out from right (cathode) to left (anode) at 5 V/cm for 2 h at 15°C, and then electrophoresed into antiserum charged agarose from bottom to top at 2 V/cm for 15 h at 15°C. After electrophoresis, the gel was pressed, dried, and stained in 0.25% Coomassie brilliant blue. For quantitative analysis, tracings of two loops of precipitate were made from two-fold enlarged copies of the immunoelectrogram, and then the areas under them were measured by tablet digitizer on the HP 1000 system.

Results

C3 Cleavage in Vitro

C3 cleavage was investigated by crossed immunoelectrophoresis (CIE). The appearance of beta 1A in aged blood incubated at 37°C is shown in Fig. 1. Fresh serum, prepared for electrophoresis before incubation of whole blood, showed only beta 1C [*1*]. With longer incubation time, beta 1C decreased, and beta 1A, which has apparent faster mobility, is increased gradually [*2–7*].

Time Courses of C3 Cleavage at Different Temperatures

To examine the conversion of beta 1C to beta 1A, whole blood was incubated for various times at 37, 23, and 14°C. Time lines showing the percentages of C3 cleavage at different temperatures are in Fig. 2. From these results, it was observed that incubation at a higher temperature resulted in a higher rate of C3 cleavage so that C3 cleavage at 37°C was about five times faster than that at 14°C.

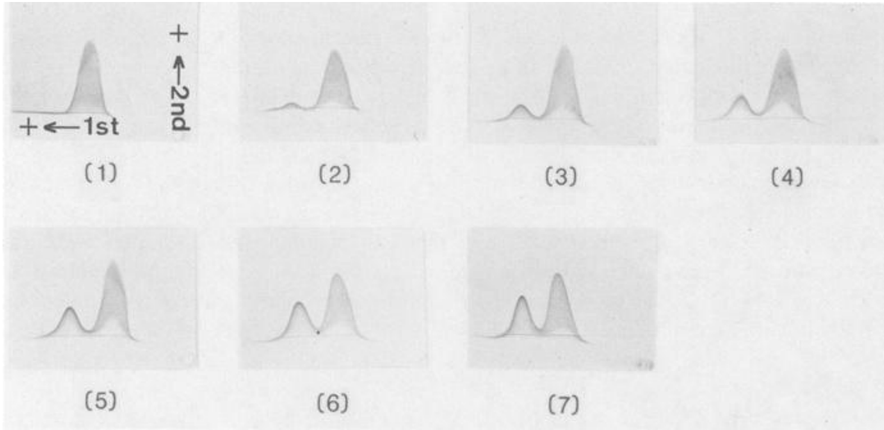

FIG. 1—*Crossed immunoelectrophoresis of aged blood at 37°C. Whole blood was incubated at 37°C for 0* (1), *12* (2), *24* (3), *36* (4), *48* (5), *60* (6), *or 72 h* (7). *Sera were harvested and stored at −80°C. Samples were subjected to crossed immunoelectrophoresis. Gels were stained with Coomassie brilliant blue.*

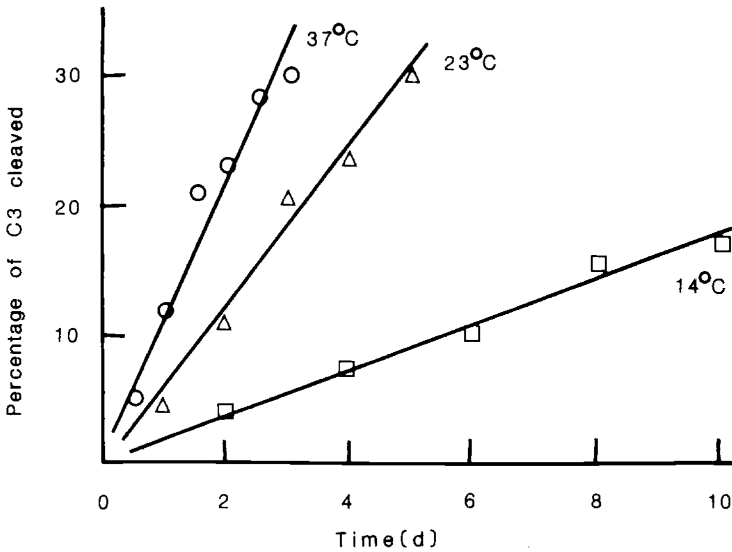

FIG. 2—*Time line of C3 cleavage at different temperatures. Whole blood was incubated at 37°C* (open circle), *23°C* (open triangle), *and 14°C* (open square). *Sera from each sample were collected at various times to be applied to crossed immunoelectrophoresis. The areas under two loops corresponding to beta 1C and beta 1A were measured, and the percentage of C3 cleaved was calculated by using the relationship:*

Percentage of C3 cleaved (%) = 100 (area under beta 1A)/(area under beta 1A and beta 1C)*

C3 Cleavage in Cadaverous Blood

The percentage of C3 cleavage in cadaveric blood was determined by calculating the proportion of the area under beta 1A to the total area. All data, including the cause of death, rectal temperature, PMI (time between death and storage of blood sample at 0°C), and the percentages of C3 cleavage are given in Table 1. Figure 3 shows a positive correlation (r = 0.832) between PMI and the percentage of C3 cleavage. The 95% confidence [9] of predicting PMI from experimentally determined percentages of C3 conversion was ±8.0 h for all data. The cause of death did not appear to influence the relationship between PMI and the percentage of C3 cleavage. The rectal temperature affected the ratio of C3 conversion. For example, in Cases 8 and 23, the ratio was higher at higher rectal temperature than that at lower rectal temperature.

Discussion

By incubating whole blood, it can be demonstrated that the percentage of C3 cleavage increases with time, and that the cleavage is influenced by the temperature of incubation. Recently, it has been shown that the intramolecular thioester bond of C3 is altered by water hydrolysis changing it to C3(H_2O) with the loss of hemolytic activity [10]. This is followed by two proteolytic steps: the first cleavage is from C3(H_2O) to iC3(H_2O) with factor I and factor H [11], and the second slow cleavage is from iC3(H_2O) to C3c + C3d,g with proteases such

TABLE 1—*Age, cause of death (COD), postmortem interval (PMI), rectal temperature, and percentage of C3 cleavage.*

Case	Age, Years	COD[a]	PMI, Hours	Rectal Temperature, °C	Percentage of C3 Cleaved
1	66	OHD	4	31	2.4
2	20	OHD	20	34	10.6
3	86	OHD	4	33	5.1
4	73	OHD	21	23	8.2
5	50	DA	10	33	5.9
6	47	un	14	31	6.8
7	54	Tx-aorta	22	21.5	10.7
8	70	pneumonia	23	28	13.3
9	76	OHD	16	23	10.5
10	50	un	21	23	13.0
11	53	OHD	17	29	9.8
12	74	pneumonia	19	31	9.7
13	42	un	12	32	6.7
14	39	un	27	26	15.7
15	55	OHD	12	32	8.5
16	14	un	4	32	3.3
17	62	SAH	24	20.5	9.3
18	82	DA	16	30	7.3
19	70	OHD	19	26	10.7
20	62	OHD	21	26	15.6
21	63	drowning	15	30	10.1
22	86	OHD	22	25.5	16.3
23	65	OHD	23	24	10.8
24	43	crush	25	16	12.3
25	42	OHD	7	32	7.5
26	43	SAH	21	28	9.3

[a]Cause of death abbreviations: OHD = organic heart disease, DA = dissecting aneurysm, Tx = transection, SAH = subarachnoid hemorrhage, and un = unknown cause.

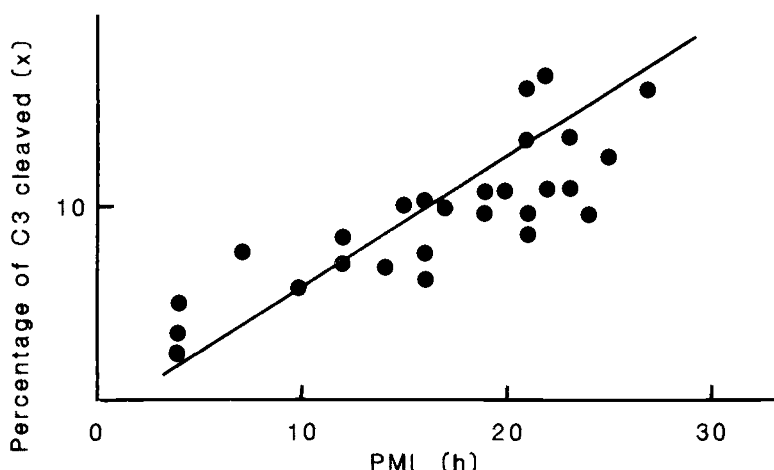

FIG. 3—*Percentage of C3 cleavage in cadaverous blood. Measured percentages of C3 cleavage* (x) *in cadaveric blood are plotted against their respective PMI (hours). Regression analysis of the points creates a line with the equation:*

$$PMI\ (hours) = 1.64 + 1.56\ x$$

The correlation coefficient is r = *0.832.*

as plasmin [*12,13*]. Since the rates of proteolytic cleavages may be faster than that of water hydrolysis, the first step could determine the rate in the fragmentation of native C3 to C3-derived fragments. Because the water hydrolysis is a first order reaction, the cleavage rate of beta 1C to beta 1A depends on the concentration of beta 1C. Therefore, kinetics of C3 cleavage may be described as $dC/dt = -kt$, where C is the concentration of native C3 at time t, and k is a rate constant, which is the conversion rate of C3 to its derived fragments with no hemolytic activity.

In vivo, the modified C3 is probably removed from circulation. Therefore, C3-derived fragments are not present with the exception of patients with complement activation such as acute glomerular nephritis [*14*], systemic lupus erythematosus, and sepsis, which are not listed in Table 1. After circulation stops, C3 products can accumulate with an increasing period of time. Actually, we have shown that in cadaveric blood the percentage of C3 derived fragments in relation to native C3 rises with time after death.

Of the various objective methods employed, postmortem accumulation of potassium (K^+) in vitreous humor is a widely used gauge for assessing PMI. A number of studies on the relationship between PMI and vitreous humor K^+ established that K^+ accumulates with increasing PMI, but the accuracy is quite variable. Some investigators concluded that the standard error of the estimate of time of death was 4.7 h [*15*], while others reported that there was such individual variation that the confidence limits of the method exceeded ± 10 h [*16*]. The method described here demonstrates that the 95% confidence of predicting PMI by experimentally measuring the percentage of C3 cleavage was ± 8.0 h. This is ascribed to the fact that C3-derived fragments cannot be detected in antemortem blood. It is therefore probable that a method for estimating PMI based on C3 cleavage is of some help in estimating the time of death.

In the next paper, we will show that the equation which is proposed here to represent the kinetics of C3 cleavage fits well with the experimental data, and that the percentage of C3 cleavage in cadaveric blood can be calculated by using the equation. Also, the comparison between the percentage which is measured by CIE and the percentage which is calculated from the equation may lead to the estimation of PMI.

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References

[*1*] Henry, J. B. and Smith, F. A., "Estimation of the Postmortem Interval by Chemical Means," *American Journal of Forensic Medicine and Pathology*, Vol. 1, 1980, pp. 341-347.
[*2*] Hamilton-Peterson, J. L. and Johnson, E. W. M., "Postmortem Glycolysis," *Journal of Pathology and Bacteriology*, Vol. 50, 1940, pp. 473-482.
[*3*] Hamilton, R. C., "Postmortem Blood Chemical Determinations: A Comparison of Chemical Analyses of Blood Obtained Postmortem with Degrees of Renal Damage," *Archives of Pathology*, Vol. 26, No. 6, Dec. 1938, pp. 1135-1143.
[*4*] Jetter, W. W., "Postmortem Biochemical Changes," *Journal of Forensic Sciences*, Vol. 4, 1959, pp. 330-341.
[*5*] Nillson, U. R., Mandle, R. J., Jr., and McConnell-Mapes, J. A., "Human C3 and C5: Subunit Structure and Modifications by Trypsin and C42-C423," *Journal of Immunology*, Vol. 114, 1975, pp. 815-822.
[*6*] West, C. D., Davis, N. C., Forristal, J., Herbst, J., and Spitzer, R., "Antigenic Determinants of Human Beta 1C- and Beta 1G-Globulins," *Journal of Immunology*, Vol. 96, No. 4, 1965, pp. 650-658.
[*7*] Alper, C. A. and Johnson, A. M., "Immunofixation Electrophoresis: a Technique for the Study of Protein Polymorphism," *Vox Sanguinis*, Vol. 17, 1969, pp. 445-452.
[*8*] Weeke, B., "Crossed Immunoelectrophoresis," *Scandinavia Journal of Immunology*, Vol. 2, Supplement No. 2, 1973, pp. 47-56.
[*9*] Snedecor, G. and Cochran, W., *Statistical Methods*, Iowa State University Press, Ames, IA, 1980.
[*10*] Panburg, M. K. and Muller-Eberhard, H. J., "Relation of a Putative Thioester Bond in C3 to Activation of the Alternative Pathway and the Binding of C3b," *Journal of Experimental Medicine*, Vol. 152, 1980, pp. 1102-1114.
[*11*] Crossley, L. G. and Porter, R. R., "Purification of the Human Complement Control Protein C3b Inactivator," *Biochemical Journal*, Vol. 191, 1980, pp. 173-182.
[*12*] Nagasawa, S. and Stroud, R. M., "Mechanism of Action of the C3b Inactivator: Requirement for a High Molecular Weight Cofactor (C3b-C4bINA Cofactor) and Production of a New C3b Derivative (C3b′)," *Immunochemistry*, Vol. 14, 1977, pp. 749-756.
[*13*] Carlo, J. R., Spitznagel, E. J., Conrad, D. H., and Ruddy, S., "Cleavage of Membrane Bound C3bi, an Intermediate of the Third Component of Complement, to C3c and C3d-Like Fragments by Crude Leucocyte Lysosomal Lysates and Purified Leucocyte Elastase," *Immunology*, Vol. 44, 1981, pp. 381-391.
[*14*] West, C. D., Winter, S., Forristal, J., McConville, J. M., and Davis, N. C., "Evidence for In Vitro Breakdown of Beta 1C Globulin in Hypocomplementimic Glomerulonephritis," *Journal of Clinical Investigation*, Vol. 46, pp. 539-548.
[*15*] Sturner, W. Q. and Gantner, G. E., "The Postmortem Interval," *American Journal of Clinical Pathology*, Vol. 42, No. 2, 1964, pp. 137-144.
[*16*] Coe, J. I., "Postmortem Chemistries on Human Vitreous Humor," *American Journal of Clinical Pathology*, Vol. 51, No. 6, 1969, pp. 741-750.

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