CHEMICAL, CLINICAL, AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION.

III. AMINO ACID COMPOSITION OF PLASMA PROTEINS[1]

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The determination of the proportions of the amino acids that are liberated when proteins are subjected to complete hydrolysis provides information of value from many points of view. Amino acids are the fundamental units from which the protein molecule is constructed since the basic pattern of the structure depends upon the combination of the α-carboxyl group of one amino acid with the α-amino group of its neighbor to form peptide linkages. Accordingly, the conclusion may be drawn that the accessory polar groups of the basic and dicarboxylic amino acids commonly found in proteins are for the most part either free or are available for chemical combinations of various degrees of stability. Such combinations may occur between two polar groups located sufficiently near together within the same molecule, or they may give rise to interaction between separate protein molecules, or between the protein molecule and the molecules or ions of substances likewise present in the solution, or between the protein molecule and the molecules or ions of the solvent itself.

The physical chemical analyses of the human plasma fractions which are set forth in Paper I of this series (1) treat them as more or less elongated ellipsoids. The surface of these molecules may be considered as containing numerous discrete points at which a wide variety of chemical reactions can occur. Thus, at the point at which the basic amino acid lysine enters into the structure, under ordinary physical conditions, the positively charged free amino group of this constituent will be present. At this point, therefore, combination or interaction with negatively charged ions in the solution is possible. At another point on the molecule, there may be present the negatively charged carboxyl group of glutamic or aspartic acid and here, accordingly, interaction with positive ions in the solution can occur. Distributed over the surface of the protein molecule are hydrocarbon groups such as those of valine, leucine, and phenylalanine, which are of a non-polar type and therefore do not readily interact either with ions or molecules in aqueous solution. At other points are groups of greater or less degrees of polarity, such as the hydroxyl groups of tyrosine, serine, and threonine, and the sulfur groups of cystine, cysteine, and methionine. With these, interactions of various types can occur. The properties of protein molecules are thus to a great extent functions of the relative proportions in which the several constituent amino acid radicals occur and of the pattern according to which these radicals are arranged or distributed in the surface of the molecule.

The complexities of this concept are such that interpretation of the properties of a protein in terms of its amino acid composition is at the present time possible only in a preliminary and tentative fashion. Nevertheless, some success has been achieved. Thus, the acid-combining power of a protein should be equal to the sum of the basic groups, and the base-binding power equal to the sum of the free acid groups. The first relationship does in fact hold fairly closely in a number of cases of which 3 are shown in

[1] The present analytical studies of plasma proteins have been carried out under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Columbia University. The preparations examined were, with one or two exceptions, made from blood collected by the American Red Cross by the Department of Physical Chemistry, Harvard Medical School, Boston, Massachusetts, under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

TABLE I

Composition of serum albumin of man, cattle, and horse

	Human[1] No. 42	Bovine[2] No. 456	Horse B[3]
Assumed molecular weight [4]	70,000	70,000	70,000
Average residue weight	113.3	112.5	114.4
Approximate number of amino acids per mole [5]	618	620	612
Approximate number of peptide bonds per mole [6]	582	583	577
Approximate number of sub-units (polypeptide chains) per mole [7]	36	39	35
Approximate average size of sub-units	1,900	1,800	2,000
Approximate average number of amino acids per sub-unit	17	16	18
Total number of basic groups by analysis, per mole [8]	105	111	117
Total number of basic groups by acid combining power, per mole [9]	99	103	98
Ratio of basic amino acids to total number of amino acids [8]	1:9.0	1:8.6	1:7.5
Total number of hydroxy-amino acids, per mole [10]	73	89	85
Ratio of hydroxy-amino acids to total number of amino acids	1:8.4	1:7.0	1:7.2
Total number of sulfur atoms in S-S linkages per mole	32	32	30
Ratio of sulfur atoms in S-S linkages to total number of amino acids	1:19	1:19	1:20

	Per cent			Number of residues or atoms per mole		
	Human	Bovine	Horse B	Human	Bovine	Horse B
Total nitrogen	15.95	16.07	15.90	797	803	795
Free α-amino nitrogen	0.72	0.78	0.70	36	39	35
Amide NH_3	1.07	1.05	0.90	44	42	37
Arginine	6.15	6.20	5.50	25	25	22
Histidine [11]	3.52	3.83	4.31	16	17	20
Lysine	5.8	6.5	8.4	28	30	40
Total protein sulfur	1.96	1.94	1.78	42	42	39
Cysteine	0.70	1.10	1.13	4	6	7
Half-cystine	5.58	5.41	5.23	32	32	30
Methionine	1.28	0.81	0	6	4	0
Tryptophane	0.19	0.58	0.30	0.6	2	1
Tyrosine	4.66	5.49	4.66	18	21	18
Serine	3.7	4.5	4.8	25	30	32
Threonine	5.03	6.46	5.97	30	38	35
Hydroxyproline	0			0		
Leucine	11.9	13.7	10.1	64	73	54
Glycine [12]		1.97			18	
Aspartic acid [12]	9.9	10.35		52	54	
Glutamic acid [12]	17.1	16.9		81	80	

[1] Human serum albumin No. 42, a crystalline preparation.

[2] Bovine serum albumin No. 456, a crystalline preparation.

[3] Horse serum albumin B (carbohydrate free fraction, so designated by Kekwick, R. A.: Observations on the Crystallizable Albumin Fraction of Horse Serum, Biochem. J., 32, 552–562 (1938)) prepared by Mr. Manfred Mayer of the Department of Medicine, College of Physicians and Surgeons, according to the method of Adair, G. S., and Robinson, M. E.: The Specific Refraction Increments of Serum-Albumin and Serum-Globulin, Biochem. J., 24, 993–1011 (1930).

[4] The number of residues (or atoms) are calculated on this basis.

[5] For calculation see Brand, E., and Kassell, B. (reference 5 in text).

[6] Total number of amino acids less number of free α-amino nitrogen groups.

[7] Equal to the number of free α-amino nitrogen groups, cf. text.

[8] Basic groups are equal to the sum of the free α-amino nitrogen groups and of the basic amino acids (arginine, histidine, and lysine).

[9] Acid combining power: Human and bovine serum albumin from data obtained in the Department of Physical Chemistry, Harvard Medical School. Horse serum albumin: Value quoted from Proteins, Amino Acids, and Peptides, by Edwin J. Cohn and John T. Edsall, Reinhold Publishing Corp., New York, 1942, page 355.

Table I. The measurement of total base-binding power is, however, difficult, and the accurate determination of the free acid groups in proteins has been accomplished in only a few cases. However, it appears clear that, in the physiological pH range, the negative charges carried by a protein molecule arise from the ionized free carboxyl groups of dicarboxylic acid residues (and terminal carboxyl groups, if present). Positive charges arise from the guanidinium groups of the arginine, and the ϵ-ammonium groups of the lysine residues, all of which groups may be considered to be positively charged at reactions acid to pH 8. The imidazole groups of histidine, and any α-amino groups present in the molecule, carry positive charges when acid to pH 6, and are uncharged when alkaline to pH 8.5 or 9. It is in the physiological pH range that these groups give up, or take on, positive charge; and it is therefore these groups that are almost entirely responsible for the buffering power of proteins under physiological conditions.

All of the plasma proteins carry a net negative charge in this range. This net charge is small compared with the total number of charged groups on the molecule, referred to in the preceding paragraph, and is obviously equal to the algebraic difference between the sum of the total positive and the total negative charges.

Other correlations between amino acid composition and behavior exist, but, in the present state of the theory of protein structure, are less clearly defined. As an example may be mentioned the present-day view that the protein molecule is made up of what are apparently the equivalent of a group of polypeptide chains, often referred to as "sub-units." This hypothesis is an attempt to explain the analytical fact that many proteins contain more amino nitrogen than can be accounted for as the amino nitrogen of lysine radicals. It throws much light upon many other properties of proteins, however, such for example as certain phenomena that accompany denaturation, and upon the fact that many proteins can be obtained in the form of films or fibers. A discussion of this view has recently been given by Chibnall (2).

Information concerning the amino acid composition of the plasma proteins has special significance in medicine. The formation of the plasma proteins proceeds continuously within certain tissues of the body, among which the liver appears to play a prominent part (3). At the same time, the process of protein breakdown goes on; isotope studies, for instance, have indicated that the mean half lifetime of certain serum protein molecules in the body is of the order of two weeks (4). When the plasma protein content of the body is depleted, as from burns or hemorrhage, or in certain types of disease, extra demands for the regeneration of these proteins by the body must be met (3). It is therefore of importance to know as accurately as possible the amino acid composition of plasma proteins, in order to evaluate the nutritional problems involved in regenerating them when they are deficient; and such studies form an integral part of the general program which is set forth in this series of papers.

Conversely, the injection of whole blood, plasma, or plasma protein fractions to treat certain specific clinical conditions represents also the injection of materials which, in the course of time, are broken down in the body and used for other metabolic processes. These human proteins are not dealt with as foreign substances by the human organism, but become part of its structure. For this reason, also, it is important to know accurately the nature of the contribution which they make to the total chemical economy of the organism.

As distinctive chemical differences are detected between different plasma protein fractions, analytical methods may sometimes serve as a control and guide in the process of fractionation, as do the physico-chemical methods

[10] Hydroxy-amino acids: Tyrosine, serine, threonine, and hydroxyproline.

[11] The histidine values were obtained by a recently developed procedure (15) involving separation of histidine as mercury complex and subsequent colorimetric determination. Dr. H. B. Vickery (personal communication) has found 3.22 per cent of histidine in human and 3.46 per cent in bovine serum albumin by a new isolation procedure.

[12] The values for glycine, aspartic acid, and glutamic acid were obtained by Dr. David Shemin, under the direction of Professor H. T. Clarke of the College of Physicians and Surgeons, Columbia University, by the isotope dilution method. The preparations analyzed were crystallized bovine albumin lot 17 and crystallized human albumin (COM-1).

discussed in Paper I of this series (1). Analytical methods have already served as valuable tools in the solution of various problems that have arisen and should become even more significant in the future.

ANALYTICAL METHODS

The micro methods used for the determinations of the various amino acid constituents of the human plasma proteins that have been studied can be divided into 3 groups that yield results that are accurate: (*a*) within about 2 per cent, (*b*) within about 5 per cent, and (*c*) with errors possibly greater than 5 per cent. In the first group belong the values for total nitrogen (micro Dumas), total sulfur (Pregl, cf. (5)), amide nitrogen (6), arginine (7), cysteine (5, 8, 9), cystine (8, 9), methionine (9), tryptophane (10), tyrosine (11), threonine (modifications (12) of (13)), leucine (14), and histidine (15). To the second group belongs the determination of serine (modification of (16)), the serine values being corrected for 10 per cent destruction during acid hydrolysis (17). In the third group are the results for free α-amino nitrogen and for lysine, which were estimated from the rate of liberation of nitrogen in the manometric Van Slyke apparatus (15, cf. 2, 18). Hydroxyproline was not detected in human serum albumin (see Table I), when the colorimetric test of McFarlane and Guest (19) was applied.

The values for the average residue weights may be slightly too low; they are affected mainly by the uncertainty in the distribution of the free amino nitrogen of the protein between the true free α-amino nitrogen and the ϵ-amino nitrogen of lysine.

SERUM ALBUMIN

The composition of the serum albumin of man, of cattle, and of the horse is reported in Table I. The results are given in percentage by weight and in terms of residues per mole on the basis of an assumed molecular weight of 70,000, a figure from which the actual molecular weights of the 3 albumins differ only slightly (*cf.* 1). With proteins having the degree of homogenity apparently possessed by the samples discussed in this report, the computations in terms of residues per mole are of value because they make the comparison between different proteins easier and may ultimately be of help in the correlation of chemical composition with physico-chemical properties.

In the case of the bovine albumin, about 85 grams of the split products per 100 grams of protein are identified and 430 out of 620 amino acids accounted for. For human albumin the data in Table I show 77 grams of the split products, and 381 out of 618 amino acids.

The data show that there are considerable differences in the amino acid make-up of these 3 blood proteins, although in a general way human and bovine albumin resemble each other fairly closely and differ from that of the horse. The complete absence of methionine, an essential amino acid, from horse serum albumin-B is especially noteworthy. The carbohydrate-containing horse serum albumin-A likewise contains no methionine (12). This is of interest in view of the claim of Whipple and his coworkers (20) that cystine is an essential sulfur amino acid for serum protein regeneration (in dogs) while methionine is ineffective.

The tryptophane content of all 3 albumins is low, about 1, 2, and ½ a residue per mole, respectively, in horse, bovine, and human serum albumin. The fractional value for tryptophane, which has repeatedly been obtained in different highly purified preparations of human albumin, suggests that these preparations are still not chemically homogeneous.

The hydroxy-amino acids (tyrosine, serine, and threonine) account for about 12 to 14 per cent of the weight of these proteins. Although high, this is by no means unusual. The similar high hydroxy-amino acid content of β-lactoglobulin (5, 12) may be mentioned as an example, and it would appear that about 1 out of 8 of the constituent amino acids of the serum albumins is a hydroxy-amino acid.

The number of residues per mole of cysteine, leucine, and lysine in the 3 albumins shows some variability, while the content of arginine and of histidine is fairly uniform. The present evidence suggests that the lysine content of horse serum albumin is unusually high.

The dicarboxylic amino acids account for about 27 per cent of the weight of both human and bovine serum albumin. The distribution between glutamic and aspartic acid is practically identical in these 2 proteins (*cf.* Dr. Shemin's data in Table I).

The uniformity in the free α-amino nitrogen in the 3 albumins is striking as well as the total number of basic groups and the number of disulfide linkages. The molecule of serum albumin seems to be characterized by about 16

TABLE II

Composition of human γ-globulin

Preparation No. 36, II-1 (H) [1]

Assumed molecular weight [2]	171,000
Average residue weight [3]	112.4
Approximate number of amino acids per mole [3]	1525
Approximate number of peptide bonds per mole [3]	1500
Approximate number of sub-units (polypeptide chains) per mole [4]	25
Approximate average size of sub-units	6800
Approximate average number of amino acids per sub-unit	60
Total number of basic groups by analysis, per mole [5]	177
Ratio of basic amino acids to total number of amino acids	1:10
Total number of hydroxy-amino acids per mole	370
Ratio of hydroxy-amino acids to total number of amino acids	1:4
Total number of sulfur atoms in S-S linkages, per mole	34
Ratio of sulfur atoms in S-S linkages to total number of amino acids	1:45

Constituent	Per cent	Number of residues or atoms per mole
Total nitrogen	16.03	1957
Free α-amino nitrogen	0.21	25
Amide NH_3	1.35	136
Arginine	4.80	46
Histidine	2.50	28
Lysine	6.7	78
Total protein sulfur	1.02	55
Cysteine	0.70	10
Half-cystine	2.37	34
Methionine	1.06	12
Tryptophane	2.86	24
Tyrosine	6.75	64
Serine	11.4	186
Threonine	8.36	120
Leucine	9.32	122

sulfur bridges and by about 110 basic groups per mole (105, 111, and 117 basic groups, *i.e.*, the sum of the α-amino nitrogen, arginine, histidine, and lysine groups for human, bovine, and horse, respectively). The content of free α-amino nitrogen is uniformly high (about 4 per cent of the total nitrogen) and amounts to about 35 such groups per mole. According to a recent hypothesis on the structure of corpuscular proteins (cf. 5, 2), the free α-amino nitrogen may be interpreted as indicating that these molecules do not consist of a single polypeptide chain (folded or coiled), but that they are formed out of a number of shorter chains or "sub-units." Thus, the molecule of serum albumin would be made up out of about 35 "sub-units," which on the average, consist of about 16 amino acids. The teleologist (cf. 1) may wonder whether it is not one of the inherent functions of serum albumin to make available, all through the body, building stones (sub-units) for the easy synthesis of a great variety of proteins.

γ-GLOBULIN

Data on the composition of human γ-globulin are given in Table II in percentage by weight. The number of residues per mole in this table was calculated on the basis of an assumed molecular weight of 171,000. Comparisons of this figure with those derived from physico-chemical measurements on this and other preparations of human γ-globulin are given in Table II, Footnote 2.

[1] This preparation contained not more than 0.5 per cent of a protein impurity with an electrophoretic mobility different from that of the major component.

[2] A minimum molecular weight of 171,000 can be calculated for this preparation of γ-globulin from the experimental values for the cysteine, half-cystine, methionine, arginine, histidine, threonine, tyrosine, and tryptophane content. The calculations are carried out as described in detail for chymotrypsinogen by Brand, E., and Kassell, B.: Determination of Certain Amino Acids in Chymotrypsinogen, and its Molecular Weight, J. Gen. Physiol., 25, 167–176 (Nov. 1941), and β-lactoglobulin (reference 5 in text). It should be noted that by definition the number of half-cystine residues must always be an even integer (*loc. cit.*).

Svedberg, T. and Pedersen, K. O. (The Ultracentrifuge, Table 48, p. 406, Oxford Press (1940)) have reported a molecular weight of 176,000 for human serum γ-globulin based on sedimentation and diffusion measurements upon the total γ-globulin fraction separated by electrophoresis.

Fraction II, the analysis of which is reported here, does not represent all of the γ-globulin but only a portion thereof. A certain amount of high molecular weight material is present in Fraction II which is thus not homogeneous with respect to size. However, it contains extremely little material of very high molecular weight. (See Paper II of this series.) The best mean molecular weight for this material may be tentatively taken from the osmotic pressure measurements of Scatchard, Batchelder, and Brown as 156,000. (See Paper I of this series.)

[3] For calculation, cf. Brand, E., and Kassell, B., (reference 5 in text).

[4] Equal to the number of free α-amino nitrogen groups, cf. text.

[5] Basic groups are equal to the sum of the free α-amino groups and of the basic amino acids (arginine, histidine, and lysine).

In discussing the amino acid make-up of γ-globulin, we are somewhat handicapped since the inferences must be based upon the analysis of the γ-globulin of only one species, man. As was to be expected from the differences in physico-chemical properties (cf. 1), the composition of γ-globulin differs markedly from that of the albumin. Compared to the serum albumins, human γ-globulin contains fewer basic groups (about ½ as many gram per gram) and fewer disulfide linkages (less than ½). On the other hand, the content of tryptophane and of hydroxy-amino acids is very much higher.

The content of free α-amino nitrogen is 0.21 per cent (about 1 per cent of the total nitrogen, compared to 4 per cent in the case of serum albumins), corresponding to about 25 such groups. In terms of the above discussed sub-units hypothesis, this suggests that the molecule of human γ-globulin may be made up out of about 25 sub-units, which on the average would be large (molecular weight about 7,000) and would on the average contain about 60 amino acid residues.

The high content of the hydroxy-amino acids (tyrosine, serine, and threonine) is especially noteworthy; they account for about 22.5 per cent of the weight of this protein. Accordingly, about 1 out of 4 of the constituent amino acids of γ-globulin is a hydroxy-amino acid. It may not be without significance that a similar high content of hydroxy-amino acids is found among the corpuscular proteins only in the 2 protein enzymes, pepsin and chymotrypsin (12). The high content of hydroxy-amino acids is also of interest in connection with Pauling's hypothesis on the rôle of γ-globulin in antibody formation (21).

β-GLOBULIN

A few preliminary data, recently obtained on a fraction rich in β-globulin, are shown in Table III. This sample, isolated in the course of experiments designed to concentrate prothrombin, was reported to contain 11 per cent of α-globulin, 53 per cent of β_1-globulin, 9 per cent of β_2-globulin, and 27 per cent of γ-globulin and was accordingly a complex mixture of proteins of which less than two-thirds had the mobility associated with β-globulin. The sample contained 10.5 per cent of ash for which the analytical data have been corrected.

TABLE III

Preliminary analysis of human β-globulin (prothrombin fraction)

Plasma globulin—Fraction III-2, Prep. GL291

Constituent	Per cent	Moles or atoms per gram $\times 10^5$
Total nitrogen	14.84	1060
Arginine	5.64	32.4
Histidine	2.50	16.1
Total protein sulfur	1.05	32.8
Cysteine	0.2	1.7
Half-cystine	2.5	20.8
Methionine	1.54	10.3
Tryptophane	2.06	10.1
Tyrosine	5.60	30.9
Serine	8.4	80.0
Threonine	7.26	61.0
Leucine	8.9	67.9

Little emphasis can be placed upon the present analytical results, but in general the composition resembles γ-globulin more closely than it does albumin. The hydroxy-amino acids in particular are high and account for about one-fifth of the total.

A more extended analytical study will be carried out when fractions richer in β-globulin become available.

FIBRINOGEN AND FIBRIN

Data on the composition of human fibrinogen and fibrin are given in Table IV, both in percentage by weight and in terms of moles per gram.

Since the fibrinogen preparation analyzed contained about 50 per cent of salt, the results were calculated on the basis of its nitrogen content on the assumption that the nitrogen of fibrinogen was the same as that of fibrin (16.9 per cent). In spite of this analytical difficulty, it can be seen from Table IV that for a number of amino acids, identical results were obtained. The apparent difference in the sulfur and cysteine-cystine content is of questionable significance.

The fundamental question, *i.e.*, whether the nitrogen content and the amino acid composition of fibrinogen and fibrin are identical, remains

unsolved. Conclusive evidence on this point is not as yet available, but it is prerequisite to further advance in the understanding of the chemical reactions of blood clotting.

TABLE IV

Composition of human fibrinogen,[1] human fibrin,[2] and bovine fibrin [3]

Constituent	Human fibrinogen		Human fibrin		Bovine fibrin	
	Per cent	Moles or atoms per gram X10⁵	Per cent	Moles or atoms per gram X10⁵	Per cent	Moles or atoms per gram X10⁵
Total nitrogen	(16.9)[1]		16.9		(17.0)[3]	
Arginine	7.9	45.4	7.9	45.4	8.1	46.5
Histidine	2.8	18.0	2.8	18.0	2.6	16.8
Total protein sulfur	1.26	39.3	1.23	38.4	1.26	39.3
Cysteine	0.41	3.4	0.60	5.0	0.52	4.3
Half-cystine	2.32	19.3	1.81	15.1	2.02	16.8
Methionine	2.52	16.9	2.62	17.6	2.73	18.3
Tryptophane	3.29	16.1	3.22	15.8	3.37	16.5
Tyrosine	5.75	31.8	5.75	31.8	5.70	31.5
Serine	8.3	79.0	9.8	93.3	7.5	71.4
Threonine	6.6	55.4	6.5	54.6	6.8	57.1
Leucine	7.1	54.2	7.1	54.2	7.5	51.2
Glycine [4]					5.70	76.0
Aspartic acid [4]					12.5	93.9
Glutamic acid [4]					14.5	98.6

[1] The preparation of human fibrinogen (No. 81 RI) contained about 47 per cent of protein and 53 per cent of salts; the protein was 87 per cent clottable. The values reported are calculated on the assumption that the content of total nitrogen is the same in fibrinogen as in fibrin (16.9 per cent).

[2] The fibrin (preparation 65A) used for analysis was obtained from a lot of fibrinogen which clotted spontaneously during purification. The clot was extracted with water until free from salts, dehydrated with alcohol, and dried at the laboratory of the Connecticut Agricultural Experiment Station. The nitrogen determination was made by Dr. Jane K. Winternitz of that laboratory.

[3] The bovine fibrin was a commercial preparation. The values given in this table are based on a total nitrogen content of 17.0.

[4] These results were obtained by Rittenberg, D., and Foster, G. L.: A New Procedure for Quantitative Analysis by Isotope Dilution with Application to the Determination of Amino Acids and Fatty Acids, J. Biol. Chem., **133**, 737–744 (May 1940), on the same preparation of bovine fibrin. The values reported by these authors have been corrected in Table IV on the basis of a nitrogen content of 17.0 for bovine fibrin.

TABLE V

Amino acid composition of human plasma proteins

	Albumin crystallized from Fraction V	γ-globulin purified from Fraction II	β-globulin concentrate from Fraction III-2[1]	Fibrinogen purified from Fraction I	Fibrin clotted from Fibrinogen[2]
Total nitrogen	15.95	16.03	14.84	16.9	16.9
Free amino nitrogen	0.72	0.21			
Amide nitrogen	1.07	1.35			
Arginine	6.15	4.80	5.64	7.9	7.9
Histidine	3.52	2.50	2.50	2.8	2.8
Lysine	5.8	6.7			
Total protein sulfur	1.96	1.02	1.05	1.26	1.23
Cysteine	0.70	0.70	0.20	0.41	0.60
Half-cystine	5.58	2.37	2.50	2.32	1.81
Methionine	1.28	1.06	1.54	2.52	2.62
Tryptophane	0.19	2.86	2.06	3.29	3.22
Tyrosine	4.66	6.75	5.60	5.75	5.75
Serine	3.7	11.4	8.4	8.3	9.8
Threonine	5.03	8.36	7.26	6.6	6.5
Hydroxyproline	0				
Leucine	11.9	9.32	8.9	7.1	7.1

[1] The β-globulin, unlike the albumin and γ-globulin preparations, is a concentrate and not a purified protein. It contained 62 per cent of β-globulin, 11 per cent of α-globulin, and 27 per cent of γ-globulin. It was extracted with ether to remove lipid components. The values are included in this report to permit preliminary comparison with the albumin and γ-globulin.

[2] The fibrin preparation was purified by extracting the clot extensively with water and alcohol. It contained 0.2 per cent of ash.

In order to permit ready comparison of the composition of the human plasma proteins, one with the other, the amino acid composition expressed in percentage has been set out in Table V. These data will be revised and amplified and extended to other plasma proteins as opportunity offers.

The data presented in this paper, incomplete as they are, have yielded some interesting implications, which should prove to be of practical use. The necessity for more detailed information on the chemical composition of all of the various plasma fractions is clearly indicated.

SUMMARY

As a preliminary contribution to the understanding of the composition of the human plasma

proteins, amino acid analyses have been carried out on normal human serum albumin, the human γ-globulins of importance in immunity, a fraction rich in β-globulin, and human fibrinogen and fibrin. Comparable analytical studies on the serum albumin of cattle and the horse are included for comparison. Highly significant differences in the amino acid distribution have been noted, among them being the high percentage of hydroxy amino acids in the γ-globulins, the very low content of tryptophane in the albumins, and the absence of methionine in horse serum albumin. Knowledge of the amino acid composition of the plasma proteins is essential from the point of view of nutrition, as well as for the understanding of the regeneration of plasma proteins. It is also of fundamental importance to a knowledge of the reactions that take place between proteins and other substances.

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