ELECTROPHORETIC ANALYSIS OF ANTE- AND POSTMORTEM SERUM IN DIFFERENT DISEASES[1]

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(Received for publication December 16, 1946)

Our present knowledge of the protein components of normal human plasma has been markedly advanced in recent years as a result of the blood fractionation program carried out by Cohn and his group (1). To a considerable extent the success of this program was due to the availability of large quantities of pooled plasma from normal humans. In attempting to extend the available information on normal human plasma to pathological human plasma, one is faced with the serious technical difficulty of being restricted to relatively small samples from a patient with a given disease. It therefore seemed desirable to investigate the possibility of using postmortem blood obtained at autopsy. The chief question to be answered in such a study is that of the influence of postmortem conditions on the plasma protein composition. Scudder (2) in a study of various factors influencing blood preservation stated that a serum and plasma sample obtained at autopsy were both "abnormal" on electrophoretic analysis. No reference was made as to the antemortem clinical status, nor were any antemortem analyses carried out. In view of this report it seemed important to determine what changes, if any, were to be found in samples of postmortem blood in different diseased states. Essentially the problem was that of obtaining a sample of blood antemortem and a second sample postmortem and comparing the electrophoretic patterns. A study of 9 cases is reported in the present paper, the results of which indicate that postmortem blood, which may be obtained in considerable quantity, shows no striking electrophoretic variation from that observed antemortem during the course of the disease.

METHODS

Collection of blood samples

At times varying from 8 to 240 hours before death, 25- to 30-ml. samples of blood were collected and allowed to clot at room temperature in a 50-ml. centrifuge tube. After retraction of the clot had begun, the tube and contents were centrifuged at 3,000 r.p.m. for 10 minutes. The clear serum was transferred to a 50-ml. Erlenmeyer flask which was then placed on its side in a quick-freeze unit. Blood was again collected at times varying from ½ to 13 hours after death and treated in the same manner as the antemortem samples. A number of different methods were used to obtain the postmortem blood. If the antecubital veins were in good condition it was found that by placing a tourniquet below the antecubital fossa and allowing the arm to drop off the side of the table with the opposite shoulder elevated so that the back was perpendicular to the table, 25 to 30 ml. could be procured from the distended veins. Frequently the veins in this region were not suitable and thus it was necessary to resort to either cardiac puncture or puncture of the femoral vessels. In cases of necropsy within 3 hours postmortem, the following procedure was found effective for obtaining large quantities of blood (1,500 to 2,500 ml.). The vena cava was incised and a length of tubing 10 mm. in diameter inserted and directed toward the heart. This was secured by tying a ligature about the vessel. The superior portion of the vena cava was either tied or clamped. The distal end of the tube was placed in a suitable container, and the body then elevated so that the feet were at the top of an incline of approximately 40°. It was found convenient to include a glass T-tube near the distal end of the tube to permit application of suction in instances where clots interfered with free drainage.

Electrophoretic analysis

The frozen samples were thawed out at room temperature. The resulting solutions were centrifuged and diluted with 2 parts of 0.10μ sodium diethylbarbiturate buffer, pH 8.6. The diluted samples were then dialyzed against 2 liters of buffer at 1 to 2° C. for 48 hours. The dialyzed samples were examined in the electrophoresis apparatus using the Schlieren scanning technique of Longsworth (3). Electrophoresis was carried out in 11-ml. cells for 150 minutes at a potential gradient of about 6 volts per cm. at 1.5° C. Relative composition of the serum samples was estimated by measuring the areas under the projected patterns after the areas were resolved into a series of symmetrical curves. In all instances, the percentage composition represents the average of the ascending and descending boundaries.

Mobilities were determined by measuring the migration distances of the different components from the starting

[1] Aided in part by a grant from the Wisconsin Alumni Research Foundation.

boundaries. Conductivity measurements were made on the protein-free buffer used as the dialyzing solution.

Total protein was determined by the micro-Kjeldahl technique.

RESULTS

Examination of Table I reveals that in only 1 instance (case 5) was there any significant change in the composition of the postmortem samples as compared with the antemortem samples. It appears from the analyses in case 5 that there is a postmortem decrease in beta globulin and an increase in gamma globulin. Whether this is actually due to a quantitative change in these components or to some unknown qualitative change causing a portion of the beta globulin fraction to migrate as gamma globulin is not apparent. The fact that the postmortem sample in this instance was taken only 1 hour after death suggests that the change is not due to postmortem conditions as such. It should be noted that the average values of the ratios of ante and postmortem analyses are remarkably close to unity.

With the exception of case 9 the total protein determination ante- and postmortem agreed quite closely. The high postmortem value found in this instance is in all probability an artefact due to dehydration of the sample in the freezer.

The serum protein patterns in several of the conditions studied showed marked variations from normal. The range of normal values obtained with sera from young normal adults and determined with the same technique is given in Table II.

TABLE I

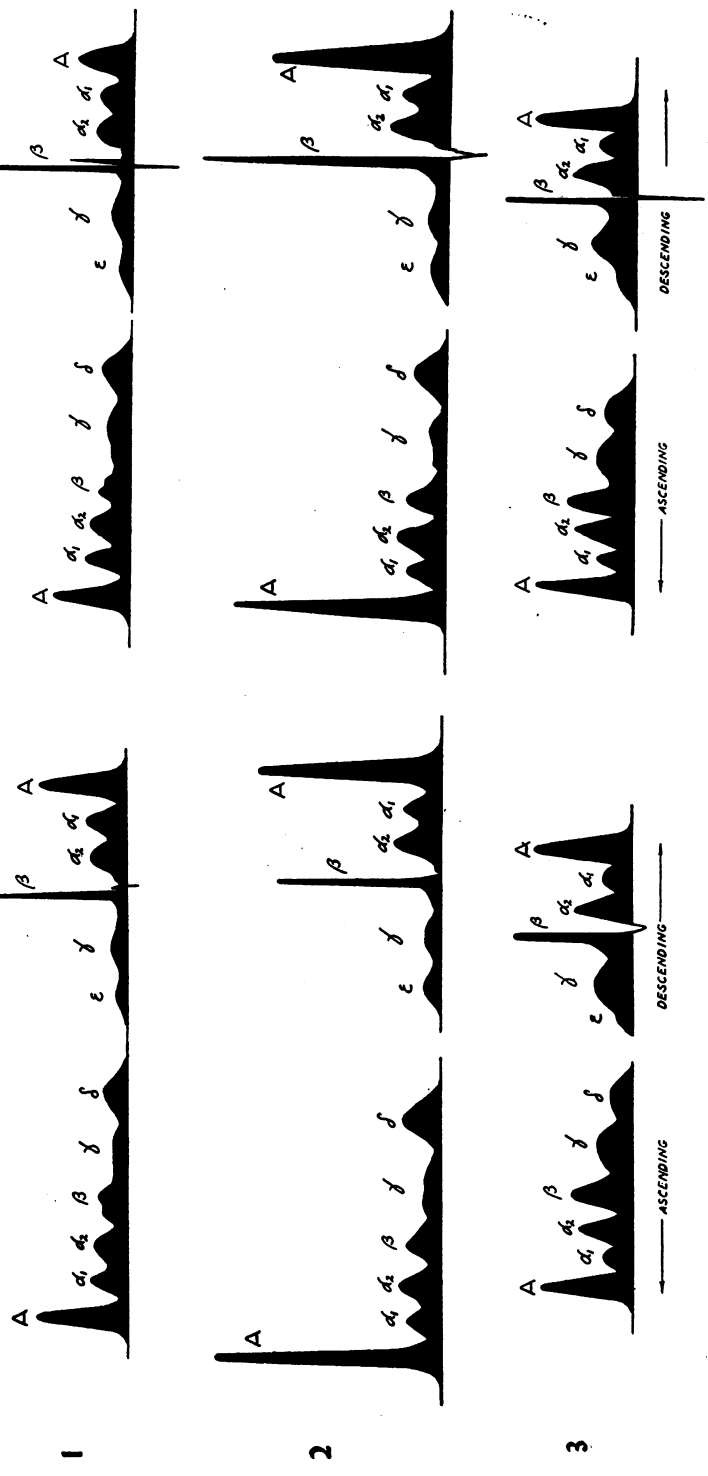
*Per cent composition of ante- and postmortem serums**

Case no.	Diagnosis	Hours	Total protein	Alb.	Ratio**	α-1	Ratio	α-2	Ratio	β	Ratio	γ	Ratio
1	Angiomatous mesothelioma of pelvis	59.0	4.53	31.40	1.17	15.90	0.96	19.20	1.05	18.00	1.05	15.50	0.72
		1.5	4.89	26.90		16.50		18.20		17.10		21.30	
2	Agranulocytosis with terminal lobar pneumonia	11.0	6.97	46.10	1.03	12.50	1.02	16.20	0.94	14.50	0.90	10.70	1.07
		3.0	6.99	44.60		12.20		17.20		16.00		10.00	
3	Carcinoma of esophagus with metastases	36.0	7.25	26.40	1.04	8.90	0.87	16.40	0.86	24.60	1.17	23.70	0.96
		13.0	7.86	25.20		10.20		19.00		21.00		24.60	
4	Nonlipoid histiocytosis (Letterer-Siwe's disease)	13.5	7.06	28.00	0.95	12.40	0.85	15.20	1.00	16.10	1.03	28.30	1.11
		2.5	6.84	29.40		14.40		15.20		15.60		25.40	
5	Localized Hodgkins with infiltration of lungs	57.0	7.02	40.30	0.97	9.70	1.03	19.70	0.83	17.90	1.92	12.40	0.76
		1.0	7.52	41.60		9.40		23.50		9.30		16.22	
6	Arteriosclerosis, generalized	7.5	6.58	44.40	1.01	13.10	1.00	12.30	0.82	17.40	1.10	12.80	1.00
		2.0	6.13	43.60		13.00		14.90		15.70		12.80	
7	Myelogenous leukemia	55.0	6.78	51.40	0.94	9.80	1.11	10.00	0.97	16.10	1.22	12.70	0.94
		0.5	6.55	54.30		8.80		10.30		13.10		13.50	
8	Hypernephroma with metastases	135.0	6.91	23.30	1.01	11.30	0.98	13.40	1.01	21.10	0.83	31.90	1.12
		1.00	6.61	22.90		11.50		13.20		24.00		28.40	
9	Hypertensive and arteriosclerotic heart disease, multiple pulmonary emboli	239.5	6.86	42.70	1.23	8.70	1.14	12.40	0.84	19.90	0.84	16.30	0.84
		3.0	8.50	34.70		7.60		14.70		23.70		19.30	
Mean of ratios:					1.04		1.00		0.92		1.12		0.95

* In each instance top line represents antemortem and bottom line postmortem values.
** Ratio of antemortem and postmortem values.

FIGURE 1

ELECTROPHORETIC DIAGRAMS



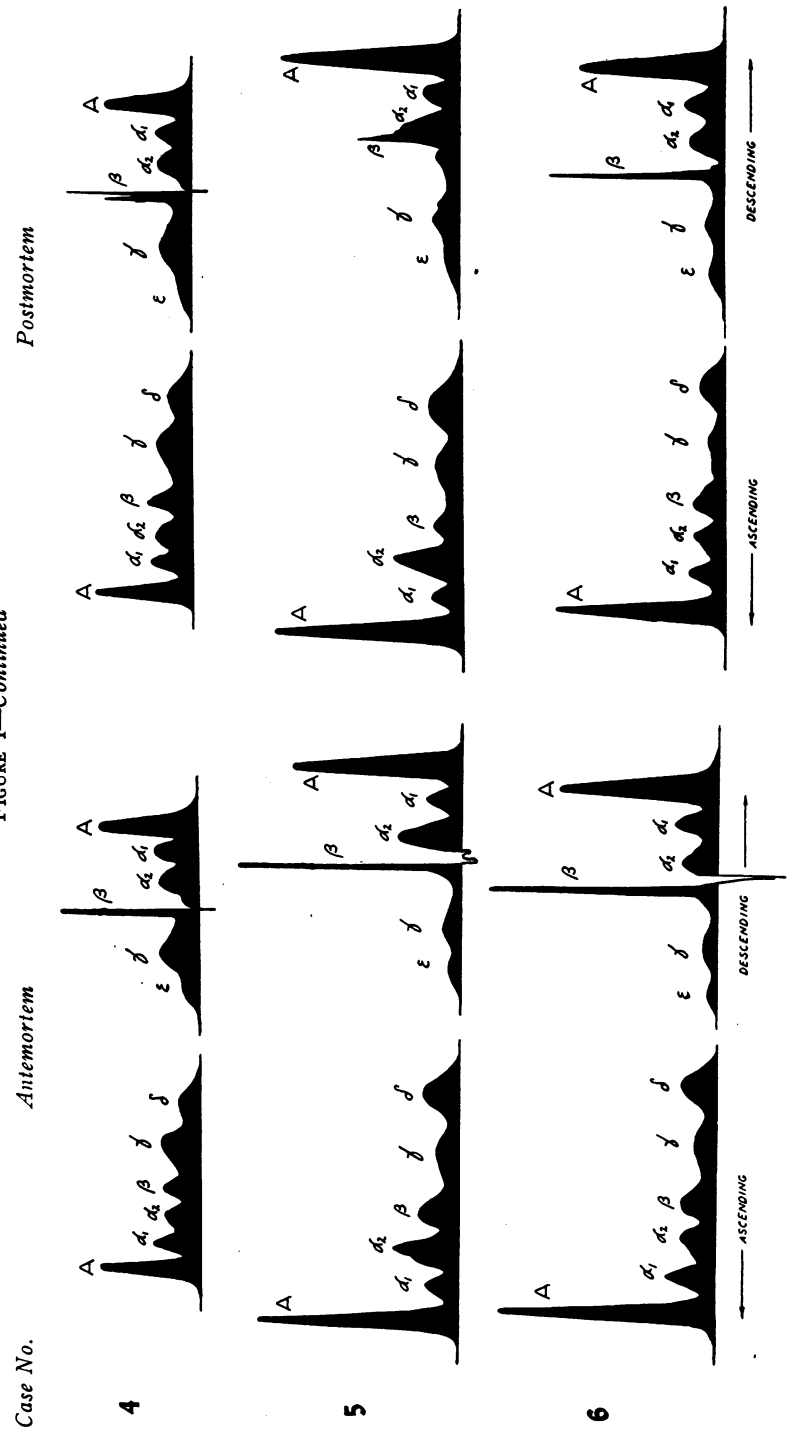


FIGURE 1—*Continued*

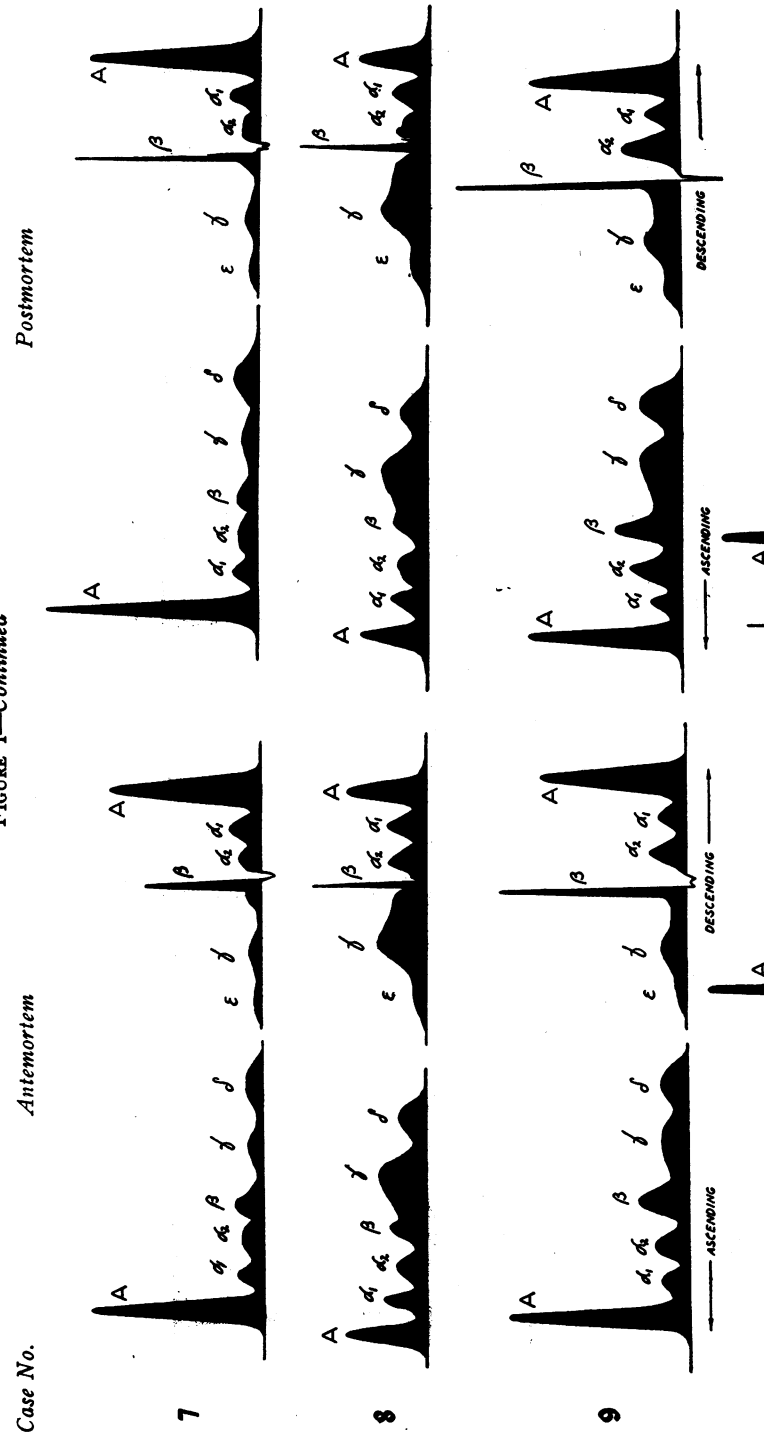


FIGURE 1—*Continued*

Normal (No. 7, table II)

TABLE II

Electrophoretic analysis of 10 sera from young normal adults

	Albumin	Alpha-1	Alpha-2	Beta	Gamma
1	53.1	5.4	11.4	16.6	13.8
2	57.8	6.0	8.5	13.7	13.8
3	55.5	5.9	9.8	13.7	15.1
4	54.1	5.0	9.1	16.6	15.2
5	53.1	4.9	8.6	17.2	16.2
6	59.8	4.8	8.0	14.6	12.8
7	58.6	4.8	7.5	13.7	15.4
8	59.0	4.2	9.6	16.6	10.6
9	54.6	5.5	9.3	14.2	16.4
10	59.0	5.2	7.8	15.2	12.8
Mean	56.5	5.2	9.0	15.2	14.2
S.D.	2.8	1.7	1.4	1.5	1.8
S.E. of mean	0.9	0.6	0.5	0.5	0.6

In cases 1, 3, 4, and 8 the serum albumin levels are extremely low. Of interest, however, is the fact that the globulin components are not uniformly increased. Thus, in case 1, marked increases are seen only in the alpha-1 and alpha-2 fractions; in case 3, in all the globulin fractions; in case 4, in the alpha-1, alpha-2, and gamma globulin fractions; and in case 8, in the alpha-1, beta, and gamma fractions. The one fraction showing the most consistent and striking variation from normal in all the conditions studied here is the alpha-1 globulin. This finding is in keeping with the observations made previously by Shedlovsky and Scudder (4).

Tracings of the electrophoretic patterns ante- and postmortem of the different cases and that of a typical normal serum are shown in Figure 1. The greatest apparent variation in the patterns of the ante- and postmortem specimens is seen to occur in the beta-anomaly of the descending patterns. However, variations of the same degree and type have been observed in determinations on a series of normals and even in a series of 3 determinations on the same individual at varying intervals of time.

The mobility values of the different fractions of the normal, antemortem, and postmortem series are shown in Table III. It is apparent that the protein components in serum from cases of disease, ante- and postmortem, have the same mobility values as those found with normal serum.

TABLE III

Mobilities ($cm.^2/volt\ sec. \times 10^{-5}$)

		Albumin	Alpha-1	Alpha-2	Beta	Gamma
Normal	Mean	6.8	5.8	4.6	3.3	1.5
	S.D.	0.23	0.25	0.19	0.22	0.21
Antemortem	Mean	6.8	5.7	4.5	3.2	1.4
	S.D.	0.30	0.30	0.32	0.29	0.26
Postmortem	Mean	6.7	5.7	4.5	3.4	1.5
	S.D.	0.30	0.32	0.31	0.32	0.28

SUMMARY

Serum samples taken ante- and postmortem from 9 cases of different diseases were examined electrophoretically. While all the cases showed some variation from a series of normals, no striking electrophoretic differences were noted in the postmortem as compared with the antemortem samples. On this basis it would appear that up to 13 hours postmortem, serum proteins undergo no significant changes. Thus, the possibility presents itself of preparing purified protein fractions from postmortem blood of diseased patients and comparing their physical, chemical, and biological properties with those obtained from normal pooled plasma.

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