CXVII. INVESTIGATIONS ON THE POLYPEPTIDE-CONTENT OF THE SERUM

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EXISTING methods for determination of the polypeptide content of serum are mainly based on two different principles. According to the first the protein is removed and estimations are made with the protein-free filtrates. It is thus essential that the protein precipitant should not precipitate any polypeptides. In such a protein-free filtrate the amino-nitrogen may be determined before and after hydrolysis, the difference is taken as an index for the polypeptide content [Hiller & Van Slyke, 1922; Becher & Herrmann, 1931, 1, 2; 1932, 1, 2; Martens 1930].

In the second type of method two different protein-free filtrates of the serum are made, of which the first contains the polypeptides whereas the second filtrate does not. The difference in some chemical constituent is taken as an index of the polypeptide content, e.g. the nitrogen content [Hahn, 1921; Wolff, 1921; Puech & Cristol, 1929] or the tyrosine content [Goiffon & Spaey, 1934].

Both principles have been strongly criticized. It is the object of this paper to demonstrate that under the correct circumstances reliable results may be obtained with the aid of a method based on the second.

Choice of method

The method used was that of Goiffon & Spaey, the proteins being first removed from the serum with the aid of two different protein precipitants.

The first filtrate is obtained by mixing 1 vol. serum with 9 vol. 3% trichloroacetic acid; after standing for at least 15 min. the solution is filtered. It will be shown that this solution still contains the polypeptides. The second filtrate is obtained by mixing 1 vol. serum with 9 vol. of a solution containing phosphotungstic acid (22 g.) and *N* HCl (30 ml.) in 1 l.; again after at least 15 min. the solution is filtered. This time the filtrate will not contain the polypeptides. Hence the filtrates will have different nitrogen and tyrosine contents, and the difference between the nitrogen content of the trichloroacetic acid filtrate (TN) and that of the phosphotungstic acid filtrate (PWN) will correspond to the nitrogen content of the polypeptides, the so-called "double nitrogen" (DN). 5 ml. of each filtrate are treated with two drops of the phenol reagent of Folin and Denis¹ and 4 ml. of 10% Na_2CO_3 ; after 30–60 min. one drop of saturated aqueous Na_2SO_3 is added and the volume made up with water to 10 ml. Comparison is made with 5 ml. of a standard solution of tyrosine, containing 0.01 g. tyrosine in 1 l. of 0.1 *N* HCl similarly treated. From the difference in colour the

¹ This reagent was prepared by dissolving 25 g. of sodium molybdate and 100 g. of sodium tungstate in 700 ml. of water; 50 ml. of 85% phosphoric acid and 100 ml. of 38% hydrochloric acid were added; after boiling for 8 hr. under reflux the solution was cooled and the volume adjusted to 1 l.

tyrosine index may be calculated which indicates the number of mg. of tyrosine contained in the polypeptides of 11. of serum. In order to prove that the double nitrogen and the tyrosine index really do depend on the presence of polypeptides, a peptidase (erepsin) was added to a number of sera; by this means the values of the double nitrogen and of the tyrosine index were reduced almost to zero. Care had to be taken that the erepsin preparation was free from proteases.

In the first experiments a commercial erepsin was used which proved useless since the preparation itself gave a strong colour with the reagents used for the determination of the tyrosine index; moreover, a few hours after its addition the serum proteins began to be broken down. The commercial preparation itself contained nitrogen compounds which were not precipitated by trichloroacetic acid, but were precipitated by phosphotungstic acid.

Erepsin was therefore prepared according to Waldschmidt-Leitz & Schöffner [1926]. 100 g. of the mucosa of the small intestine of the pig were ground up with 500 ml. of 87% glycerol. For each experiment 10–15 ml. of this suspension were mixed with 3 vol. water and centrifuged. The supernatant fluid, containing the erepsin, was precipitated with about 2 ml. 0.1 *N* acetic acid. The tube was inverted in order to remove the fluid as completely as possible. The precipitate was mixed with the serum, of which TN, PWN, DN and the tyrosine-index (TI) had just been estimated. The precipitate soon settled down. Estimations of DN and TI were carried out immediately and after several hours at 37° [Godfried, 1938]. Table I shows some of the results.

Table I

Exp. no. ...	5			6			7			8		
	Serum + erepsin			Serum + erepsin			Serum + erepsin			Serum + erepsin		
	Serum	At once	After 18 hr.	Serum	At once	After 10 hr.	Serum	At once	After 10 hr.	Serum	At once	After 10 hr.
TN	51.2	50.7	50.1	56.3	56.3	56.3	45.1	45.6	46.8	59.1	59.2	59.9
PWN	30.0	30.0	50.1	50.7	50.2	55.8	38.1	37.6	46.2	57.2	57.3	59.9
DN	21.2	20.7	0	5.6	6.1	0.5	7.0	8.0	0.6	1.9	1.9	0
TI	38	36	2	18	18	2	18	18	0	—	—	—

Exp. no. ...	9			10				11		
	Serum + erepsin			Serum + erepsin				Serum + erepsin		
	Serum	At once	After 3 hr.	Serum	At once	After 6 hr.	After 30 hr.	Serum	At once	After 5 hr.
TN	18.5	18.6	18.5	143.1	142.0	143.1	142.5	31.1	36.7	37.5
PWN	13.2	13.4	18.5	116.8	116.8	124.3	140.6	24.9	30.5	36.1
DN	5.3	5.2	0	26.3	25.2	18.8	1.9	6.2	6.2	1.4
TI	22	22	0	46	46	32	4	26	26	0

TN = trichloroacetic acid filtrate-N; PWN = phosphotungstic acid filtrate-N; DN = polypeptide-N all in mg./100 ml.; TI = tyrosine index.

From these experiments the conclusion may be drawn that the double nitrogen and the tyrosine index correspond to polypeptides which are broken down by erepsin.

In a further experiment glycyl-*L*-tyrosine and *D*-leucylglycine were added to serum; the nitrogen of these compounds could be recovered quantitatively in the trichloroacetic acid filtrate, but was absent from the phosphotungstic acid filtrate.

The results obtained by the tyrosine method did not always run parallel with those obtained by the nitrogen estimations, presumably owing to the variability of the tyrosine and nitrogen contents of different polypeptides.

Results

According to the tyrosine method the normal value of the polypeptide content of the serum is less than 25 (as mg. of tyrosine per litre of serum); according to the nitrogen method, described above, it is less than 7 mg./100 ml.

Conditions in which the serum content of polypeptides is increased may be divided into three groups. Some of the results are shown in Table II. For more detailed figures the reader is referred to the author's thesis [Godfried, 1938].

The first group contains those cases in which there is increased parenteral destruction of proteins. In gastric and duodenal ulcer hyperpolypeptidaemia has been reported [Marino & Saladino, 1937]; in the writer's experience such hyperpolypeptidaemia was only found shortly after a haemorrhage. Suppuration may be accompanied by hyperpolypeptidaemia [Hülse & Strauss, 1924]. This has been confirmed in the present work and attention is drawn to the increased content of polypeptides in ulcerative colitis. When the condition of a patient suffering from ulcerative colitis improves, the hyperpolypeptidaemia becomes less marked (Table II, no. V¹). In leukaemia, especially when the patient has been treated with X-rays, hyperpolypeptidaemia may be marked. In malignant new growth, chiefly when extensive metastases exist, very high indices for the polypeptide content may be found. The highest values are reached when metastases have formed in the liver. Among diseases of metabolism the presence of hyperpolypeptidaemia in severe diabetes [Puech, 1926, 1, 2] has been confirmed. In several cases of sprue and of gout an increase in serum polypeptides was also found.

The second group comprises diseases of the liver. It is well known, that under normal conditions polypeptides are broken down to urea or synthesized into proteins in the liver [Fiessinger, 1934]. In cirrhosis of the liver hyperpolypeptidaemia may or may not be found [Labbé & Nepveux, 1931; Becher & Herrmann, 1931, 1, 2; 1932, 1, 2; Valdigué, 1934; Bentz & Larizza, 1937]. Reference has already been made to the association of malignancy involving metastases in the liver with a high polypeptide content of the serum; the estimation of the degree of the polypeptidaemia in jaundiced patients may indeed be of great value, especially to assess the indication for cholecystogastrostomy in cases of a carcinoma of the head of the pancreas. If such a carcinoma is still small, it may nevertheless cause jaundice; in such cases cholecystogastrostomy may enable the bile to flow away from the liver along the intrahepatic bile passages. If, however, large metastases have formed in the liver, these will obstruct the intrahepatic bile passages and cholecystogastrostomy will not cause the disappearance of the jaundice. If hyperpolypeptidaemia is found in cases of carcinoma of the pancreas, the presence of large metastases is very probable and cholecystogastrostomy will remain without any effect. Cases IX³² and IX³³ demonstrate this clearly; in both patients, suffering from a carcinoma of the head of the pancreas and of the bile passages respectively, marked jaundice and hyperpolypeptidaemia were present; in both cholecystogastrostomy was performed, but in both it remained without any result. However, even if metastases in the liver exist in a jaundiced patient suffering from a carcinoma of the head of the pancreas, the polypeptide content of the serum may still be low (compare case IX³⁰).

The third group is formed by diseases of the kidneys [Pribram & Klein, 1923; Becher & Herrmann, 1931, 1, 2; 1932, 1, 2; Puech & Cristol, 1929; Martens, 1928; Larizza, 1937]. High blood pressure alone is not accompanied by a hyperpolypeptidaemia; acute and chronic nephritis, however, are; apparently the diseased kidney does not excrete sufficient polypeptides. No parallelism exists between the polypeptide content and the non-protein-nitrogen. The polypeptide content of the serum in a patient suffering from chronic nephritis does not give any prognosis of the duration of life, nor does any relation exist between the polypeptide content and the usual kidney function tests (phenosulphophthalein-test, urea clearance etc.). The raised values in cases of cardiac decompensation may be considered to be caused by disturbance of the functions of the liver and of the kidneys.

Table II. *Polypeptide content of serum in various diseases*

No.	Sex	Age	Diagnosis	TI	TN	PWN	DN
III ²	♂	39	Duodenal ulcer	42	—	—	—*
				22	—	—	—†
III ⁸	♂	35	Haemorrhage from a gastric ulcer	52	—	—	—
IV ²	♂	69	Pneumonia	50	—	—	—
IV ⁶	♂	30	Bronchiectasis	54	—	—	—
IV ⁸	♂	24	Abscess of the lung	62	32.5	9.8	22.7
V ¹	♀	28	Ulcerative colitis	70	—	—	—
				30	35.6	28.8	6.8‡
V ²	♀	26	Do.	42	—	—	—
V ³	♀	11	Do.	104	—	—	—
V ⁹	♀	23	Do.	46	22.4	11.5	10.9
V ¹²	♀	35	Do.	44	71.4	61.3	10.1
V ¹³	♀	51	Do.	44	33.0	16.2	16.8
VI ²	♀	49	Lymphatic leukaemia	34	63.6	54.0	9.6
VI ⁵	♀	50	Do.	86	—	—	—§
VII ⁴	♀	64	Tumour of the lung	128	—	—	—
VII ⁸	♀	59	Metastases of a carcinoma of the chest	36	29.7	18.7	11.0
VIII ¹	♀	47	Diabetes	80	—	—	—
VIII ²	♀	40	Do.	88	—	—	—
VIII ¹²	♀	53	Gout	80	—	—	—
VIII ⁶	♀	53	Sprue	68	—	—	—
VIII ⁷	♀	65	Do.	40	30.5	20.4	10.1
VIII ⁹	♀	51	Do.	—	51.8	30.0	21.1
IX ²	♀	57	Cirrhosis of the liver	100	—	—	—
IX ⁹	♀	67	Cirrhosis of the liver; gall stones	44	23.0	11.8	11.2
IX ¹¹	♀	26	Do.	4	24.9	23.2	1.7
IX ¹²	♀	53	Do.	10	27.4	25.8	1.6
IX ²⁴	♀	40	Gall stones; abscess of the liver	56	—	—	—
IX ²⁶	♀	52	Metastases of a carcinoma of the chest	120	—	—	—
IX ³⁰	♀	48	Carcinoma of the head of the pancreas; metastases in the liver	30	—	—	—
IX ³¹	♀	52	Do.	124	—	—	—
IX ³²	♀	59	Do.	80	—	—	—
IX ³³	♀	60	Carcinoma of the bile passages	138	—	—	—
X ³	♀	46	Hypertension	16	26.6	22.4	4.2
X ⁵	♀	54	Do.	26	31.1	24.9	6.2
XI ⁸	♀	40	Acute nephritis	56	—	—	—
XI ¹⁰	♀	17	Do.	40	31.1	21.8	9.3
XI ²⁴	♀	40	Chronic nephritis	50	160.4	144.7	15.7
XI ²⁶	♀	36	Do.	26	48.7	41.4	7.3
XI ³³	♀	60	Do.	38	21.6	9.5	12.1
XI ³⁴	♀	21	Do.	40	43.7	30.2	13.5
XI ³⁵	♀	25	Do.	46	143.1	116.8	26.3
XIII ¹²	♀	46	Cardiac decompensation	78	73.9	53.7	20.2
XIII ³	♀	30	Do.	88	—	—	—

* Immediately after a haemorrhage.

† 18 days afterwards.

‡ A year after the first estimation.

§ Treated with X-rays.

|| See text.

SUMMARY

It has been shown that the differences in nitrogen content or in tyrosine content of two filtrates of the same serum, obtained after the addition of 3% trichloroacetic acid and phosphotungstic acid respectively, correspond to the polypeptides of the serum. Hence these differences form a reliable index of the polypeptide content of the serum. The circumstances where the serum contains excessive amounts of polypeptides are discussed.

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