ELECTROPHORETIC STUDIES IN LIVER DISEASE. I. COMPARISON OF SERUM AND PLASMA ELECTROPHORETIC PATTERNS IN LIVER DISEASE, WITH SPECIAL REFERENCE TO FIBRINOGEN AND GAMMA GLOBULIN PATTERNS

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The development of electrophoresis has permitted the study of body-fluid protein changes in health and disease. Most reports on electrophoretic analysis in hepatic diseases are based upon findings in serum rather than plasma. The chief reasons for the preference of serum over plasma are: (a) it avoids the use of anticoagulants such as heparin which may affect the electrophoretic pattern (1), (b) it permits comparison of electrophoretic data with the usual flocculation tests performed on serum, (c) it avoids the difficulty of differentiating protein complexes having a mobility similar to that of fibrinogen and forming with it a single peak which is incapable of resolution into its constituent components (2).

In a recent study of electrophoretic plasma protein partition of patients with hepatic diseases, especially those with cirrhosis, unexpected fibrinogen and gamma globulin values were noted. The "fibrinogen" peaks were markedly elevated and the gamma globulin elevations were not as high as expected from previous serum electrophoresis and chemical partition. To clear up these discrepancies, electrophoretic tracings on a series of simultaneously collected serum and plasma specimens of normal persons and patients with various hepatobiliary and other diseases were compared with each other as well as with chemical partition of blood proteins. This study was planned to give general information on possible differences in the electrophoretic pattern of serum and plasma of normal persons as well as patients with hepatic and other diseases.

MATERIALS AND METHODS

Clinical Material

The subjects for the study came from three groups: (1) a group with liver disease made up of 23 cirrhotics (22 of the Laennec type and one post-necrotic), six cases of infectious and six of toxic hepatitis (two arsenic, one post-pneumonic and three of unknown etiology whose clinical and biopsy pictures resembled toxic hepatitis of known toxic etiology) and three cases of extrahepatic obstruction (two pancreatic neoplasms and one case of common duct lithiasis); (2) a miscellaneous group of 15 cases of rheumatoid arthritis, two of gastric ulcer and one of multiple myeloma; and (3) a normal group composed of 10 doctors and technicians working in the laboratory.

Hepatic tests were performed upon all subjects. In addition all but five of the patients with hepatic disease had needle or surgical biopsies performed for confirmation of the diagnoses. All diagnoses were made by clinical, laboratory or surgical means.

Method

Approximately 50 ml. of blood were drawn from every subject in the fasting state. Ten ml. were placed in a dry clean test tube and allowed to clot, after which the serum was separated by centrifugation. Another 10 ml. were placed in a test tube containing 30 mg. of potassium oxalate as the anticoagulant and centrifuged for separation of plasma. The remainder was utilized for chemical determination of the total serum proteins, fibrinogen (in 27 cases), or protein partition, and other hepatic function tests.

Electrophoretic analysis of the pairs of serum and plasma was performed in a Tiselius apparatus at pH 8.6 in sodium diethyl barbiturate and ionic strength of 0.1

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with barbiturate buffer according to the Longsworth procedure (3). All runs were made at 1.5° C. for 120 minutes. Schlieren diagrams, photographically enlarged, were traced with a planimeter according to the method of Pedersen and Svedberg (4), to obtain the percentage of the total area assigned to each protein fraction. Mobility measurements were made from the boundary anomaly of the pattern. Only descending patterns were used. The relative concentrations, calculated from the proportions of the component peaks of the patterns, were converted to absolute concentrations expressed in grams/100 ml. of the total serum protein which had been determined chemically. Total serum proteins were determined by the Weichselbaum modification of the biuret reaction (5). The nitrogen to protein conversion factor used was 6.25. In 27 instances fibrinogen was determined chemically by standard laboratory methods of calcium salt precipitation and nitrogen determination (6).

Total plasma proteins were not determined chemically. The normal values for serum are practically identical with those of plasma despite a fibrinogen content of 200–400 mg./100 ml. in the latter (7, 8). The addition of an anticoagulant increases the plasma salt concentration and causes diffusion of water out of the erythrocytes. The presence of fibrinogen in plasma is thus nullified by the increased dilution of the plasma (9). When comparing absolute plasma and serum findings it is desirable to reduce this source of error. A formula was utilized to calculate the total plasma proteins from the absolute serum protein values and the relative percentages of the serum and plasma electrophoretic components. The formula and its derivation are as follows:

N = total plasma protein in grams/100 ml.
S = total serum protein in grams/100 ml.
X = plasma fibrinogen content in grams/100 ml.
$S = N - X$
γ' = grams of γ' component found in serum
$g = \dfrac{\% \ \gamma' \text{ component in serum (electrophoretic)}}{100}$
$\qquad\qquad$ = fraction of γ' found in serum
$\gamma' = gS$
$f = \dfrac{\% \ \phi \text{ (electrophoretic)}}{100}$ = fraction of ϕ found in plasma.
$X = fN - \gamma'$
$S = N - (fN - \gamma')$.
$N = \dfrac{S - \gamma'}{1 - f}$

RESULTS

The detailed analyses of our electrophoretic findings in the various liver diseases and their correlation with the clinical, chemical and biopsy findings are being reported elsewhere. This report is concerned primarily with the difference between serum and plasma in different liver diseases. These are apparent in Figure 1 which depicts the

γ SERUM = γ' + γ''
γ PLASMA ≈ γ'' SERUM
CORRECTED γ PLASMA = γ PLASMA + γ' or γ SERUM
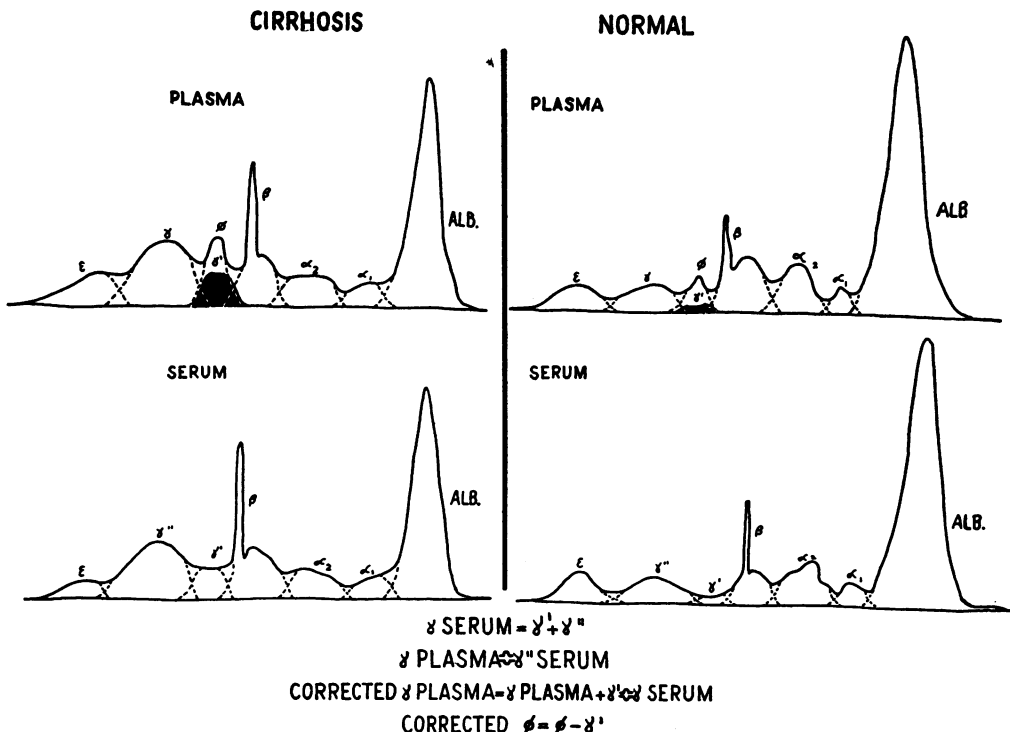CORRECTED $\phi = \phi - \gamma'$

FIG. 1. ELECTROPHORETIC PATTERNS ON PLASMA AND SERUM OF A CIRRHOTIC AND NORMAL INDIVIDUAL

typical serum and plasma electrophoretic patterns in normal and cirrhotic subjects. The usual findings of decreased albumin and increased gamma globulin (10–15) are seen in the cirrhotic pattern and the only differences between the serum and plasma patterns are in the gamma globulin and fibrinogen fractions. The normal patterns show similar serum-plasma differences except they are smaller. These serum-plasma differences in the globulin and fibrinogen components will be described and discussed later. The descending patterns are used. In addition to the presence of the usual peaks denoting the albumin, alpha$_1$, alpha$_2$, beta, gamma, fibrinogen, and epsilon boundary anomaly, the gamma globulin component is composed of a fraction labelled gamma$_2$, and a gamma$_1$ fraction of greater mobility. The gamma$_1$ portion of the plasma gamma globulin is buried in the "fibrinogen" peak so that the plasma gamma globulin peak corresponds to the gamma$_2$ component of the serum. We have adopted the nomenclature of Deutsch (2) in describing the globulin components.

The mean percentage distributions, absolute concentrations and mobilities of the protein components as determined electrophoretically in serum and plasma are summarized in Tables I and II.

TABLE I

Electrophoretic analyses of serum and plasma in normal individuals and in patients with liver and miscellaneous diseases

Diagnosis	No. of cases	S/P	TP$_s$	TP$_p$ calc.	Albumin gm	Albumin %TP	Globulin gm	Globulin %TP	Globulin corr gm	Globulin corr %TP	α_1 gm	α_1 %TP	α_2 gm	α_2 %TP	α TOTAL gm	α TOTAL %TP	β gm	β %TP	γ' gm	γ' %TP	γ'' gm	γ'' %TP	γ_s TOTAL gm	γ_s TOTAL %TP	γ_p gm	γ_p %TP	γ_p corr gm	γ_p corr %TP	ϕ gm	ϕ %TP	ϕ corr gm	ϕ corr %TP	A/G	A/G corr
CIRRHOSIS	23	S	7.06		2.47	35.0	4.59	65.0			0.40	5.7	0.80	11.3	1.20	17.0	1.22	17.3	0.50	7.1	1.67	23.6	2.17	30.7									0.55	
		P		7.62	2.57	33.7	3.99	52.4	4.49	59.0	0.39	5.1	0.79	10.4	1.18	15.5	1.19	15.6							1.62	21.3	2.12	27.9	1.06	13.9	0.56	7.3	0.64	0.57
INF. HEPATITIS	6	S	7.70		3.47	45.1	4.23	54.9			0.40	5.2	0.83	10.8	1.23	16.0	1.28	16.6	0.26	3.4	1.46	18.9	1.72	22.3									0.82	
		P		8.15	3.43	42.1	4.01	49.2	4.27	52.4	0.44	5.5	0.94	11.5	1.38	17.0	1.21	14.8							1.42	17.4	1.68	20.6	0.71	8.7	0.45	5.5	0.86	0.80
TOXIC HEPATITIS	6	S	7.24		3.10	42.8	4.14	57.2			0.43	6.0	0.77	10.6	1.20	16.6	1.23	17.0	0.31	4.3	1.40	19.3	1.71	23.6									0.75	
		P		7.60	3.11	41.0	3.82	50.2	4.13	54.3	0.44	6.4	0.81	10.7	1.30	17.1	1.29	17.0							1.23	16.1	1.54	20.2	0.67	8.8	0.36	4.7	0.81	0.75
OBST JAUNDICE	3	S	7.20		3.13	43.5	4.07	56.5			0.41	5.7	1.18	16.4	1.59	22.1	1.21	16.8	0.34	4.7	0.93	12.9	1.27	17.6									0.77	
		P		7.81	3.24	41.5	3.62	46.4	3.96	50.7	0.49	6.3	1.22	15.6	1.71	21.9	1.15	14.7							0.76	9.8	1.10	14.1	0.95	12.1	0.61	7.7	0.94	0.82
MISC	18	S	7.26		3.64	50.1	3.62	49.9			0.45	6.3	0.74	10.2	1.19	16.5	1.01	14.7	0.21	2.9	1.15	15.8	1.36	18.7									0.96	
		P		7.70	3.62	47.0	3.43	44.5	3.62	47.3	0.44	5.7	0.86	11.1	1.29	16.8	1.12	14.5							1.02	13.2	1.23	15.8	0.65	8.5	0.44	5.7	1.06	1.00
NORMALS	10	S	7.32		4.40	60.1	2.92	39.9			0.37	5.0	0.68	9.3	1.05	14.3	0.84	11.5	0.18	2.5	0.85	11.6	1.03	14.1									1.48	
		P		7.65	4.55	59.5	2.59	33.8	2.77	36.2	0.33	4.3	0.67	8.7	1.00	13.0	0.79	10.3							0.81	10.5	0.99	12.9	0.51	6.7	0.33	4.3	1.75	1.64

(gm columns = gm/100 ml)

TABLE II

Comparison of electrophoretic mobilities of protein components in serum and plasma

Diagnosis	No. of cases	Serum plasma	Av. alb.	Mobilities (cm²/volt sec. ×10⁻⁵)						
				α_1	α_2	β	ϕ	γ_1	γ_2	γ_p (uncorr.)
Cirrhosis	23	S	6.64	5.49	4.42	3.35	—	2.54	1.48	—
		P	6.65	5.53	4.43	3.39	2.51	—	—	1.49
Infectious hepatitis	6	S	6.56	5.44	4.40	3.42	—	2.51	1.46	—
		P	6.56	5.45	4.41	3.38	2.49	—	—	1.45
Toxic hepatitis	6	S	6.58	5.45	4.42	3.40	—	2.53	1.45	—
		P	6.55	5.47	4.43	3.42	2.51	—	—	1.47
Obstructive jaundice	3	S	6.63	5.47	4.35	3.30	—	2.48	1.49	—
		P	6.59	5.32	4.40	3.30	2.45	—	—	1.48
Miscellaneous diseases	18	S	6.64	5.47	4.40	3.41	—	2.54	1.50	—
		P	6.62	5.44	4.41	3.38	2.49	—	—	1.48
Normal	10	S	6.66	5.48	4.43	3.43	—	2.59	1.58	—
		P	6.63	5.46	4.45	3.46	2.54	—	—	1.58
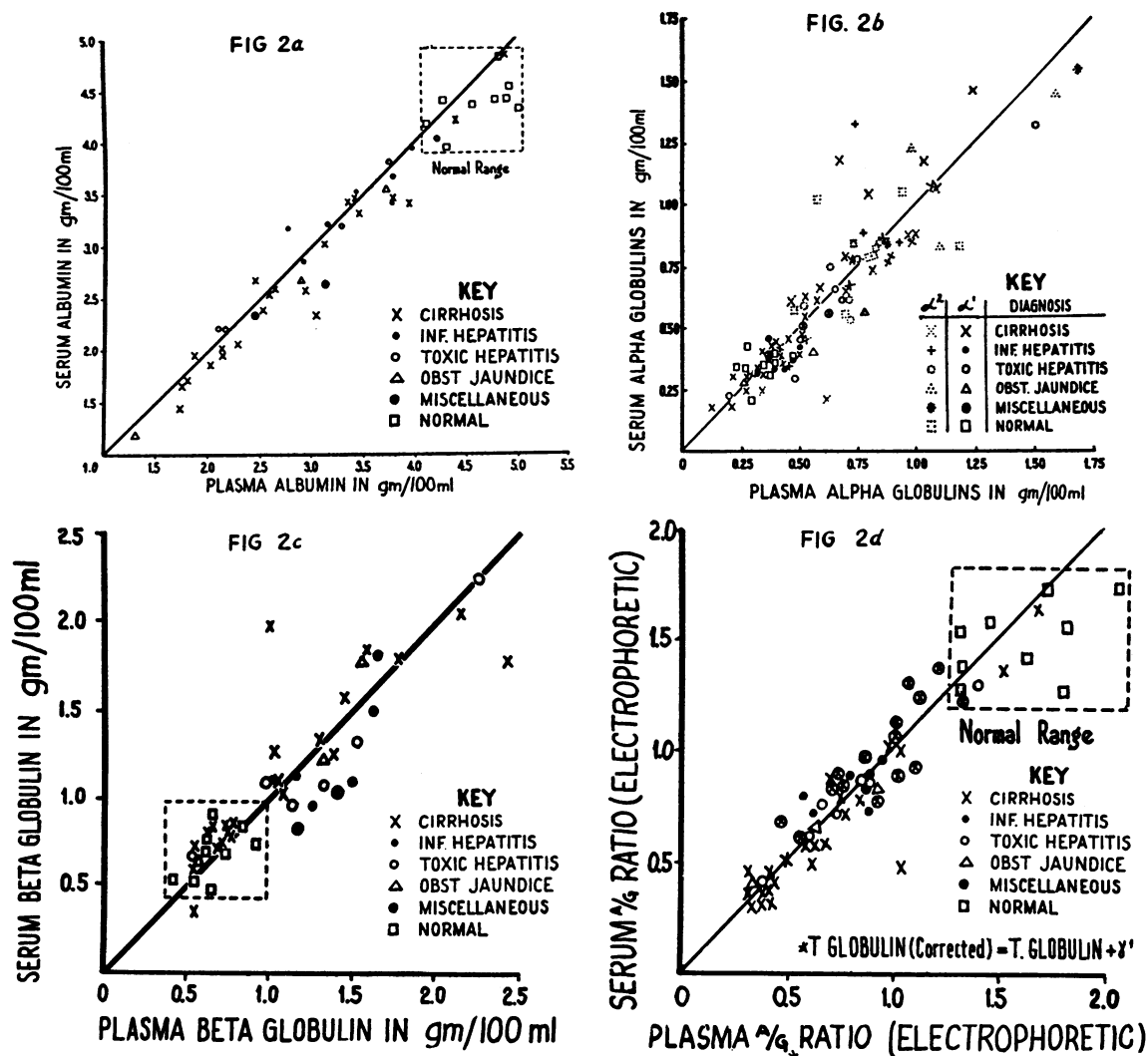
FIGS. 2a, b, c, d. RELATION OF ELECTROPHORETIC SERUM AND PLASMA ALBUMINS, ALPHA GLOBULINS, BETA GLOB-
ULINS AND ALBUMIN-GLOBULIN RATIOS

The decrease in the albumin component when com-
pared to the values in the normal group was most
marked in the cirrhotics, less in the other liver dis-
eases, and least in the miscellaneous group. The
cases of rheumatoid arthritis which formed most
of the miscellaneous group are responsible for the
albumin and globulin changes in this group and
are similar to those found in liver disease though
smaller. They are in agreement with those re-
ported by others (16–18). All groups showed
slightly higher serum than plasma values but these
differences, both in percentage and absolute con-
centrations, were not significant. The correlation
between serum and plasma albumin is good (Fig-

ure 2a) and the observed scatter is due largely to
the inherent error in the electrophoretic procedure.

There was a tendency to slight increase of the
alpha globulins in all the pathologic cases and most
marked in obstructive jaundice (Table I) where
the frequent association of lipids with alpha (as
well as beta globulin) may account for this ele-
vation. Elevated values of lipid were present in the
three cases of obstructive jaundice. The alpha
globulins were the only protein components in
which the plasma values were greater than were the
serum values, both relatively and absolutely. This
was found to be true in all groups except the nor-
mal and cirrhotic groups. However, these differ-

ences between alpha globulins in serum and plasma were not significant. Figure 2b reveals that the spread on either side of the theoretical line of perfect correlation is greater than that of any of the other components. This was particularly true of the alpha$_2$ fraction.

Beta globulins (Table I) were increased in liver diseases as others have found (13–15). The relative percentage values in serum and plasma vary significantly but after they are converted to absolute values the variation loses its significance and does not exceed the limits of error of the method. Figure 2c reveals a good correlation between absolute values of beta globulin in serum and plasma.

The characteristically elevated gamma globulin in liver disease and rheumatoid arthritis is well illustrated in Tables I and III. This increase was most marked in cirrhosis, less so in hepatitis and least in obstructive jaundice and miscellaneous group. These findings are in agreement with those of previous authors (10–19). The normal serum gamma globulin values (average 14.1%) in this study are slightly higher than those reported by others (9, 20) because of our inclusion of the gamma$_1$ fractions of the total gamma values. This gamma$_1$ component normally forms approximately 2–3% of the total protein (2) and is often not included in the electrophoretically determined gamma globulin. The serum gamma globulins of all groups of cases studied including the normals were much higher than the plasma values (Tables I and III).

Figure 1 reveals the cause of this significant discrepancy between serum and plasma gamma globulin values. The serum gamma globulin is composed of two fractions of different mobilities, a fraction labelled gamma$_2$ and a fraction with a greater mobility labelled gamma$_1$. This latter fraction has a mobility similar to fibrinogen and is therefore buried in the "fibrinogen" peak in the plasma pattern (Figure 1). Thus the apparent plasma gamma is the equivalent of the gamma$_2$ serum fraction (Tables I, III, Figures 1, 3). If the serum gamma$_1$ is determined in grams/100 ml. and this absolute value is then added to the absolute plasma gamma globulin value, the resultant corrected plasma gamma globulin value approaches the serum value. The difference between the serum gamma globulin and the corrected plasma gamma globulin then falls within the inherent error of the electrophoretic method (Tables I, III, Figures 3, 4). In cirrhosis this gamma$_1$ fraction is increased almost threefold from an average value of 2.5% in the normal group to 7.1%. There is a moderate increase in gamma$_1$ in the three obstructive jaundice cases. Only a slight increase in this component was present in the hepatitis cases and normal values were obtained in the miscellaneous group despite somewhat increased total gamma globulins in this latter group. After correction, the plasma total gamma globulin values were still lower than the serum values although the differences were much less than before correction. Prior to correction of

TABLE III

Comparison of electrophoretic serum and plasma gamma globulins

| DIAGNOSIS	NO. CASES	% TOTAL PROTEIN				gm/100ml				
		γ'	γ^2	TOTAL γ_s	$\gamma'\%$ OF Tot.γ_s	δ'	γ^2	TOTAL γ_s	TOTAL γ_p UNCORRECTED	TOTAL γ_p CORRECTED
CIRRHOSIS	23	7.1	23.6	30.7	23.6	0.50	1.67	2.17	1.62	2.12
RANGE		(2.1 – 11.5)	(11.9 – 55.6)	(15.1 – 65)	(9.5 – 36.1)					
INF HEPATITIS	6	3.4	18.9	22.3	15.2	0.26	1.46	1.72	1.42	1.68
RANGE		(2.3 – 4.5)	(14.8 – 22.0)	(16.9 – 27.5)	(12 – 20.3)					
TOXIC HEPATITIS	6	4.3	19.3	23.6	18.6	0.31	1.40	1.71	1.23	1.54
RANGE		(1.8 – 6.0)	(10.7 – 40.0)	(12.5 – 44.9)	(10.9 – 34.4)					
OBST. JAUNDICE	3	4.7	12.9	17.6	26.6	0.34	0.93	1.27	0.76	1.10
RANGE		(3.3 – 8.5)	(12.4 – 15.0)	(14 – 18.4)	(18.6 – 28.4)					
MISCELLANEOUS	18	2.9	15.8	18.7	15.5	0.21	1.15	1.36	1.02	1.23
RANGE		(1.6 – 47)	(9.1 – 61.4)	(12.0 – 66.1)	(11.6 – 23.4)					
NORMAL	10	2.5	11.5	14.1	17.7	0.18	0.85	1.03	0.81	0.99
RANGE		(1.7 – 3.4)	(8.5 – 17.2)	(12.6 – 19.1)	(10.0 – 17.3)					
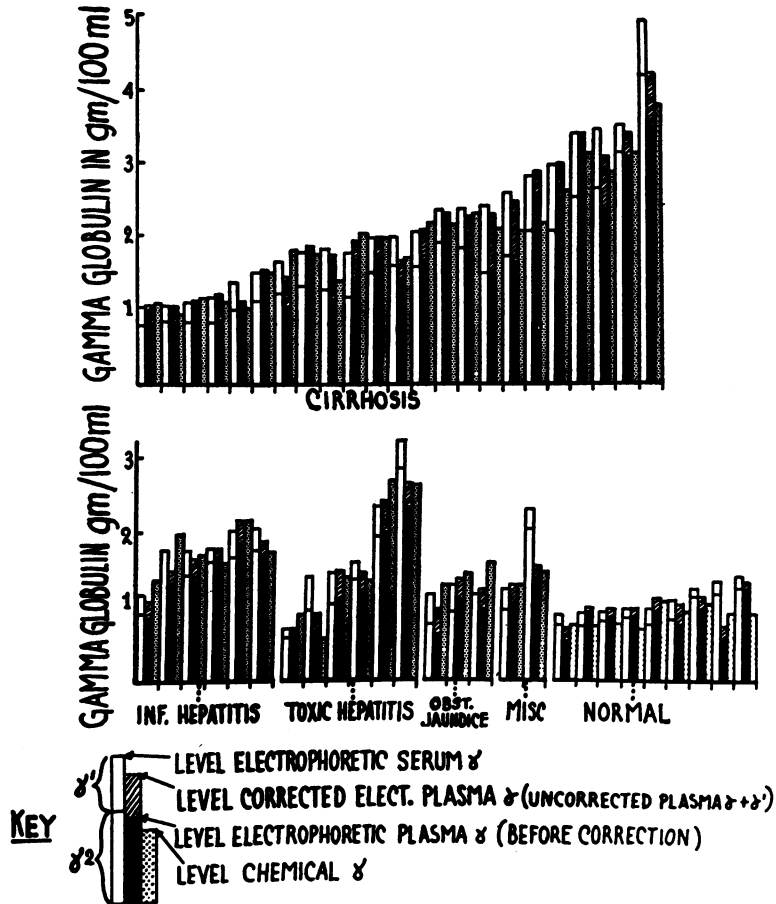
FIG. 3. RELATION OF ELECTROPHORETIC SERUM, PLASMA AND CHEMICAL
GAMMA GLOBULINS

the plasma gamma globulin, particularly in the pathologic cases, the chemical gamma globulin values more closely resembled the serum electrophoretic results. After correction there was essentially no difference between the relation of the serum and plasma electrophoretic gamma globulin results and the chemical gamma globulin.

An analysis of fibrinogen data (Tables I, IV) reveals greatly elevated values in cirrhosis (average 13.9%, with a range between 6.07% and 28%). The obstructive jaundice cases also had elevated fibrinogen values and the other groups revealed smaller increases in this component. Figure 1 indicates the reason for these abnormal elevations in fibrinogen, particularly in cirrhosis where the plasma pattern contains a markedly elevated "fibrinogen" peak. In both the cirrhotic and normal electrophoretic patterns there is incomplete disappearance of the "fibrinogen" peaks in the cor-

responding serum patterns. In the normal pattern, a small plateau-shaped component is still present between the gamma and beta globulin complexes. In the cirrhotic pattern this remaining component is of much greater magnitude and accounts for almost 50% of the area underneath the plasma "fibrinogen" peak. This component, lying between the gamma and beta peak, is the $gamma_1$ globulin fraction of the serum and has electrophoretic mobility similar to fibrinogen. Inclusion of this $gamma_1$ fraction in the "fibrinogen peak" thus accounts for most of the marked fibrinogen elevations in the cirrhotics as well as the lesser elevations of this component in the other pathologic plasma specimens. The electrophoretic fibrinogen values can be corrected by subtracting from the originally elevated values, the absolute $gamma_1$ value as determined in the corresponding serum samples. The corrected values are much lower
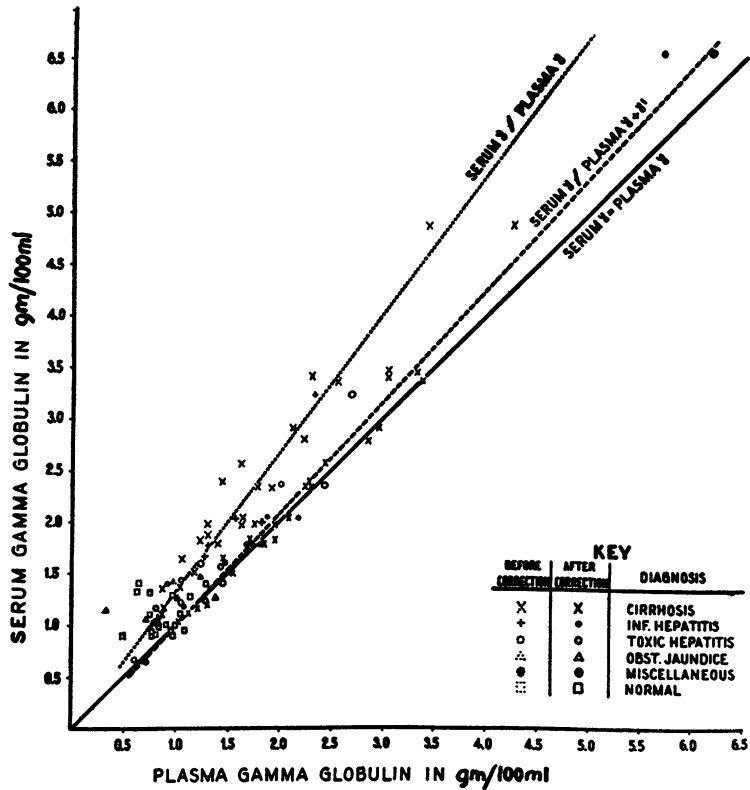
FIG. 4. RELATION OF ELECTROPHORETIC SERUM AND PLASMA GAMMA
GLOBULINS BEFORE AND AFTER CORRECTION

TABLE IV

*Electrophoretic fibrinogen before and after correction compared with
chemically determined fibrinogen*

DIAGNOSIS	NO. CASES	% TOTAL PROTEIN			gm/100ml			
		ϕ BEFORE CORRECTION	ϕ' % OF T.P.p	CORRECTED ϕ	ϕ BEFORE CORRECTION	ϕ'	CORRECTED $(\phi + \phi')$	CHEMICAL ϕ
CIRRHOSIS	23	13.9 (6.0 – 28.0)	6.6 (2.0 – 10.8)	7.3 (3.0 – 20.5)	1.06	0.50	0.56	0.34 (9 CASES)
INF. HEPATITIS	6	8.7 (6.7 – 10.2)	3.2 (2.3 – 4.0)	5.5 (3.5 – 6.8)	0.71	0.26	0.45	0.29 (4 CASES)
TOXIC HEPATITIS	6	8.8 (5.9 – 11.1)	4.1 (1.8 – 5.7)	4.7 (1.7 – 7.2)	0.67	0.31	0.36	0.25 (1 CASE)
OBST. JAUNDICE	3	12.1 (10.8 – 12.4)	4.4 (3.0 – 5.1)	7.7 (5.7 – 9.4)	0.95	0.34	0.61	0.55 (2 CASES)
MISCELLANEOUS	18	8.5 (6.0 – 12.4)	2.8 (1.6 – 4.4)	5.7 (3.6 – 9.4)	0.65	0.21	0.44	0.36 (1 CASE)
NORMAL	10	6.7 (4.4 – 8.4)	2.4 (1.6 – 3.3)	4.3 (1.8 – 6.6)	0.51	0.18	0.33	0.34 (10 CASES)

than the original ones. The corrected absolute values approach the chemical values; in the normal group the electrophoretic results are still somewhat higher than those secured by the chemical method (Tables I, IV, Figure 5).

There is a good correlation between the albumin-globulin ratio as determined electrophoretically in the serum and plasma especially after the plasma total globulin has been corrected by the addition of $gamma_1$ globulin (Table I, Figure 2d).

In comparing the serum-plasma electrophoretic patterns, an unexpected finding was the fact that the greatest variations in the protein components were in the normals and this was true of all components.

DISCUSSION

Electrophoretic analysis has been employed widely in recent years to study blood protein changes in liver disease (10–15, 19, 21). Usually this has been performed upon serum for the reasons previously mentioned. Dole (9) reported significant differences between serum and plasma protein electrophoretic values in a normal person primarily in the gamma and beta globulin fractions. Only the relative concentrations expressed in per cent of total protein were compared. Comparison of serum and plasma electrophoretic results are more meaningful if absolute rather than percentage values are compared. The percentage values are not strictly comparable, being fractions of total proteins which are dissimilar because of fibrinogen content in plasma. Chemical determinations may reveal similar values for total serum and plasma proteins because of dehydration of erythrocytes with consequent dilution of the plasma because of the anticoagulant (7, 8). Were it not for this di-

lution one would expect the fractions of total proteins to be about 4–5% greater in serum than in plasma with its fibrinogen (9). For a proper evaluation of any possible differences between serum and plasma electrophoretic values, the comparison should be made between absolute values determined from total serum and plasma proteins corrected for anticoagulant dilution of the plasma. Heparin alters the electrophoretic pattern (1). Theoretically, potassium oxalate might possibly cause changes due to chemical denaturation of the protein. However, in previous studies (22) we found no appreciable changes using standard amounts of potassium oxalate as an anticoagulant.

The albumin changes in liver disease and rheumatoid arthritis (Table I) were similar to those reported by others (10–19).

Reports in the literature differ as to the fate of alpha globulins in liver disease, some workers reporting irregular increases (13, 14) while others found no change (15, 21). Gutman (23) believes that there is an association between increased alpha globulin and decreased gamma globulin values. In this study the alpha globulins showed a slight increase in all the pathologic cases, the greatest increase being in obstructive jaundice. The elevated lipids on our cases of obstructive jaundice
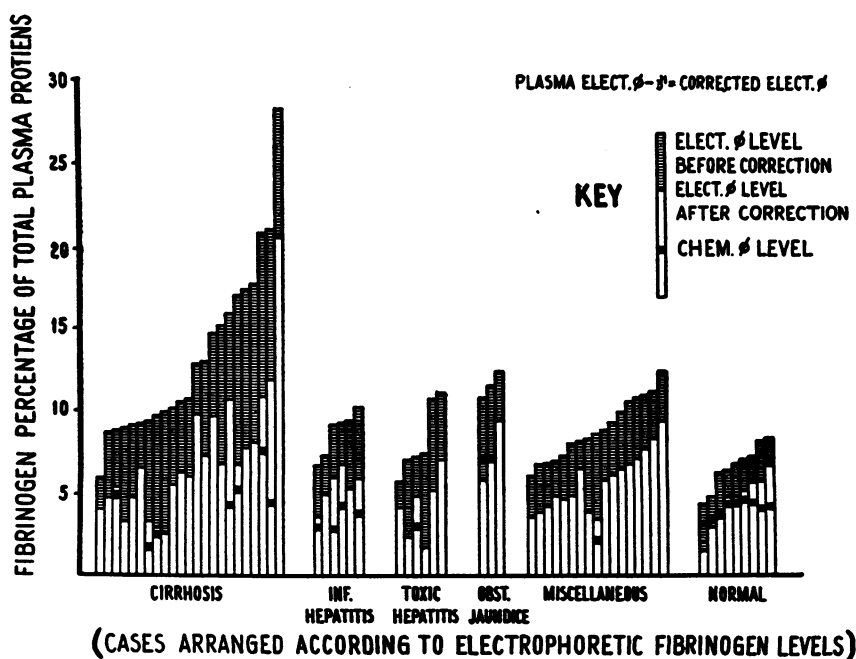

FIG. 5. COMPARISON OF ELECTROPHORETIC FIBRINOGEN BEFORE AND AFTER CORRECTION WITH CHEMICALLY DETERMINED FIBRINOGEN

may account for this increase in alpha globulin. Lipids frequently may be associated with alpha as well as beta globulins (23). Dole (9) has suggested that the lower alpha values in serum as compared with plasma may be due to removal of this component in the clot. While the total serum alpha globulins in this study were less than that of plasma, it was not significantly so. However, as Table I reveals, serum alpha$_1$ globulin was lower than the corresponding plasma values while the reverse was true for the alpha$_2$ fraction. The greater spread on either side of the theoretical line of perfect correlation in this component (Figure 2b) also indicates a greater variation in this component than in any other protein fraction. The increased beta globulins in the liver diseases groups are in agreement with the findings of others (13–15, 24). There appears to be a significant variation in the relative percentage values of serum and plasma beta globulins. However, if these percentage values are converted to absolute values in grams/100 ml. that variation loses its significance and falls within the inherent limits of error of the method. The serum-plasma absolute values correlate well (Figure 2c). Dole (9), in comparing the serum and plasma of a normal individual, similarly found the beta serum value elevated and believed this to be due to overlapping of the beta globulin and "fibrinogen" peaks. However, he did not compare absolute values. When this was done in our cases it tended to nullify the serum and plasma differences.

The serum gamma globulin findings in the disease groups are in agreement with those recorded by previous authors (10–19, 21, 24). The significant difference between the serum-plasma gamma globulin values (Tables I and III), particularly in cirrhosis, is due to burial of the gamma$_1$ fractions in the "fibrinogen" peak of the plasma pattern. The greater the gamma$_1$ fraction the greater will be the serum-plasma difference in total gamma values. Electrophoresis, particularly in the pathologic cases, yields plasma gamma globulin values 15–20% lower than serum values. It is important, therefore, to determine whether gamma$_1$ globulin is increased in other diseases, lest misleading gamma globulin values be secured in plasma determinations in these diseases. The true picture of gamma globulin changes is found in serum determinations. The nature of gamma$_1$

globulin and the possible significance of its elevation in liver disease are discussed elsewhere (25).

An analysis of the reported observations on the plasma fibrinogen content in liver disease reveals a diversity of opinion (8, 11, 15, 21). The liver is probably the prime site of fibrinogen formation (26), and diminution of this protein has been reported in acute hepatic insufficiency such as in the damage experimentally produced by chloroform and other toxins (27). Diminished fibrinogen may also be found in humans in the severe hepatic insufficiency of acute yellow atrophy (8). Elevated fibrinogen values have been reported in cirrhosis (11, 21). Whitman and his co-workers (11) consider the possibility that the elevated "fibrinogen" peak in cirrhosis is due to an increase in gamma globulin as well as to an actual fibrinogen increase. Our data show this to be correct. The markedly elevated "fibrinogen" peaks often found in the cirrhotic electrophoretic pattern occur because the gamma$_1$ component has a similar mobility to that of fibrinogen and is concealed in the "fibrinogen" peak. Similarly, Zeldis and Alling (28) had noted spurious elevations in fibrinogen values in plasma from dogs and had attributed this to increments from adjacent components. Even after correction of the fibrinogen values by subtracting the amount contributed by the gamma$_1$ complex to the "fibrinogen" peak, the electrophoretic values for that component were still elevated above those determined chemically in the cirrhotic patients (Tables I, IV, Figure 5). Fibrinogen is the plasma protein which forms fibrin under the action of thrombin. Fibrinogen analysis by chemical means may give erroneously low values because of incomplete precipitation of fibrin or false high values due to occlusion of other plasma components to the clot (29a). The effect of variation of fibrinogen and thrombin concentrations and pH and of the presence of other proteins must be taken into consideration in the chemical determination of fibrinogen. The occlusion of other protein components, particularly lipoproteins and certain enzymes such as thrombin, is sufficient to introduce error into fibrinogen chemical analysis (29b). Fibrinogen is also more sensitive to thermal and chemical changes than are proteins (30). The question then arises as to whether the more reproducible method of electrophosesis, particularly after subtraction of gamma$_1$

globulin, is not a more accurate method of fibrinogen analysis than is the chemical method. Against such a supposition is the possibility of other protein moieties besides gamma$_1$ globulin having a similar mobility as fibrinogen. It is also questionable labeling an electrophoretic "fibrinogen" peak as fibrinogen in the sense of protein clottable with thrombin. Nevertheless, we believe that the difference between the electrophoretic and chemically determined fibrinogen indicates an actual increase of this component in cirrhotic liver disease.

The plasma electrophoretic pattern as such appears on inspection to be more characteristic of chronic liver disease than the serum electrophoretic pattern. In addition to the characteristically depressed albumin and elevated gamma globulin peaks seen in the cirrhotic serum pattern, there is also the markedly elevated "fibrinogen" peak present in the plasma electrophoretic pattern. We have not seen this triad of findings to such a marked degree in the electrophoretic pattern of any other pathologic condition. However, serum rather than plasma is preferable where it is desired to study the electrophoretic quantitative partition of the protein complexes in chronic liver disease. The elevation of gamma$_1$ globulin and its inclusion in the "fibrinogen" peak in the plasma pattern give a false impression of extreme fibrinogen elevation and at the same time cause the gamma globulin elevations to be lower than those found in serum. A more correct picture of the fibrinogen alterations in chronic liver disease requires simultaneous electrophoretic analyses on serum and plasma and correction for the increased gamma$_1$ globulin frequently associated with that pathologic state.

It is fully realized that the electrophoretic pattern, particularly in cirrhosis, may vary depending upon the stage and course of the disease. Our cases of cirrhosis varied from those in the inactive stage to those with marked liver damage. We were primarily interested not in the type of pattern in the different liver diseases nor in the changes taking place during the course of the disease, but in the comparison of the patterns in the serum and plasma determinations. Stage and course of disease had no effect upon these serum-plasma differences. In fact, the only significant differences were those in the gamma globulin and these did not depend upon the stage of disease but

the type of disease. The gamma$_1$ globulin relation to hepatic damage as measured by the cephalin flocculation, thymol turbidity and zinc sulphate turbidity tests is shown in Figure 4 of the following article (25).

SUMMARY

1. Paired serum and plasma samples of 66 persons with various hepatic or miscellaneous diseases and normal subjects were analyzed electrophoretically. The liver disease group included cases of cirrhosis, infectious and toxic hepatitis, and obstructive jaundice. The miscellaneous disease group was comprised of patients with rheumatoid arthritis, peptic ulcer, and multiple myeloma.

2. Serum protein changes in the various disease groups were similar to those previously reported by other authors.

3. Fibrinogen values were markedly elevated particularly in the cirrhotic group. This was due to the inclusion of a gamma$_1$ globulin fraction in the "fibrinogen" peak. This was also responsible for the serum gamma globulin being significantly greater than the plasma gamma globulin. The addition of gamma$_1$, as determined in the serum, to the total plasma gamma globulin yielded total gamma globulin values essentially similar to those of the serum. At the same time subtraction of gamma$_1$ from the elevated fibrinogen values, as revealed by the plasma patterns, reduced these values approximately by 35–45%. After this correction, there was still a significant difference between electrophoretic and chemically determined fibrinogen, indicating an actual increase of this component in cirrhotic liver disease. The gamma$_1$ burial in the "fibrinogen" peak was also responsible for the depressed values of total globulin and albumin/globulin ratio in plasma determinations. These likewise could be corrected for by addition of gamma$_1$ to the plasma globulin.

4. Aside from the significant serum-plasma difference in gamma globulin, the other protein components were essentially similar in serum and plasma, particularly when expressed as grams/100 ml. The beta globulin content in relative percentage was somewhat more elevated in serum than plasma. After conversion to absolute values however, this difference was not significant.

5. The routine use of plasma electrophoresis for the study of protein alterations in hepatic dis-

eases is not recommended. It is impossible to resolve and separate completely the components of the "fibrinogen" peak. Inaccurate gamma globulin and fibrinogen values result.

REFERENCES

1. Chargaff, E., Ziff, M., and Moore, D. H., Studies on the chemistry of blood coagulation. XII. An electrophoretic study of the effects of anticoagulants on human plasma proteins, with remarks on the separation of the heparin complement. J. Biol. Chem., 1941, 139, 383.
2. Deutsch, H. F., Alberty, R. A., and Gosting, L. J., Biophysical studies of blood plasma proteins. IV. Separation and purification of a new globulin from normal human plasma. J. Biol. Chem., 1946, 165, 21.
3. Longsworth, L. G., Recent advances in study of proteins by electrophoresis. Chem. Rev., 1942, 30, 323.
4. Svedberg, T., and Pederson, K. O., Ultracentrifuge. Oxford University Press, London, 1940.
5. Weichselbaum, T. E., An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. Am. J. Clin. Path., 16 (Tech. Sec.), 1946, 10, 401.
6. Simmons, J. S., and Gentzkow, C. J., Laboratory methods of the United States Army. Lea & Febiger, Philadelphia, 1944, Ed. 5.
7. Marrock, J. R., and Hoch, H., Serum proteins. J. Clin. Path., 1949, 2, 161.
8. Cantarow, A., and Trumper, M., Clinical Biochemistry. W. B. Saunders Co., Philadelphia, 1949, Ed. 4.
9. Dole, V. P., The electrophoretic patterns of normal plasma. J. Clin. Invest., 1944, 23, 708.
10. Rafsky, H. A., Weingarten, M., Krieger, C. I., Stern, K. G., and Newman, B., Electrophoretic studies in liver disease. Gastroenterology, 1950, 14, 29.
11. Whitman, J. F., Rossmiller, H. R., and Lewis, L. A., Protein alteration in portal cirrhosis, as determined by electrophoresis. J. Lab. & Clin. Med., 1950, 35, 167.
12. Ricketts, W. E., Sterling, K., Kirsner, J. B., and Palmer, W. L., Electrophoretic studies of the serum proteins in portal cirrhosis. Gastroenterology, 1949, 13, 205.
13. Gray, S. J., and Barron, E. S. G., Electrophoretic analyses of the serum proteins in diseases of the liver. J. Clin. Invest., 1943, 22, 191.
14. Kabat, E. A., Hanger, F. M., Moore, D. H., and Landow, H., The relation of cephalin flocculation and colloidal gold reactions to the serum proteins. J. Clin. Invest., 1943, 22, 563.
15. Luetscher, J. A., Jr., Electrophoretic analysis of plasma and urinary proteins. J. Clin. Invest., 1940, 19, 313.
16. Dole, V. P., and Rothbard, S., Electrophoretic changes in the serum of a patient with rheumatoid arthritis. J. Clin. Invest., 1947, 26, 87.
17. Malmros, H., and Blix, G., The plasma proteins in cases with high erythrocyte sedimentation rate. Acta med. Scandinav., Suppl., 1946, 170, 280.
18. Perlmann, G. E., and Kaufman, D., Electrophoretic distribution of proteins in serum, plasma and synovial fluid of patients with rheumatoid arthritis. J. Clin. Invest., 1946, 25, 931.
19. Martin, N. H., The components of the serum proteins in infective hepatitis and in homologous serum jaundice (an electrophoretic study). Brit. J. Exper. Path., 1946, 27, 363.
20. Armstrong, S. H., Jr., Budka, M. J. E., and Morrison, K. C., Preparation and properties of serum and plasma proteins. XI. Quantitative interpretation of electrophoretic schlieren diagrams of normal human plasma proteins. J. Am. Chem. Soc., 1947, 69, 416.
21. Thorn, G. W., Armstrong, S. H., Jr., and Davenport, V. D., Chemical, clinical and immunological studies on the products of human plasma fractionation. XXXI. The use of salt-poor concentrated human serum albumin solution in the treatment of hepatic cirrhosis. J. Clin. Invest., 1946, 25, 304.
22. Franklin, M., Paul, W. D., and Routh, J. I., Unpublished data.
23. Gutman, A. B., The plasma proteins in disease, in Advances in Protein Chemistry, edited by Anson, M. L., and Edsall, J. T. Academic Press, New York, 1948, Vol. 4, p. 155.
24. Cohen, P. P., and Thompson, F. L., Mechanism of the thymol turbidity test. J. Lab. & Clin. Med., 1947, 32, 475.
25. Franklin, M., Bean, W. B., Paul, W. D., Routh, J. I., de la Huerga, J., and Popper, H., Electrophoretic studies in liver disease. II. Gamma₁ globulin in chronic liver disease. J. Clin. Invest., 1951, 30, 729.
26. Meek, W. J., Relation of the liver to the fibrinogen content of blood. Am. J. Physiol., 1912, 30, 161.
27. Whipple, G. H., and Hurwitz, S. H., Fibrinogen of the blood as influenced by the liver necrosis of chloroform poisoning. J. Exper. Med., 1911, 13, 136.
28. Zeldis, L. J., and Alling, E. L., Plasma protein metabolism—electrophoretic studies. Restoration of the circulating proteins following acute depletion by plasmapheresis. J. Exper. Med., 1945, 81, 515.
29a. Morrison, P. R., On the determination of fibrinogen. Federation Proc., 1946, 6, 280.
b. Morrison, P. R., Preparation and properties of serum and plasma proteins. XV. Some factors influencing the quantitative determination of fibrinogen. J. Am. Chem. Soc., 1947, 69, 2723.
30. Stenhagen, E., Electrophoresis of human blood plasma. Electrophoretic properties of fibrinogen. Biochem. J., 1938, 32, 714.