AMINO-ACID COMPOSITION OF SERUM PROTEINS IN HEALTH AND DISEASE

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Electrophoresis of serum proteins in disease frequently discloses abnormalities. The alterations are rarely diagnostic; thus a diffuse increase of the γ -globulin is common to many clinically unrelated conditions. It would be of considerable value therefore if it were possible to distinguish between the proteins which are responsible for a common abnormality in the electrophoretic pattern. It was with a view to relating the composition of the serum proteins to disease that we have undertaken a study of the amino-acid composition of the electrophoretic fractions in liver disease, kidney disease, and myelomatosis, conditions in which it is well known that the serum proteins show great abnormality.

Existing data on the amino-acid composition of serum proteins are very meagre, and we have been unable to find any literature dealing with the amino-acid content of electrophoretically pure serum protein fractions. The composition of the plasma proteins in healthy subjects has been investigated by Brand, Kassell, and Sidel (1944), Shemin (1945), Brand (1946), and Brand and Edsall (1947), who give data for the percentage composition of certain amino-acids in albumin and various globulin fractions separated by precipitation methods. Brand and Edsall (1947) include results of complete amino-acid analyses of α - and β -globulins as well as of albumin and γ -globulin, but the fractions were not electrophoretically homogeneous. The findings have been reviewed by Marrack and Hoch (1949).

The content of a few amino-acids in plasma proteins has been studied in pathological conditions. Tuchman and Sobotka (1932) found discrepancies in Wu and Kjeldahl methods of serum protein determination in cases with nephrosis or nephritis with oedema, and concluded that in these conditions the albumin tyrosine content is increased while the globulin tyrosine is diminished. Alving and Mirsky (1936) studied the cystine content of the plasma proteins in eight cases of kidney disease and in five healthy individuals; part of the albumin fraction from patients with the nephrotic syndrome was

found to have an abnormally low cystine content. Bálint and Bálint (1942 and 1943) estimated tyrosine, tryptophan, cystine, arginine, and histidine in the plasma proteins from patients with acute infections and in cases of anaemia. In acute infections they found tyrosine and tryptophan decreased, and cystine increased, in the albumin, and the reverse changes in the globulin. Emmrich (1950) investigated the cystine content of normal and pathological serum proteins and found abnormal levels in protein deficiency, cirrhosis of the liver, chronic kidney disease, tuberculosis, and other chronic inflammatory processes. Schuck (1950) found variation in the cystine and methionine content of the plasma proteins in pregnancy with abnormal levels in the toxæmia of pregnancy. Müting (1951), who estimated only methionine, showed that the level of this amino-acid in serum protein was lower than normal in parenchymatous liver disease and high in cases of the nephrotic syndrome. Schreier (1951) estimated biologically threonine, valine, leucine, isoleucine, phenylalanine, lysine, histidine, cystine, and methionine in serum albumin from four cases of the nephrotic syndrome and single cases of liver disease, Hodgkin's disease, leukaemia, and carcinomatosis, but found no significant variation from normal.

Clinical Material

Data are presented here of the composition of the serum proteins of six healthy young adults, six patients with the nephrotic syndrome, six with liver disease, and six with multiple myeloma. Of the patients suffering from liver disease, four had cirrhosis, one had subacute viral hepatitis, and one was a case of acute hepatic necrosis. Except for one patient with the nephrotic syndrome and one with cirrhosis, all the patients were adults, and all except two were attending University College Hospital. In each case the clinical features of the condition were typical and the diagnosis was supported by appropriate pathological investigations or confirmed at necropsy. The sera from all the

patients showed abnormalities in the electrophoretic pattern of the proteins; notably there was an increase of the γ -globulin and a decrease of the albumin in the cases of liver disease, an increase of the α_2 -globulin and a decrease of the albumin and γ -globulin in those with the nephrotic syndrome, and a marked compact increase of the γ -globulin in the cases with myeloma.

Method

The proteins were fractionated by electrophoresis on filter paper, the fractions hydrolysed, their constituent amino-acids separated and identified by paper chromatography and then estimated colorimetrically. The α_1 -globulin was not analysed because insufficient was available.

Electrophoresis.—The proteins were fractionated by paper electrophoresis as described by Flynn and de Mayo (1951). Serum, 0.2 ml., was applied to each of eight or 10 filter paper strips (Whatman No. 31 extra thick) and a potential of 120 volts applied for 18 hours. The strips were heated at 80° C. until just dry before staining with bromphenol-blue.

Isolation of Proteins.—The protein fractions so detected were cut out and steeped in approximately 150 ml. of 0.5 N-sodium hydroxide for 24 hours with occasional shaking. The solutions were filtered, and the filtrate adjusted to pH 5 with sulphuric acid. Mercuric chloride, 1 g., was added and the whole heated until just boiling. The coagulated proteins were allowed to sediment for 24 hours and the supernatant fluid decanted as completely as possible. The remaining sludge was transferred to a centrifuge tube, being washed in with water, and the proteins collected by centrifuging; they were then washed twice by mixing with water and re-centrifuging.

Preparation of the Hydrolysate.—To the protein in each centrifuge tube 5 ml. of 6 N-hydrochloric acid was added. The tube was sealed and incubated at 100° C. for 15 hours. The hydrolysate was then evaporated to dryness under reduced pressure and the residue dissolved in 2 ml. of 10% (v/v) iso-propyl alcohol as recommended by Block (1950). This solution was desalted electrolytically and subsequently a sample taken for a total nitrogen determination by a micro-Kjeldahl procedure.

Chromatography.—Two-way chromatography was carried out as described by Dent (1948) using Whatman No. 4 filter paper. A volume of hydrolysate equivalent to 0.1 mg. of nitrogen was applied to each chromatogram. The developed chromatograms were lightly sprayed with 0.2% (v/v) ninhydrin in *n*-butanol and allowed to stand at room temperature until the colour developed; usually they were left overnight.

Determination of Amino-acids.—From each chromatogram the amino-acid spots were cut out with a margin of unstained paper, together with paper blanks of the same size. In practice all the amino-acid spots could often be contained within circles of 1½ in. diameter and then a single blank of the same dimensions would be

used. The cut-out amino-acid spots were transferred to test-tubes and 1 ml. of 5% (v/v) ninhydrin in 90% iso-propyl alcohol (Block, 1950) added to each. The tubes were shaken well and incubated in a water bath at 80° C. for 10 minutes. After cooling, 5 ml. of 70% (v/v) acetone in water was added to each tube. The tubes were shaken to dissolve the dye and then stood for 20 minutes to complete the solution (Fowden, 1951). Certain of the amino-acids gave too intense a colour for convenient reading in the photometer; in these instances further dilution with 70% acetone was carried out. The optical densities were measured at 570 m μ . The chromatography and amino-acid estimations were carried out in duplicate. Isoleucine and leucine were estimated together, since their separation cannot be effected with the solvents used. Proline was not estimated as the recovery was very poor, and in the case of cystine and other amino-acids the amount was too small to determine.

Calculation.—Factors were obtained correlating the colorimetric readings with the amounts of individual amino-acids present by subjecting proteins of known composition to the above procedure. Casein, gelatine and electrophoretically pure normal serum albumin were used for this purpose. The data given by Hawk, Osier, and Summerson (1947) for casein and gelatine, and by Brand and Edsall (1947) for serum albumin, were used for the calculation of the factors; the data given by these authors were first corrected to express the percentage amino-acid composition per 100 g. amino-acids. By this method of calculation allowance is made for variations in the amount of colour produced in the reaction with ninhydrin, as well as for any lack of quantitative recovery of the individual amino-acids. The colorimetric readings were multiplied by the appropriate factors and the values thus obtained for the actual amounts of individual amino-acids present were then expressed as a percentage of the total amount of amino-acids estimated.

Reproducibility.—The final figure for each individual amino-acid value was shown to be reproducible to within 10% for all the acids except lysine and histidine, which were reproducible to within 15%, and for arginine which was reproducible to within 20%. The amount of arginine detected appears to be related to the length of time required for desalting; if this is prolonged arginine is converted to ornithine (Stein and Moore, 1951).

Results

The results are shown in Figs. 1–12, and values for the average composition of the normal serum proteins are given in Table I.

Table I shows that the different fractions differ somewhat in composition, the content of serine, alanine, threonine, and lysine being particularly variable. These findings are in accord with those of other authors using different methods of analysis (Brand and Edsall, 1947).

Analysis of the results from the pathological sera shows that in no case are the serum proteins grossly

FIG. 1

FIG. 2

FIG. 3

FIG. 4

FIGS. 1-4.—Amino-acid composition of serum protein fractions in six cases of parenchymatous liver disease. Values expressed as g. per 100 g. amino-acid estimated. Shaded area represents range of values found in six normals. Fig. 1 is for albumin, Fig. 2 for α_2 -globulin (five cases only), Fig. 3 for β -globulin, and Fig. 4 for γ -globulin.

FIG. 5

FIG. 6

FIG. 7

FIG. 8

FIGS. 5-8.—Amino-acid composition of serum protein fractions in six cases of nephrotic syndrome. Values expressed as g. per 100 g. amino-acid estimated. Shaded area represents range of values found in six normals. Fig. 5 is for albumin, Fig. 6 for α_2 -globulin, Fig. 7 for β -globulin, and Fig. 8 for γ -globulin.

FIG. 9

FIG. 10

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FIG. 11

FIG. 12

FIGS. 9-12.—Amino-acid composition of serum protein fractions in six cases of multiple myeloma. Values expressed as g. per 100 g. amino-acid estimated. Shaded area represents range of values found in six normals. Fig. 9 is for albumin, Fig. 10 for α_2 -globulin, Fig. 11 for β -globulin, and Fig. 12 for γ -globulin.

TABLE I
MEAN VALUES* FOR THE COMPOSITION OF NORMAL
SERUM PROTEINS

	Albumin	α_2 -globulin	β -globulin	γ -globulin
Glutamic acid	15.9	14.1	15.1	11.8
Leucine	13.0	16.5	14.7	12.8
Alanine	11.6	9.4	8.7	6.6
Lysine	9.4	6.0	6.1	6.0
Aspartic acid	8.4	11.0	10.6	8.1
Valine	8.3	9.2	8.8	10.5
Phenylalanine	7.5	5.5	5.1	4.7
Tyrosine	5.1	5.6	5.8	5.6
Arginine	5.3	3.4	4.3	3.9
Threonine	5.0	6.3	5.8	8.4
Serine	3.7	5.3	6.0	10.8
Histidine	2.2	1.7	1.5	1.5
Glycine	1.5	3.4	3.7	3.9

* Values expressed as g. per 100 g. amino-acid estimated.

abnormal in composition, at least in terms of those amino-acids estimated. More critical examination of the data, however, shows that minor variations may occur in individual cases. In Table II the frequency and direction of deviations from the normal range which are considered to be definitely outside the range of experimental error are indicated.

Discussion

Paper electrophoresis of proteins has several advantages over the classical Tiselius technique, an important one being that all the fractions are entirely separated from one another in a single operation. This technique therefore provided us with a simple method of isolating electrophoretically pure protein fractions for the purposes of this study.

Abnormality of the chemical composition of the serum proteins might be expected in disease, since the proteins often show considerable variation from normal in their physical properties. Some of these changes, however, may result from an alteration in the structure of the protein molecules rather than

from an abnormal chemical composition. A recent report shows that great deviation from normal may occur in the chemical composition of proteins under artificial circumstances: thus, in infants fed on lysine-deficient diets, proteins appeared in which lysine was replaced by arginine (*Lancet*, 1953).

Our findings establish that there is no gross deviation in the proportions of at least 13 amino-acids in the serum proteins, even when there is a marked disturbance of protein metabolism. The minor changes we have found must be interpreted with caution. The fact that data are available from only six normals must be kept in mind, and it is this in particular that has prevented statistical analysis of the results. It must also be noted that when the percentage of one amino-acid is increased the increase occurs at the expense of others which must therefore be decreased. The experimental error is regrettably large and indeed estimation of some of the amino-acids present in smaller quantity was abandoned because of this; however, as noted in Table II, some of the results fall well outside this error. Despite all these reservations our findings in the pathological sera require some comment.

The results show more frequent deviations from normal composition in the cases of liver disease than in the other conditions studied. It is also of interest that in liver disease and myelomatosis abnormalities occurred most often in the γ -globulin fraction, which was the fraction showing the greatest increase in concentration. In the cases of the nephrotic syndrome, however, although the α_2 -globulin showed the greatest increase, abnormalities of composition were found more frequently in the β -globulin.

Variations in the composition of the serum protein fractions in disease may result either from the production of proteins of abnormal composition or

TABLE II
FREQUENCY OF DEVIATION OF AMINO-ACID COMPOSITION FROM THE NORMAL RANGE

	Liver Disease				Kidney Disease				Multiple Myeloma			
	Albumin	α_2	β	γ	Albumin	α_2	β	γ	Albumin	α_2	β	γ
Leucine					↑							
Alanine				↓			↓↓					
Aspartic acid				↑↑		↓	↓					↑↑
Phenylalanine			↓			↑	↑↑	↑				↑
Tyrosine		↑	↑↑	↑↑↑				↓				↑↑
Threonine	↓↓		↑	↓↓					↓			
Serine	↓↓↓		↑	↓								
Glycine		↑↑↑						↑				

↑ = Increase and ↓ = decrease in concentration. Number of arrows corresponds to number of cases showing abnormality.

from alteration in the relative proportions of proteins normally present. With regard to the latter possibility it is probable that many different proteins together make up the electrophoretic spectrum of serum, and since the major bands of the spectrum show differences in chemical composition it is likely that the different proteins which together constitute a particular fraction will also differ slightly in composition. If this is so, then a marked increase or decrease of the concentration of a particular component protein could cause the overall composition of that fraction to differ from normal. Variation in the proportion of component proteins could be held responsible for the small variation in composition found in the serum proteins of healthy individuals.

In all the cases of liver disease studied, electrophoresis showed a diffuse increase of the serum γ -globulins, the increase affecting the faster moving γ_1 -globulins to the greatest extent. The chemical abnormalities found in the whole fraction might therefore be due to a selective increase in the γ_1 -globulins. On the other hand this finding, and the abnormalities found in the other fractions, could be the result of synthesis of abnormal proteins.

In the nephrotic syndrome the predominant proteins to appear in the urine are albumin and α_1 - and β -globulins (Slater and Kunkel, 1953; Squire, 1953; Flynn, 1954). This differential loss of protein can be related to the molecular weight of the proteins (Squire, 1953), and may serve to explain why we encountered variation in protein composition most frequently in the β -globulin. The normal serum β -globulin fraction is composed of two main groups of proteins, one with a molecular weight of around 90,000, the other with a molecular weight of about 1,300,000. Differential loss of the lower molecular weight component, should it differ somewhat in amino-acid composition from that of the other component, could then be responsible for the serum β -globulin appearing abnormal in composition. Lack of abnormality in the serum albumin could be attributed to all the albumin components passing into the urine with equal facility. The findings of Albanese, Davis, Smetak, and Lein (1949) suggested that the albumin lost in the urine in the nephrotic syndrome may differ in composition from normal plasma albumin. Their results, taken in conjunction with our finding that the serum albumin is normal in composition, suggest that abnormal albumins are synthesized but are immediately excreted in the urine. However,

it must be noted that Schreier's (1951) results on the urine from two cases do not confirm the findings of Albanese *et al.* (1949).

In myelomatosis the presence of a chemically abnormal serum protein might well have been expected in view of the findings of Dent and Rose (1949) that Bence-Jones protein entirely lacks methionine. However, our findings with 13 amino-acids show little alteration in the composition of the γ -globulin fraction despite its great increase in concentration.

Taking all the above results into consideration one may conclude that amino-acid analysis of serum protein fractions is unlikely to be of diagnostic value.

Summary

The amino-acid composition of electrophoretically homogeneous serum protein fractions has been studied in six healthy young adults and in six cases each of liver disease, the nephrotic syndrome, and myelomatosis. Thirteen amino-acids were estimated quantitatively.

No gross or characteristic alteration from normal was found in the pathological sera in the albumin, α_2 -, β -, and γ -globulins.

Minor deviations from normal composition were found in individual cases. The possible significance of these findings is discussed.

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