LVI. THE DETERMINATION OF THE COMPOSITION OF THE DIFFERENT PROTEINS OF OX AND HORSE SERUM BY THE METHOD OF VAN SLYKE.

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Recent investigations have shown that serum albumin and serum globulin exhibit well marked differences in chemical composition. Abderhalden [1903, 1905] working with the proteins of horse serum found that albumin yielded no glycocoll on hydrolysis, while globulin gave 3·5 % of this amino acid. Haussmann [1899] and Gümbel [1904] studied the albumin of horse serum by Haussmann's nitrogen distribution method and Gibson [1912] using the same method determined the nitrogen distribution in the pseudo-globulin from horse serum. The results obtained by these three authors show that albumin contains a greater proportion of basic or diamino nitrogen than globulin. Bywaters and Tasker [1913] determined the sulphur and carbohydrate content of the albumin and globulin of horse serum. They found that albumin contains about 2 % of sulphur and 0·25 % of carbohydrate, while globulin yields less sulphur (1·17 %) and more carbohydrate (3·23 %). They also demonstrated the presence of phosphorus in globulin but could find none in albumin.

The globulin occurring in serum is of two kinds, viz. euglobulin and pseudo-globulin. The physical properties and solubilities of these two substances are very different, and recently evidence has been brought forward to show that they differ also in chemical composition. Hardy [1905] was the first to show that globulin contained phosphorus. He was unable to prepare a phosphorus-free globulin by extraction with alcohol and ether and concluded that the phosphorus of globulin is not due to entangled lecithin. Hardy also

succeeded in preparing an osazone from the insoluble phosphorus-containing globulin but failed to do so from the soluble phosphorus-free globulin. Haslam [1912, 1913] prepared pseudo-globulin free from phosphorus and showed that euglobulin contains about 0·1 % of phosphorus, about half of which he was able to extract by means of alcohol and ether. Hardy and Haslam worked with the proteins of ox serum.

In the experiments described in this paper the different proteins of ox and horse serum have been prepared and their chemical composition has been determined by the method of protein analysis recently introduced by van Slyke [1911]. This method has the advantages of yielding approximately quantitative results and requiring the use of relatively small quantities of material, and indicates the nature of the nitrogenous products resulting from complete acid hydrolysis. The whole proteins of the serum, the albumin, the total globulin, the pseudo-globulin and the euglobulin have been prepared in as pure a condition as possible. Each protein was finally obtained in the dry condition and weighed quantities of the air-dried preparations were analysed. Duplicate analyses were carried out in all cases in which sufficient material was available. Details of the preparation of the various proteins and the figures obtained in the analyses of the same are recorded at the end of the paper.

Results.

The proteins of ox serum were studied first. Unfortunately, the amount of pure protein available was not sufficient in any case for duplicate analyses to be carried out, and in one case (viz. the euglobulin prepared from ox serum by one-third saturation with ammonium sulphate) only 2·5 g. of material were used. After hydrolysis the solution employed for analysis in this instance contained 235 milligrams of nitrogen: of this, only 52 milligrams were present as diamino nitrogen and the determination of the four diamino acids was carried out on this small quantity. On account of the small amount of material used, less confidence is felt in the result of this particular analysis than in the others. It has been found by experience that the analysis of proteins by van Slyke's method is conveniently carried out on solutions containing about 400 milligrams of nitrogen; in the case of the serum proteins such solutions contain about 100 milligrams of diamino nitrogen. In the case of the proteins of horse serum larger quantities of material were prepared and in three cases enough material was available for duplicate analyses to be carried out.

The results of the analysis of the different proteins of ox and horse serum are summarised in the following tables:

TABLE I.

Summary of the analyses of the different proteins of ox serum, in percentages of the total nitrogen.

	Whole Protein		Albumin		Total Globulin		Pseudo-Globulin		Euglobulin ($(NH_4)_2SO_4$ method)		Euglobulin (Panum's method)	
Ammonia N ..	7·05		5·8		7·7		7·5		9·3		8·0	
Melanin N ..	1·60		1·1		2·0		1·9		2·0		2·5	
Cystine N ..	2·75	31·75	3·5	36·9	2·0	28·2	1·9	27·1	2·0	26·6	1·4	28·7
Arginine N ..	11·05		10·4		10·9		10·8		11·6		11·7	
Histidine N ..	4·40		6·7		6·3		4·8		3·8		6·5	
Lysine N ..	13·55		16·3		9·0		9·6		9·2		9·1	
Amino N of the Filtrate	56·65	58·80	54·2	56·5	59·8	62·0	61·7	63·3	57·9	60·7	58·0	59·4
Non-amino N of the Filtrate	2·15		2·3		2·2		1·6		2·8		1·4	
Sum	99·20		100·3		99·9		99·8		98·6		98·6	

TABLE II.

Summary of the analyses of the different proteins of horse serum, in percentages of the total nitrogen.

	Whole Protein		Albumin		Total Globulin		Pseudo-Globulin		Euglobulin ($(NH_4)_2SO_4$ method)		Euglobulin (Panum's method)	
Ammonia N ..	7·25		6·65		7·95		7·7		7·90		8·0	
Melanin N ..	1·60		0·95		2·30		2·2		2·25		2·3	
Cystine N ..	2·10	30·00	3·10	34·75	1·65	25·25	1·7	26·2	1·65	25·30	1·8	26·5
Arginine N ..	9·50		9·90		8·00		8·9		8·25		9·4	
Histidine N ..	5·60		5·85		4·80		5·8		5·45		5·2	
Lysine N ..	12·80		15·90		10·80		9·8		9·95		10·1	
Amino N of the Filtrate	59·95	61·95	56·55	58·15	62·65	64·80	61·3	64·2	62·20	64·35	61·0	62·8
Non-amino N of the Filtrate	2·00		1·60		2·15		2·9		2·15		1·8	
Sum	100·80		100·50		100·30		100·3		99·80		99·6	

Discussion of Results.

From a consideration of the figures given in the two preceding tables it is evident that the composition of serum albumin is very different from that of any of the globulins. Albumin yields on hydrolysis a greater proportion of diamino acids than the globulins, a result which is in agreement with those obtained by Haussmann [1899], Gümbel [1904] and Gibson [1912], who used the nitrogen distribution method devised by Haussmann. Further, albumin

yields a greater proportion of cystine than globulin. In the case of ox serum albumin 3·5 % of the total nitrogen occurs as cystine, while the average for the four globulins is 1·8 %. The corresponding figures for the albumin and globulin from horse serum are 3·1 % and 1·7 %. The results for cystine are in agreement with those of Abderhalden [1903, 1905], who obtained a greater amount of cystine from albumin than from globulin, and of Bywaters and Tasker [1913], who found that the sulphur content of albumin is greater than that of globulin. Estimations of the lysine, arginine, and histidine content of the serum proteins have not hitherto been carried out. From the figures given above for these amino acids it is clear that in the case of both sera examined the albumin differs from the globulins in containing a much larger amount of lysine. It is also to be observed that albumin yields a smaller amount of ammonia and melanin on hydrolysis than the globulins.

Other experimental evidence regarding the lysine content of albumin and globulin has been obtained. Van Slyke and Birchard [1914] determined the free amino nitrogen of a number of proteins and showed that on treatment with nitrous acid in van Slyke's apparatus for half an hour the volume of nitrogen gas evolved corresponded to one half the lysine nitrogen of the protein. Similar experiments have been carried out with the albumin and globulin prepared from ox serum. It was found that albumin yielded about twice as much nitrogen as globulin on treatment with nitrous acid and that in each case the nitrogen obtained was almost exactly one half the lysine nitrogen of the respective proteins. Full details of these experiments will be published shortly.

The marked difference in composition of the proteins of the serum is of interest when considered with reference to the question of the conversion of albumin into globulin, a change which according to Moll [1904, 1906] occurs when serum is heated for an hour at 60° after the addition of a little dilute alkali. Gibson [1912] and Bywaters and Tasker [1913] brought forward evidence which showed that albumin solutions treated in the manner described by Moll yielded a protein which is not identical with naturally occurring globulin and Abderhalden [1903, 1904] showed that globulin yields glycocoll on hydrolysis while albumin does not. The figures given in the above tables show other important differences in the chemical composition of albumin and the globulins of serum, particularly a striking difference in the relative amounts of lysine. When the whole of the evidence dealing with the chemical nature of the serum proteins is considered it is not easy to understand the mechanism of the process by which such simple treatment

of one protein can lead to the formation of another possessing such a widely different chemical composition.

The figures obtained in the analyses of the different globulins indicate that these substances are very similar in chemical composition. Euglobulin was prepared by two methods: (1) by the method of Panum [1851], and (2) from the previously prepared and purified "total globulin[1]" by one-third saturation with ammonium sulphate. A portion of this "total globulin" was set aside for analysis and the remainder was separated into pseudo-globulin and euglobulin by repeated salting out with ammonium sulphate. Analysis of these different globulin preparations by the method of van Slyke failed to reveal any notable differences in chemical composition. The agreement is particularly close in the case of the globulins prepared from horse serum. In the case of the ox serum globulins, the euglobulin prepared by one-third saturation with ammonium sulphate shows a slightly higher ammonia and lower histidine nitrogen value than the other three, but in other respects the agreement is fairly close. As pointed out above, the analytical errors in this particular estimation are probably greater than in the other cases as the analysis was carried out on a much smaller quantity of material. Larger quantities of this protein were prepared from horse serum and the figures obtained on analysis are almost identical with those obtained for the pseudo-globulin and the "total globulin."

The compositions of the different globulins are of interest when considered in connection with the recent work of Chick [1914]. She suggests that the relationship between euglobulin and pseudo-globulin is a very close one, and that under certain conditions pseudo-globulin may undergo a process of "denaturation" whereby a substance is formed the properties of which are very similar to those of euglobulin. She suggests that this artificial euglobulin may be a mechanical complex resulting from the interaction and mutual precipitation of two colloidal systems (viz. pseudo-globulin solution and lipoid emulsion) and that the euglobulin of serum may be a protein-lipoid complex of similar origin. The evidence brought forward in this paper regarding the composition of these two proteins lends support to the idea that euglobulin may be related to pseudo-globulin in some such way as Chick suggests. The conversion of pseudo-globulin into euglobulin is a process which, on chemical grounds, would be more easily understood and more capable of explanation than the conversion of albumin into globulin.

[1] The term "total globulin" refers to the protein precipitated from the diluted serum by half saturation with ammonium sulphate. It consists of pseudo-globulin and euglobulin.

Experimental Details.

Preparation of the different serum proteins.

Euglobulin was prepared by the method of Panum [1851], and also by means of ammonium sulphate. Albumin, total globulin, and pseudo-globulin were prepared by salting out with ammonium sulphate. The albumin and globulin were first separated by half saturation with ammonium sulphate. The serum was diluted with four volumes of distilled water and an equal volume of saturated ammonium sulphate solution added. The precipitated globulin was centrifuged off and the solution containing the albumin was saturated with powdered ammonium sulphate. The albumin was filtered off, dissolved in distilled water, and the albumin solution brought to a volume equal to that of the original *diluted* serum. An equal volume of saturated ammonium sulphate solution was added, the precipitated globulin was filtered off, and the solution containing the albumin was again saturated with powdered ammonium sulphate. The albumin was dissolved and reprecipitated five times in all. The albumin solution was always brought to the same volume as that of the original diluted serum before the addition of the saturated ammonium sulphate solution.

The total globulin of the serum was dissolved and reprecipitated five times in the case of ox serum and six times in the case of horse serum. The globulin after each precipitation was dissolved in distilled water and the solution was diluted until the volume was the same as that of the original diluted serum. A portion of the total globulin was then set aside for analysis and the remainder was separated into euglobulin and pseudo-globulin by Haslam's method [1913]. Each globulin was dissolved and reprecipitated five times in the case of ox serum and six times in the case of horse serum globulin.

The different proteins prepared as described above were finally dissolved in distilled water and dialysed against running tap water until free from ammonium sulphate. The aqueous solutions of the different proteins were evaporated to dryness in a modified form of the apparatus described by Martin [1896], a dropping funnel, the exit tube of which was drawn out to a fine capillary, being substituted for the Berkefeldt filter. The bath containing the two drying bottles was maintained at a temperature of 40° and the larger bottle which acts as a receiver was immersed in ice. The dried proteins obtained in this way were reduced to a fine powder and analysed.

Euglobulin was also prepared by the method of Panum [1851]. The serum was diluted ten times with distilled water and normal acetic acid added in sufficient amount to precipitate the euglobulin. The amount of acetic acid necessary for complete precipitation was previously determined by tests on small quantities of the diluted serum. After standing overnight the clear supernatant fluid was decanted and the precipitated euglobulin centrifuged. It was purified by being dissolved in a minimum and measured quantity of decinormal sodium hydrate and reprecipitated by the addition of an exact equivalent of decinormal acetic acid. This process was repeated four times and the euglobulin was finally washed twice in the centrifuge tubes with distilled water.

A sample of the original serum was dried in Martin's apparatus.

Methods of Analysis.

In carrying out the analyses of the different serum proteins the experimental conditions were kept as constant as possible. From four to five grams of material were boiled with 80 cc. of 20 % hydrochloric acid and the rate of hydrolysis was followed by testing samples of the fluid at intervals in van Slyke's apparatus. The hydrolysis was continued for 20 to 22 hours.

After hydrolysis of the protein the excess of hydrochloric acid was removed *in vacuo* and the hydrolysed material washed into a 250 cc. flask. Duplicate quantities of 20 cc. of the solution were used for the determination of the total nitrogen, and 200 cc. of the solution were used in all cases for analysis. The sum of the ammonia nitrogen, melanin nitrogen, total nitrogen of the filtrate, and the total nitrogen of the bases (the last-named being determined in two parts) should be the same as the amount originally present, as determined separately in the 20 cc. portions. The experience gained in the study of these serum proteins shows that with the exercise of ordinary care throughout the course of the analysis it is not difficult to recover the whole of the nitrogen.

In separating the diamino acids from the monamino acids the same amount of phosphotungstic acid (15 g.) was used, and the precipitation was carried out at a volume of 200 cc. in all cases. After the addition of the phosphotungstic acid the mixture was heated on the water bath until most of the precipitate had redissolved, and after cooling to room temperature the vessel was allowed to stand for 48 hours in the cold room. The precipitate of the bases was washed with an ice-cold solution of phosphotungstic acid in hydrochloric acid, 120 to 140 cc. of solution being used.

TABLE III.

Details of observations.

Material	Quantity taken g.	Total N: cc. N/10 acid per 20 cc.	Total N: g. N per 200 cc.	Ammonia: cc. N/10 acid per 200 cc.	Ammonia: g. N per 200 cc.	Melanin: cc. N/10 acid per 200 cc.	Melanin: g. N per 200 cc.	Cystine: g. $BaSO_4$ per 40 cc.	Cystine: g. N per 200 cc.	Arginine: cc. N/10 acid per 100 cc.	Arginine: g. N per 200 cc.
A. Ox serum											
1. (*a*) Whole protein	4	24·2 24·1	0·3381	16·9	0·0237	3·8	0·0053	0·0240	0·0072	6·2	0·0347
(*b*) Whole protein	4	24·5 24·6	0·3437	17·6	0·0246	4·0	0·0056	0·0209	0·0063	6·1	0·0342
2. Albumin	4	27·9 28·0	0·3913	16·2	0·0277	3·3	0·0046	0·0380	0·0114	6·7	0·0375
3. Total globulin	4	26·5 26·4	0·3703	20·4	0·0286	5·5	0·0077	0·0170	0·0051	6·7	0·0375
4. Pseudo-globulin	4	27·2 27·2	0·3808	20·5	0·0287	5·2	0·0073	0·0160	0·0048	6·8	0·0381
5. Euglobulin $(NH_4)_2SO_4$ method	2·6	16·8 16·8	0·2352	15·6	0·0218	3·5	0·0049	0·0075	0·0022	4·3	0·0241
6. Euglobulin Panum's method	4	28·05 28·50	0·3958	22·7	0·0318	7·0	0·0098	0·0102	0·0031	7·7	0·0431
B. Horse serum											
7. (*a*) Whole protein	5·04	33·0 32·9	0·4613	23·7	0·0332	5·6	0·0078	0·0245	0·0073	7·3	0·0409
(*b*) Whole protein	4·95	33·0 33·2	0·4634	24·3	0·0340	5·0	0·0070	0·0241	0·0072	7·3	0·0409
8. (*a*) Albumin	4	29·5 29·5	0·4130	19·8	0·0277	3·1	0·0043	0·0341	0·0102	6·85	0·0384
(*b*) Albumin	4	29·4 29·7	0·4137	19·5	0·0273	2·9	0·0041	0·0345	0·0103	6·7	0·0375
9. (*a*) Total globulin	4	28·4 28·2	0·3962	22·6	0·0316	6·4	0·0090	0·0170	0·0051	5·2	0·0291
(*b*) Total globulin	4	29·2 29·4	0·4102	23·1	0·0323	6·9	0·0097	0·0115	0·0034	5·2	0·0291
10. Pseudo-globulin	5	35·7 35·6	0·4991	27·5	0·0385	7·8	0·0109	0·0203	0·0061	7·4	0·0414
11. (*a*) Euglobulin $(NH_4)_2SO_4$ method	4·59	32·3 32·3	0·4522	26·1	0·0365	7·3	0·0102	0·0176	0·0053	6·3	0·0353
(*b*) Euglobulin $(NH_4)_2SO_4$ method	4·62	32·8 32·9	0·4599	25·7	0·0360	7·6	0·0106	0·0162	0·0048	6·0	0·0336
12. Euglobulin Panum's method	4·5	29·5 29·5	0·4130	23·6	0·0330	6·9	0·0097	0·0159	0·0048	6·4	0·0358

TABLE III.

Details of observations.

Total N of Bases			Amino N of Bases		Amino N of Filtrate		Total N of Filtrate		Total N recovered g. per 200 cc.
cc. N/10 acid per 100 cc.	cc. N/10 acid per 100 cc. + arginine titration	g. N per 200 cc.	cc. N per 40 cc.	g. N per 200 cc.	cc. N per 200/15 cc. mm.	g. N per 200 cc.	cc. N 10 acid per 200 6 cc.	g. N per 200 cc.	
29·0	35·2	0·0985	22·1; 16°; 768 mm.	0·0648	22·6, 22·7; 17°; 760	0·1962	24·6, 24·7	0·2071	0·3346
28·9	35·0	0·0980	22·5; 18°; 765 mm.	0·0651	22·7, 22·9; 16°; 768	0·2005	25·45, 25·50	0·2140	0·3422
41·4	48·1	0·1347	31·0; 16°; 774 mm.	0·0916	18·2, 18·4; 15°; 774[1]	0·2172	20·6, 20·7	0·2313	0·3933
27·4	34·1	0·0955	18·7; 17°; 763 mm.	0·0542	26·3; 19°; 762	0·2265	28·6, 28·4	0·2394	0·3712
26·7	33·5	0·0938	19·2; 18°; 764 mm.	0·0555	28·0, 27·8; 19°; 762	0·2400	29·9, 29·9	0·2512	0·3810
14·5	18·8	0·0526	10·6; 15°; 764 mm.	0·0310	16·1, 16·0, 16·2; 15°; 764	0·1415	18·3, 18·1	0·1529	0·2322
29·4	37·1	0·1039	19·5; 16°; 764 mm.	0·0568	20·0, 20·3; 16°; 764[1]	0·2349	22·0, 21·8	0·2453	0·3908
39·2	46·5	0·1302	29·5; 22°; 770 mm.	0·0843	32·4, 32·6; 20°; 773	0·2820	35·2, 35·2	0·2957	0·4669
38·5	45·8	0·1282	29·2; 21°; 765 mm.	0·0832	32·9, 33·0; 21°; 768	0·2829	35·5, 35·4	0·2978	0·4670
41·4	48·25	0·1351	31·6; 17°; 766·5 mm.	0·0184	27·3, 27·3; 17°; 766	0·2385	30·0, 29·9	0·2516	0·4188
40·5	47·2	0·1321	31·3; 17°; 766·5 mm.	0·0182	27·5, 27·6; 18°; 766	0·2394	29·7, 29·7	0·2495	0·4130
28·0	33·2	0·0930	20·8; 20°; 770 mm.	0·0600	28·8, 28·9; 19°; 773	0·2517	31·7, 31·7	0·2663	0·3999
27·5	32·7	0·0916	20·9; 20°; 764 mm.	0·0598	30·5, 30·4; 19°; 770	0·2640	32·9, 33·0	0·2768	0·4104
35·9	43·3	0·1212	25·8; 21°; 764 mm.	0·0734	36·7, 36·7; 22°; 763	0·3114	39·4, 39·4	0·3309	0·5015
30·8	37·1	0·1039	22·8; 18°; 752 mm.	0·0648	33·6, 33·4; 18°; 752	0·2856	35·8, 36·0	0·3015	0·4521
32·4	38·4	0·1075	23·4; 20°; 762 mm.	0·0667	34·2, 34·4; 18°; 752	0·2925	36·4, 36·5	0·3062	0·4603
29·1	35·5	0·0994	21·9; 24°; 757 mm.	0·0608	31·1, 31·1; 25°; 755	0·2572	32·0, 32·2	0·2696	0·4117

[1] Solution brought to 200 cc.

One of the more difficult of the estimations in the process is the determination of the total nitrogen of the bases, which is carried out on the same solution used for the determination of the arginine. In these analyses the practice was adopted of continuing the digestion for some time after the solution becomes clear, gently moving the fluid round the sides of the flask from time to time in order to wash down into the acid any particles which may have crept on to the upper parts of the digestion flask. The figures for the histidine nitrogen show the widest variation in duplicate analyses. This value is not determined directly but is calculated from the results of three other determinations, viz. the arginine nitrogen, the amino nitrogen of the bases, and the total nitrogen of the bases. As van Slyke points out these three values can all be determined accurately, and the duplicate analyses for the histidine should not vary by more than one per cent. of the total nitrogen.

The following estimations were carried out on the solution of hydrolysed protein:

(1) Total nitrogen was estimated in 20 cc. of the original solution.

(2) Ammonia nitrogen and melanin nitrogen were estimated in 200 cc. of the original solution.

(3) After removal of the ammonia and melanin the separation with phosphotungstic acid was carried out.

(4) The filtrate from the phosphotungstic acid precipitate of the bases was made to 150 cc. and the following estimations made:

(*a*) Total nitrogen of the filtrate in 25 cc.

(*b*) Amino nitrogen of the filtrate in 10 cc.

(5) The solution of the bases was made to 50 cc. and the following estimations made:

(*a*) Cystine nitrogen in 10 cc.

(*b*) Amino nitrogen in 10 cc.

(*c*) Arginine nitrogen and total nitrogen of the bases in the same sample of 25 cc.

The detailed figures obtained in the analysis of the different serum proteins are given in the tables. Table III (pp. 548, 549) contains a record of the actual observations, the volumes given in the headings referring in every case to the amount of the original solution corresponding to the volume actually employed as explained above. In Table IV the results are expressed as percentages of the total nitrogen.

TABLE IV.

Results of analyses expressed in percentages of total nitrogen.

Material	Ammonia N	Melanin N	Cystine N	Arginine N	Histidine N	Lysine N	Amino N of Filtrate	Non-Amino N of Filtrate	Total N recovered
A. Ox serum									
1. (*a*) Whole protein	7·0	1·6	2·1	10·2	3·4	13·3	58·0	3·2	98·8
Corrected for solubility of bases			2·9	11·2	4·5	13·4	56·5	1·8	
(*b*) Whole protein	7·1	1·6	1·8	9·9	3·2	13·5	58·3	3·9	99·3
Corrected			2·6	10·9	4·3	13·7	56·8	2·5	
2. Albumin	5·8	1·1	2·9	9·6	5·7	16·2	55·5	3·6	100·4
Corrected			3·5	10·4	6·7	16·3	54·2	2·3	
3. Total globulin	7·7	2·0	1·4	10·1	5·3	8·9	61·1	3·4	99·9
Corrected			2·0	10·9	6·3	9·0	59·8	2·2	
4. Pseudo-globulin	7·5	1·9	1·2	10·0	3·9	9·5	63·0	3·0	100·0
Corrected			1·9	10·8	4·8	9·6	61·7	1·6	
5. Euglobulin $(NH_4)_2SO_4$ method	9·3	2·0	0·9	10·2	2·2	9·0	60·1	4·9	98·6
Corrected			2·0	11·6	3·8	9·2	57·9	2·8	
6. Euglobulin Panum's method	8·0	2·5	0·8	10·9	5·6	9·0	59·3	2·6	98·7
Corrected			1·4	11·7	6·5	9·1	58·0	1·4	
B. Horse serum									
7. (*a*) Whole protein	7·2	1·7	1·6	8·8	4·9	12·8	61·1	3·0	101·1
Corrected			2·1	9·5	5·8	12·9	60·0	1·9	
(*b*) Whole protein	7·3	1·5	1·5	8·8	4·6	12·6	61·0	3·2	100·5
Corrected			2·1	9·5	5·4	12·7	59·9	2·1	
8. (*a*) Albumin	6·7	1·0	2·4	9·3	5·1	15·7	57·7	3·1	101·0
Corrected			3·1	10·0	6·1	16·0	56·5	2·0	
(*b*) Albumin	6·6	0·9	2·5	9·0	4·6	15·7	57·8	2·4	99·5
Corrected			3·1	9·8	5·6	15·8	56·6	1·2	
9. (*a*) Total globulin	8·0	2·3	1·3	7·3	4·1	10·6	63·5	3·7	100·8
Corrected			1·9	8·1	5·1	10·7	62·2	2·4	
(*b*) Total globulin	7·9	2·3	0·8	7·1	3·6	10·7	64·3	3·1	99·8
Corrected			1·4	7·9	4·5	10·9	63·1	1·9	
10. Pseudo-globulin	7·7	2·2	1·2	8·3	5·0	9·7	62·4	3·9	100·4
Corrected			1·7	8·9	5·8	9·8	61·3	2·9	
11. (*a*) Euglobulin $(NH_4)_2SO_4$ method	8·0	2·2	1·1	7·8	4·2	9·8	63·1	3·5	99·7
Corrected			1·7	8·5	5·0	9·9	62·0	2·4	
(*b*) Euglobulin $(NH_4)_2SO_4$ method	7·8	2·3	1·1	7·3	5·1	9·9	63·6	3·0	100·1
Corrected			1·6	8·0	5·9	10·0	62·4	1·9	
12. Euglobulin Panum's method	8·0	2·3	1·1	8·7	4·3	10·0	62·3	3·0	99·7
Corrected			1·8	9·4	5·2	10·1	61·0	1·8	

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