THE PROTEINS OF BLOOD SERA DURING CIRRHOSIS OF LIVER AND MITRAL DISEASE OF HEART

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As is well known, many pathological processes, because of the constant exchange between the protein of the blood and the proteins of the organs, lead to considerable fluctuations in the composition of the blood albumins.

Taking into consideration the important role played by the liver in the fulfillment of numerous biochemical processes, among which is the formation of serum proteins it can be supposed that during diseases of the liver, the blood proteins must change.

The disturbance in cardiac activity and circulation connected with cardiac disease must lead in the same manner to changes in the protein fraction of the blood.

The present communication is devoted to a study of the changes in the protein fraction of the blood serum in cirrhosis of the liver and mitral disease of the heart.

One of the most elegant methods, permitting the separation of the blood proteins into five fractions (albumins, α_1, α_2, β-and γ globulins), is the method of electrophoresis.

In a whole series of works [1, 2, 3, 4 and 5] it has been shown that in certain liver diseases, especially in cirrhosis, there is a marked change in the composition of the protein fractions of the serum. The total amount of protein is decreased, while the quantity of β - and γ - globulins is increased. Some authors consider that these changes can serve as a diagnostic test, and also as an indicator of the severity of the disease.

On comparing the changes in the serum proteins occuring during cirrhosis of the liver with other tests of the functional capacity of the liver, it has been shown that not one of them changes as constantly and as clearly as does the composition of the serum protein. It has also been established that the composition of the serum proteins becomes normalized much later and only after there is the obvious picture of clinical recovery and the return of all other biochemical indicators to normal.

In our work we used the electrophoretic method upon paper with the modifications suggested in 1953 [6]. In spite of the simplicity of the method, we obtained a clear separation of all five fractions of the serum proteins which appeared upon the paper after the usual treatment of the last dilution of the dye - bromophenol blue.

For the quantitative determination of the proteins on the filter paper the spots were cut out, stained with bromophenol blue, and adsorbed in accord with the given protein fraction. The stained pieces of paper were extracted in six ml of 4% $NaHCO_3$ in 50% methanol in the course of 1 - 1½ hours, after which they were observed photometrically in a Pulfrich refractometer with a light filter of 574 mu. In proportion to the light adsorption it was possible to calculate the relative content of the protein fraction in percent of the total quantity of protein in the serum (the sum of the adsorption of all five fractions).

For purposes of comparison we simultaneously conducted an electrophoresis of a control serum, obtained from a healthy human. Upon a strip of paper 6 cm wide, we usually carried out three tests (each 0.005 ml):

in the middle - the control serum of the healthy man, on the edges - from two patients.

Altogether we studied 60 sera from healthy men. The average figures for the protein fractions of normal men were found to be close to the figures reported in the literature. This gives us a basis for considering our method for comparing the composition of the various protein fractions as being sufficiently accurate.

After this, we studied 32 patients with cirrhosis of the liver, the contents of their protein fractions in the sera are given in Table 1.

TABLE 1

The Protein Fractions in the Sera of Healthy People and in Certain Pathological Conditions
(Average Figures)

Diagnosis	Number of patients	Albumins / γ-Globulins	Albumins / Globulins	Albumins (in %)	Globulins (in %)			
					α_1	α_2	β	γ
Healthy people	60	3.6	1.65	61.8	3.2	7.0	10.2	17.5
Cirrhosis of the liver	32	18	0.66	38.4	3.7	7.1	13.2	31.6
"Cirrhosis of the liver"[1]	13	2.7	1.4	55	4.0	8.8	11.6	19.8
Mitral disease of heart	43	2.5	1.3	58.8	3.4	7.1	10.5	20.0

[1] Patients with unclear diagnosis.

Almost half of the patients showed a decrease in the serum protein. The decrease in the protein occurred by the decrease (from 20 - 50%) of the albumin fraction, which in these patients must have favored the accumulation of ascitic fluid, as the albumins together with the electrolytes participate in maintaining the normal colloid osmotic pressure in the vascular bed and their absence can lead to transudation from the vessels.

Especially constant are the changes in the globulin fractions as manifested in the increase of the γ - globulins, which we have already observed in all those ill. This increase in several instances reached 100 - 150% and was frequently an index of the severity of the clinical picture of the disease.

In these patients we also noted an increase in the β - globulins, this, however, never being so great as the γ - globulin increase, and in 30% of the patients was not observed at all.

Table 1 and Table 2 give the data concerning the percentage composition of the albumins to γ - globulins and albumins to total globulins. As can be seen the ratio of albumin to γ - globulin, being normally 1.65, during cirrhosis of the liver drops to 0.66. In considering the significance of this relationship (which is frequently given also as the chemical determination of the albumin and total globulin) account is taken of the quantities of all globulin fractions, those which change during cirrhosis of the liver (β - and γ - globulins), and those which remain unaltered (α_1-, α_2, and in part, β - globulins). The most marked change in cirrhosis of the liver occurs in the ratio of the albumin to γ - globulins, as this relationship not only changes at the expense of the albumins (decrease in the numerator), but also must account for a rise in the amount of γ - globulins (increase in the denominator). Thus when the ratio of the albumin to the globulin is decreased (on the average) 3 times the ratio of the albumin to γ - globulins is 1.5 times less.

These changes can be easily visualized by examination of Fig. 1.

The determinations of the protein fractions in the serum, made by the chemical method of salting out, as compared with the electrophoretic method, gives an increased quantity of albumins and a decreased quantity of globulins, as a consequence of which the decline in the protein fraction from the normal during cirrhosis appears more accentuated. These differences are also to be explained in some measure by the fact that by the chemical methods a part of the α_1-globulins precipitate with the albumins. It is also possible that the velocity of movement of the proteins in the electric field (which is the basis of the electrophoretic fractionation method) becomes altered in pathological conditions, this change overshadowing those qualities which are the basis of

the chemical methods of protein separation. In some of our observations on patients the salting-out method showed no changes in the serum proteins, whereas the electrophoretic method always revealed a fall in the albumin and a rise in the γ - globulin content.

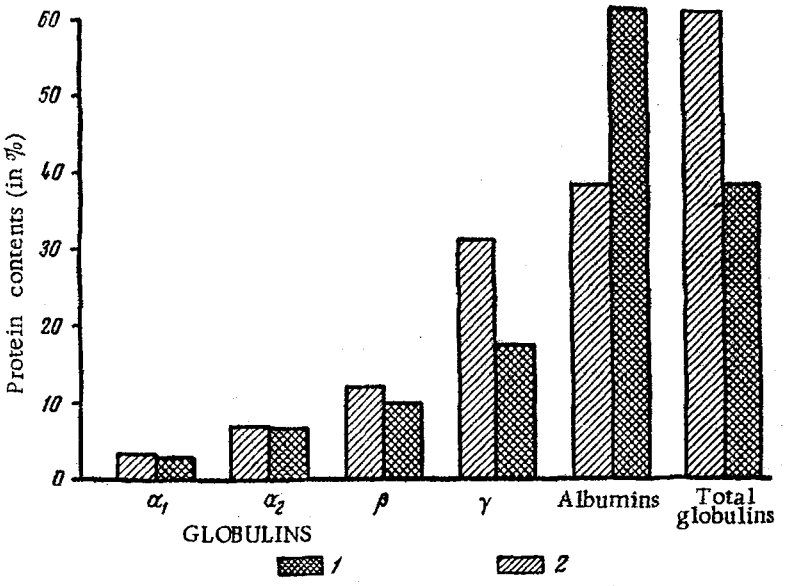

Fig. 1. Average content of protein fractions in blood sera of healthy men and patients with cirrhosis of liver.
1) Healthy, 2) diseased.

TABLE 2

Protein Fractions of the Sera of Certain Patients Having Cirrhosis of the Liver and Other Patients with an Unconfirmed Diagnosis of Cirrhosis of the Liver

Diagnosis	Patients	Albumins / Globulins	Albumins	Globulins	Albumins (in %)	Globulins (in %) α1	α2	β	γ	Bilirubin (in mg %)
Cirrhosis	M-va*	0.8	0.45	32	6.6	10.6	15.4	31.1	0.62
	A-pov	0.8	0.53	35	3.7	6.2	12.5	42.5	0.7
	T-kov	0.9	0.5	36	5.0	6.2	14.0	38.0	0.5
	B-na*	0.8	0.3	30	8.7	12.0	14.0	36.0	0.9
Absence of liver cirrhosis	Sh-va*	1.4	1.08	52	2.0	4.8	5.6	36.0	
	U-va *	1.56	0.47	40	11.7	20	16.4	20.0	0.3
	M-k	2.0	1.1	53	6.4	11.8	11.2	18.2	0.18
	K-kov	3.9	1.4	59	2.1	3	15.3	20.5	0.7
	S-oy	2.8	1.0	51	3.0	7.2	21.5	18.2	0.5

* Female

On the basis of the studies made, it has thus been proven that in 100% of cirrhosis of the liver patients there occur well defined changes in the serum proteins a decrease in the quantity of albumins, accompanied by a rise in β - globulins and also an increase in γ - globulins.

However there was a group of patients (13 people), who had entered with a diagnosis of "cirrhosis of the liver," who did not have the changes in the protein fractions that we pointed out as being so characteristic. The data on these patients are shown in Table 1; the serum proteins of some of these patients, representing this group,

are shown in Table 2. Thus, for example, patient U-va entered the institute with the diagnosis of cirrhosis of the liver. However, as can be seen from the table, electrophoretic investigations of the serum failed to show any important rise of the γ - globulin. It was assumed that the liver of this patient was not involved, and indeed a laparotomy revealed an abdominal malignancy without liver involvement. It is interesting to observe that this patient had a high α_2 - globulin fraction. In the literature are observations indicating that malignancy leads very often to a rise of this fraction.

In patients M-k, K-kov and S-oy, there was the clinical picture of a cirrhotic liver but the electrophoretic studies of the sera did not show γ - globulin elevation; the decrease in albumin, and also the change of the γ - globulin/albumin ratio was much less than is usual in true cirrhoses. In this situation the electrophoretic studies of the proteins did not support the diagnosis of a true cirrhosis of the liver and it turned out that all these patients had adhesive pericarditis, for which surgery was then performed. On the other hand, patient Sh-va had the diagnosis of malarial splenomegaly and yet the changes in her protein fractions were typical of cirrhosis of the liver. Splenectomy established the diagnosis of cirrhosis of the liver.

In this fashion there became possible a differential diagnosis in many patients and we were able to separate patients with cirrhosis of the liver from those in whom liver damage played no role.

In addition to the material obtained by protein fraction investigations in cirrhosis of the liver, our observations were confirmed in the literature, which states that during cirrhosis of the liver, there arise characteristic changes in the proteins of the sera. These changes can significantly aid the diagnosis in doubtful cases.

The next group of patients (43 people), on whom we conducted electrophoretic investigations of their sera proteins, consisted of individuals suffering mitral disease, chiefly mitral stenosis of the heart. This affliction results in patients who, in the majority of cases, had had in the past rheumatic fever (we made no observations on any one in the acute phase).

The protein fractions of the sera of these patients are shown in Table 1 and Fig. 2.

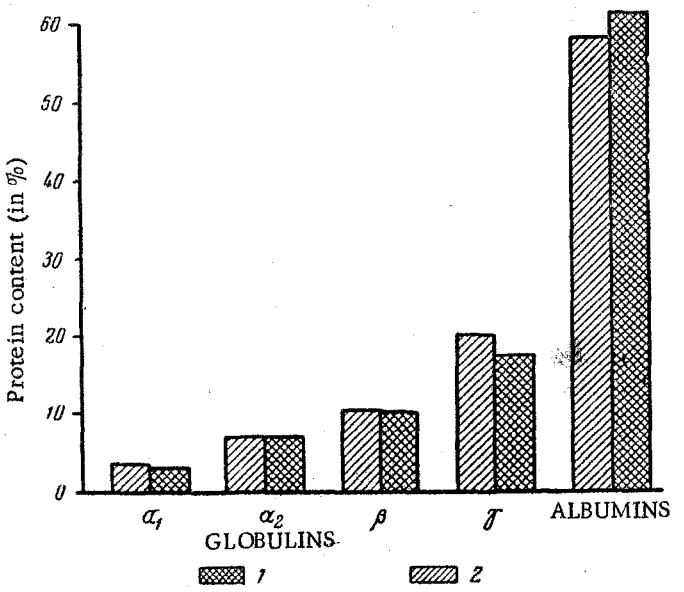

Fig. 2. Average protein content of the sera fractions in the blood of healthy people and those suffering from mitral disease.
1) Healthy, 2) ill.

It may be observed that the direction of the noticed changes is very similar in these patients as in those suffering from cirrhosis of the liver. In both instances there is quite often a decrease of the total protein, decrease of the albumin and increase of the γ - globulin. Still, in cardiac patients these changes are less intense. Thus the total albumins diminish only in some 50% of the patients, α_1-, and α_2- globulins being normal.

As far as the γ- globulins are concerned, on the average (in the entire group of cardiac patients) they rise but little; however, when we take individual cases, then it appears that in 32% of these patients the γ- globulin is not above normal, while in 25% the elevation is insignificant. The ratio of the albumins to γ- globulin and to globulins also is lowered very little, but less than in patients with cirrhosis of the liver.

Thus, among the groups investigated by us of patients both with stenosis and insufficiency of the mitral valve, half of the cases show no significant changes in the serum protein content or, if there is any change, the deviation is insignificant.

However, in 12 people the protein content of the serum was markedly different from the normal and the character of these changes was very similar to that seen in cases of cirrhosis of the liver; however, these changes were much less sharp. The protein content of the sera of some of the members of this group of patients is shown in Table 3.

TABLE 3

Protein Fractions in the Sera of Patients Having Mitral Disease of the Heart

Patient	Albumin / Globulin	Albumin	Globulin	Albumin in %	Globulins in %			
					α_1	α_2	β	γ
P-ev	1.6	0.9		49.5	3.0	6.1	11.8	29.7
L-ko	1.6	0.78		44	5.3	9.8	14.4	26.5
P-ra*	1.3	0.71		41	3.0	7.5	15.7	31.5
I-kin	1.65	0.85		46	4	9.9	12.3	27.7
S-na*	1.2	0.70		42	5.0	9.0	10.8	33.5

* Female

These patients always had clinical evidence of liver enlargement, frequently ascites and jaundice which all points in the direction of liver damage and compels us to associate the changes in the proteins of the serum with the cirrhotic process. Our suppositions were confirmed when several of these patients were later autopsied.

The analysis of the changes in the sera protein fractions in patients having diseases of the heart (stenosis, frequently accompanied by mitral valve insufficiency) revealed that the majority of these patients did not have large changes in their sera and that these changes were observed in those patients principally in whom there could be supposed to exist a cardiac cirrhosis of the liver. All this gives reason for us to express the opinion that the study does not show that disease of the heart itself leads to material changes in the sera proteins. When there are changes, these might be explained as due to secondary changes in other organs, occurring as a result of the disturbances in the circulation. If the disease is compensated, these changes do not occur.

In this fashion the electrophoretic studies of the blood sera in patients having stenosis and insufficiency of of the mitral valve enable us to correctly diagnose those cardiac patients who have a disturbed liver function and changes in the sera protein fractions which are similar to those seen in true cirrhosis patients.

In this connection the pathological changes in the liver can serve as a counter-indication to surgical interference in patients having diseases of the heart, for this method enables the discovery of those liver changes which are of significance for surgical clinics interested in cardiac surgery.

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