PARAPROTEINS IN BLOODSERUM

by

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The determination of the total serumprotein and albumin-globulin-relation is an everyday's work of a clinical laboratory to-day. The salt precipitation of the different subfractions of globulin is often carried out simultaneously. But the closer qualitative examination of the serumproteins in health and in different pathological conditions is no simple task. Nevertheless there are conditions under which it has been possible to demonstrate qualitative abnormalities in serumproteins, called paraproteins (APITZ, 1940). They show an abnormal behaviour from physico-chemical and sometimes from a purely chemical point of view.

The electrophoretic method while showing three globulins (alpha, bêta and gamma) in normal sera and determining their mutual relation in health and disease, fails to give any further information as to the occurrence of qualitative changes in one or other fractions. The ultracentrifuge gives valuable information about the size of the particles of the serumproteins and in some cases there is evidence of proteins with abnormally high molecular weight. In clinical laboratories a series of simpler tests are employed, which by giving information mostly about the colloid-lability of the whole serum, produce an indirect evidence of the pathologically changed globulins. The positive formol-gel test, the TAKATA-ARA test, the serum gold-sol test are generally indicating the rise in globulin content and some of them, so as the coagulation-band and the nephelogramm (WUHRMANN and WUNDERLY, 1946) indicate the change in the relative proportion of the three fractions in the globulin. WALDENSTRÖM's (1945) new "Alcacid"-test does not seem to run parallel with the already mentioned tests. It has been found to be of some value in detecting qualitatively changed globulins in different cases of hyperglobulinemia.

Many attempts have been made to investigate the amino-acid composition of the various sera under pathological conditions, but our knowledge is still relatively poor. The first known paraprotein is the BENCE-JONES-protein, generally regarded as evidence of myeloma multiplex. LANG (1935) established the aminoacid composition of the BENCE-JONES-protein in urine and serum. TUCHMANN and SOBOTKA found some differences in the tyrosine content of albumin and globulin in cases of hypoproteinemia. BIANCHI (1947) and others maintain, that in rheumatic conditions the tryptophane content of serum is higher than usually. One of us (BÁLINT, 1943) published a case of hungeredema, in which the aminoacid composition of serum albumin showed a definite abnormality.

The principal cause of the scarcity of our knowledge lies in methodical difficulties, as the generally employed methods require a considerable quantity of blood, hardly available from one given patient, whereas the newest methods, based on microbiological principles are hardly workable in the routine of the ordinary clinical laboratory.

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Our aim was to devise a method enabling the determination of at least some aminoacids in a small volume of bloodserum. We intended to examine the aminoacid composition of serumproteins of normal individuals and to seek for pathological pattern in various diseased conditions.

METHODS

One of us (BÁLINT, 1938) was able to determine the tyrosine and tryptophane content in 50 mg protein, by a slight modification of the original method of FOLIN and MARENZI (1929). The modified cystine determination of LUGG (1932), the slightly altered histidine determination of LANG (1933) and the arginine determination of JORPES and THOREN (1932) require a further 50 mg of protein. Thus 1.5 ml of serum was enough for the five aminoacid determinations and KINGSLEY's (1940) modification of the original HOWE procedure needed only 0.5 ml serum for the determination of total protein and albumin-globulin relation.

For the estimation of tyrosine and tryptophane we added to 0.5 ml serum 0.5 ml of water and 1 ml of 40 % NaOH and kept in a waterbath for 14–16 hours. The determinations were carried out exactly in the way described in BÁLINT's paper. For the determination of cystine, histidine and arginine 1 ml of serum was taken and boiled with 1.2 ml hydrochloric acid (dens. 1.19) with a reflux condensor over 20 hours. Afterwards the fluid was washed over to a testtube and filled up with water to the 20 ml mark. Furtheron we proceeded as described in the above mentioned papers.

RESULTS

At first we examined the above mentioned five aminoacids in normal sera. To that end we took blood from five healthy, well fed persons, four times from each in weekly intervals. The averages, s.d. and ranges shown in Table I indicate only slight variations in the aminoacid pattern of normal serumprotein.

TABLE I

No.	Date	Total protein g.p.c.	Albumin g.p.c.	Globulin g.p.c.	A/G	Tyrosine g.p.c.	Tryptophane g.p.c.	Cystine g.p.c.	Arginine g.p.c.	Histidine g.p.c.
1a	26-II	7.75	5.05	2.70	1.9	4.5	1.4	3.5	3.1	3.4
1b	5-III	7.64	4.52	3.12	1.5	4.9	1.2	3.5	2.6	3.9
1c	20-III	8.02	4.92	3.70	1.6	4.3	1.0	3.6	2.6	3.7
1d	6-V	7.75	4.52	3.23	1.4	4.3	1.1	3.3	2.7	3.5
2a	27-II	7.80	4.94	2.86	1.7	4.6	1.6	3.7	3.0	3.3
2b	8-III	7.90	4.98	2.92	1.7	4.3	1.3	3.5	2.5	3.7
2c	18-III	8.12	5.18	2.94	1.7	4.8	1.1	3.3	2.5	3.3
2d	1-V	8.23	5.01	3.22	1.6	4.3	1.1	2.6	2.8	3.1
3a	27-II	7.30	4.98	2.32	2.2	4.3	1.0	3.5	2.9	3.3
3b	7-III	7.48	4.96	2.52	2.0	4.8	1.0	3.8	2.6	3.6
3c	18-III	7.45	4.78	2.67	1.8	4.9	0.9	3.7	2.5	3.5
3d	7-V	7.20	4.68	2.52	1.9	4.7	1.0	3.5	3.2	3.8
4a	3-III	8.00	5.08	2.97	1.7	4.4	1.1	3.6	2.3	3.8
4b	10-III	8.09	5.12	2.97	1.7	4.4	1.0	3.5	2.3	4.6
4c	20-III	8.36	5.21	3.15	1.7	4.5	1.0	3.4	2.6	3.6

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No.	Date	Total protein g.p.c.	Albu-nin g.p.c.	Globu-lin g.p.c.	A/G	Tyro-sine g.p.c.	Trypto-phane g.p.c.	Cystine g.p.c.	Argi-nine g.p.c.	Histi-dine g.p.c.
4d	6-V	8.70	5.80	2.90	2.0	4.1	1.0	3.4	2.4	3.0
5a	3-III	8.15	5.29	2.86	1.9	4.4	1.0	3.6	2.6	3.7
5b	10-III	7.48	4.70	2.78	1.7	4.9	1.0	3.7	2.4	3.5
5c	20-III	7.56	5.05	2.51	2.0	4.2	1.3	3.7	2.6	3.4
5d	7-V	7.41	4.53	2.88	1.6	4.3	1.1	2.9	2.7	3.6
				Average		4.5	1.1	3.5	2.6	3.6
				S. d.		0.3	0.1	0.3	0.3	0.3
				Minimal value . . .		4.1	0.9	2.6	2.4	3.0
				Maximal value . . .		4.9	1.6	3.8	3.2	4.6

Our next attempt has been to determine the aminoacid composition of sera of patients in different illnesses. In the course of our experiments we have analysed 50 sera, all from cases, where some disturbance in the composition of serumprotein could be expected. (Liver cases, chronic nephritis, hypoproteinemia, malignant tumors. . .) The results of 42 cases have fallen between the ranges of Table I. Table II contains the data of sera in which at least one kind of aminoacid shows a positive or negative significant deviation from the normal state. In the cases with diminished A/G some increase in tyrosine, tryptophane and decrease in cystine could be expected, owing to the greater resp. smaller content of the globulin fraction in the aminoacids mentioned. The table relates only data exceeding the difference due to the change in A/G ratio.

TABLE II

No	Date	Diagnosis	Total protein g.p.c.	Albumin g.p.c.	Globulin g.p.c.	A/G	Tyrosine g.p.c.	Tryptophane g.p.c.	Cystine g.p.c.	Arginine g.p.c.	Histidine g.p.c.
1	10-III	Hungeredema . .	3.69	1.28	2.41	0.5	4.4	2.1	4.4	3.3	3.6
2	3-II	Hypoproteinemia.	3.31	1.72	1.59	1.1	5.6	2.3	2.5	4.2	2.6
3	10-II	,,	5.12	2.63	2.49	1.1	5.8	1.0	3.6	3.6	5.2
4	1-II	Nephritis chr. . .	3.75	1.68	2.07	0.8	2.8	0.6	3.3	4.1	5.1
5	17-II	,,	3.68	1.25	2.43	0.5	4.8	1.8	4.0	4.0	2.8
6	14-VIII	Hypoproteinemia	2.66	1.03	1.63	0.6	6.8	1.8	—	—	—
7	10-IX	,,	5.25	2.38	2.87	0.8	5.9	1.9	2.8	—	—
8	6-V	Cirrhosis hepatis .	9.88	2.13	7.75	0.3	4.2	1.0	1.0	3.0	2.5
9	20-IV	Myeloma multiplex	12.74	2.57	10.17	0.3	4.2	1.7	3.0	5.9	3.7
		Fibrinogen 1.00 g.p.,c.					—	—	1.9	8.1	—
		Euglobulin 6.36 g.p.c.					5.2	1.8	1.2	7.5	—

DISCUSSION

As can be seen from Table II, all cases showing an abnormal aminoacid pattern have a total protein content under or above the normal values.

No. 1 (hungeredema) has a greater content of cystine.

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No 2 and 3 are from the same patient with one week interval: we have found an increase in the total protein, with unchanged A/G. The first sample showed a higher content in arginine, which was still increased in the second sample. Histidine, on the contrary, was first diminished, and then abnormally high. Comparing the data of this case with the normal series of Table I, it is evident that there is some lability in the composition of serumproteins, even if the increase of the total protein must be regarded as a sign of recovery.

The same applies for No. 4 and 5. The first sample showed an abnormally low content of tyrosine and tryptophane, which values are becoming normal within 17 days. This time no significant change occurred in the total protein content.

No. 6 and 7, again from the same patient with 4 weeks' interval shows data, which are registered for the sake of completeness. It was described by one of us formerly (BÁLINT, 1943) and the analysis produced, contrary to the above cases, an extremely high tyrosine content, tending to the normal in the second blood-sample.

No. 8 is a hyperproteinemic case of cirrhosis hepatis, were we had got the blood sample before the patient's death. The extremely low cystine content is the outstanding feature in the aminoacid composition.

No. 9 was sent to us by the Medical Clinic of Debrecen. The clinical data will be published by the above clinic in a separate paper, we only got the plasma and serum for analysis. The extremely high protein content was due to an increase of fibrinogen and euglobulin (salt precipitation according to HOWE), the remaining fractions being rather diminished in absolute quantity. The analysis of total protein revealed abnormally much arginine, caused by the high arginine content of fibrinogen and euglobulin. In the table we are registering the aminoacid composition of these two fractions and should stress, that the arginine ranges of fibrinogen are 3.5–4.8 %, with an average of 4.2 $\pm$ 0.4 and of euglobulin 2.6–3.5 %, average 3.0 $\pm$ 0.3. (These data are results of the analysis of some 150 cases. The analysis were done by one of us and published in a series of communications. BÁLINT and BÁLINT, 1940–1943). The fact must be emphasized, that our procedure does not reveal whether this increase is due to the higher arginine content of the normal proteins, or to a newly formed pathological fraction rich in arginine.

We are fully aware that the number of our pathological cases is too small to draw any conclusions for single pathological cases, but paraproteins are likely to be found with our method described above.

SUMMARY

We determined the tyrosine, tryptophane, cystine, arginine and histidine content of 20 normal serumproteins, which was found to be constant except for some small individual variations. From the 50 pathological cases analysed 42 ones have shown a normal composition and in only 8 (from 5 patients) cases were significant changes in the aminoacid composition demonstrable. The results are clearly showing that it is possible to detect paraproteinemic conditions by means of aminoacid analysis. The whole analysis requires only 1.5 ml serum.

RÉSUMÉ

Nous avons analysé des protéines sériques humaines en vue de la détermination de leur teneur en tyrosine, tryptophane, cystine, arginine et histidine. 20 analyses complètes d'hommes sains accusaient une stabilité dans la composition des protéines sériques. Parmi 50 cas pathologiques, nous n'avons trouvé que 8 cas seulement présentant une composition anormale. Nos recherches

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prouvent qu'il est possible de trouver par l'analyse de 1.5 ml de sérum des protéides sériques différant de la composition normale. On les nomme d'après APITZ, "paraprotéines".

ZUSAMMENFASSUNG

Wir untersuchten den Gehalt an Tyrosin, Tryptophan, Cystin, Arginin und Histidin der Serumeiweisskörper, indem wir zuerst 20 normale Sera von 5 gesunden Männern viermal, in wöchentlichen Abständen analysierten. Die Zusammensetzung erwies sich als normal. Unter den weiterhin untersuchten 50 Sera, die von verschiedenen Kranken stammten, fanden wir bloss 8 (von 5 Kranken herstammend), deren Zusammensetzung als pathologisch angesehen werden kann. Die Analysen weisen darauf hin, dass es möglich ist, durch die sog. Bausteinanalyse in einigen Fällen Serumeiweisskörper pathologischer Zusammensetzung nachzuweisen, die, nach APITZ, Paraproteine genannt werden. Die Bestimmung der 5 Aminosäuren geschah insgesamt in 1.5 ml Serum.

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