IMMUNOCHEMICAL INVESTIGATION OF THORACIC DUCT LYMPH IN RATS

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The relative content of β globulins in the thoracic duct lymph of August rats is higher while that of albumin is lower than in the blood serum. During drainage of lymph from the body, prealbumins and α globulins disappear from the lymph sooner than other proteins. The antigenic spectrum of blood serum is richer than that of lymph.

In the study of the metabolism and circulation of serum proteins in the body, comparison of the proteins of the blood and lymph is important. In its content of salts and other low-molecular-weight compounds, lymph differs very little from blood plasma [3], but its spectrum and relative content of proteins show several features which distinguish it from blood serum [4]. During the investigation of such complex multicomponent systems of proteins as blood serum and lymph, the method of immunochemical analysis is particularly useful, for it combines the high sensitivity of immunological procedures with preparative biochemical fractionation of the components.

In the investigation described below, proteins of thoracic duct lymph and blood serum of animals were compared by immunochemical and enzyme-electrophoretic methods.

EXPERIMENTAL METHOD

August rats weighing 180-200 g were used. The thoracic duct was drained by Bollman's method [5], and the animals were anesthetized with chloral hydrate (0.3 g/kg body weight). After the operation the animals were fixed in frames [6] and lymph was collected in separate flasks. Portions of lymph were collected during the first 3 h (A), from 3 to 6 h (B), from 6 to 18 h (C), and from 18 to 24 h (D) after drainage. The number of lymphocytes in the samples was counted (in a Goryaev's chamber), and after sedimentation of the cells (15 min, 150 g) the protein content in the supernatant was estimated [7]. The supernatant was subjected to electrophoresis in 1% agar (Difco) gel in a voltage gradient of 9 V/cm, separation being carried out along the shorter parameter on plates measuring 13 × 18 cm. The electrolyte used during fractionation was medinal-HCl buffer, pH 8.2, μ 0.05. After electrophoresis the plates were stained for proteins, glycoproteins, and lipoproteins [8], after which densitometry was carried out on a Zeiss (Jena) Eri-10 extinctiometer. To detect fractions of certain enzymes (esterases and alkaline phosphatases), the lymph was subjected to enzyme-electrophoresis in 1.5% agar gel, under conditions described previously [2]. The substrate in this case was β-naphthyl acetate or β-naphthyl phosphate, respectively. The electrophoretic mobility of the individual fractions of proteins and enzymes was determined relative to that of human serum albumin (Cyclo Chemical Corporation).

The conditions of electrophoretic fractionation of the lymph proteins in the immunoelectrophoretic experiments were the same as those described above. By special staining of the plates after immunoelectrophoresis, precipitation lines formed by glycoproteins, lipoproteins, haptoglobin, ceruloplasmin, and esterases were discovered. The plates were developed by rabbit antisera against rat blood and lymph

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TABLE 1. Relative Percentage, Relative Electrophoretic Mobility, and Concentration of Protein and Number of Cells in Different Samples of Lymph and Blood Serum

	Fraction	Blood serum		Thoracic duct lymph							
				sample A		sample B		sample C		sample D	
		1	2	1	2	1	2	1	2	1	2
Proteins	Prealbumins	1,17*	4,9±0,45	1,19	2,6±0,74	1,10	2,7±1,16	—	—	—	—
	Albumin and α_1-globulin	0,90 1,07	65,5±0,82	0,99	57,6±2,1	0,86	58,0±3,23	0,88	61,0±4,41	0,99	64,3±4,81
	α_2-globulins	0,65	2,7±0,40	0,73	3,8±1,30	—	—	—	—	—	—
	β_1-globulins	0,57	2,46±0,20	0,55	11,0±1,52	0,57	12,3±1,69	0,57	12,0±0,58	0,58	18,0±5,9
	β_2-globulins	0,41	15,5±0,76	0,40	16,6±1,21	0,43	18,0±1,86	0,43	19,6±3,2	0,42	12,6±2,69
	γ-globulins	0,18	9,1±0,72	0,21	7,4±0,65	0,27	8,9±0,94	0,25	7,6±1,69	0,24	5,0±1,54
	Total protein concentration, mg/ml	76,40±0,1		26,0±1,4		19,0±1,1		15,5±1,9		8,3±3,3	
Lipoproteins	Prealbumins	1,17	28,0±1,01	1,20	16,0±2,5	—	—	—	—	—	—
	α_1-globulins	— 0,96	— 38,59±1,19	1,11 1,01	13,0±2,0 26,0±5,4	1,10 0,93	16,0±4,0 48,0±1,2	— —	— —	— —	— —
	α_2-globulins	— 0,70	— 32,7±1,40	0,86	46,0±9,0	0,84	36,0±1,4	0,73	100	0,80	100
	Number of lymphocytes per mm^3 lymph			21 592±659		15 223±966		II 300±656		10 580±620	

*$\pm m$ for values of relative electrophoretic mobility did not exceed 0.01.

Note. 1) Negative electrophoretic mobility, 2) relative percentage.

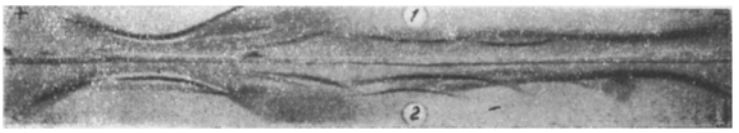

Fig. 1. Immunoelectrophoresis of rat lymph proteins. Lymph from sample D, with protein content 8 mg/ml (1), and same sample after a threefold increase in its protein content (2) used as antigen. Rabbit antiserum against rat lymph proteins used as developer.

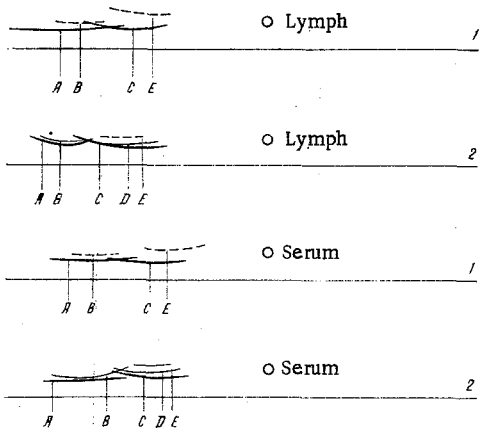

Fig. 2. Immunoelectrophoresis of esterases of thoracic duct lymph and blood serum of rats: 1) antiserum against lymph proteins; 2) antiserum against blood serum proteins.

proteins. To obtain the antisera, the rabbits were immunized by schemes published previously, with rat lymph and serum proteins [1]. To detect differences in the antigen spectrum of the lymph and blood serum, cross-exhausted antisera were used. Exhaustion was carried out by the repeated addition of lyophilically dried preparations of the corresponding proteins to the antisera, under the control of results of the ring-precipitation, agar-diffusion, and immunoelectrophoresis tests.

RESULTS

During electrophoresis the lymph proteins, like rat serum proteins, were separated into 6 fractions of different electrophoretic mobility, consisting of prealbumin, albumin, and α_1, α_2, β, and γ globulins. Except for the albumin fraction, the electrophoretic mobility of all lymph protein fractions was the same as the mobility of the corresponding rat blood serum fractions (Table 1). The lymph albumins moved more rapidly than rat blood serum albumins, and their electrophoretic mobility was approximately the same as that of human serum albumin. Lipoproteins in the lymph were represented by four, and in the blood serum by three fractions, the fourth fraction appearing only inconstantly after electrophoresis of blood serum. The electrophoretic mobility of lymph and serum lipoproteins differed slightly. Unlike those of the blood serum, the glycoproteins of lymph consisted of only one fraction, discovered in all lymph samples. Compared with serum, the lymph contained more α and β globulins and slightly less albumin (Table 1). In the course of removal of the lymph by drainage, the content both of cells and of total protein in the lymph decreased. The concentration of lymphocytes fell sharply during the first 6 h after drainage, and later fell more slowly. Meanwhile the total protein content in the lymph fell particularly sharply in the first and last stages of lymph drainage, whereas lymph samples B and C showed no significant difference in their protein level. In the last stage of drainage of lymph (sample D) the protein concentration in the lymph fell more rapdily than the number of cells, so that at this time the cell count was reduced by half and the protein concentration by two-thirds. These differences may be attributable either to differences in the character of lymph drainage from different organs and also to differences in the degree of participation of the abdominal organs of animals in protein biosynthesis and in formation of cells in rat lymph and serum.

Comparison of the protein profile for the lymph collected at different times showed that the concentration of α globulins of prealbumins fell more rapidly than the rest in the lymph. For instance α globulins could not be detected in lymph of the second sample (sample B), or prealbumins in the third and fourth samples of lymph. As a result of this, there was a relative increase in the percentage of albumin and β globulins in the protein profiles. The decrease in the content of prealbumins and α globulins in the lymph was not due to the decrease in total lymph proteins taking place during drainage of lymph. Even in the concentrated lymph samples collected in the last periods of drainage, prealbumins and some components of α globulins could not in general be detected immunochemically or they showed up only as weak, blurred precipitation arcs, whereas the other antigens (albumin, β globulin, transferrin) showed up quite clearly in these same tests (Fig. 1).

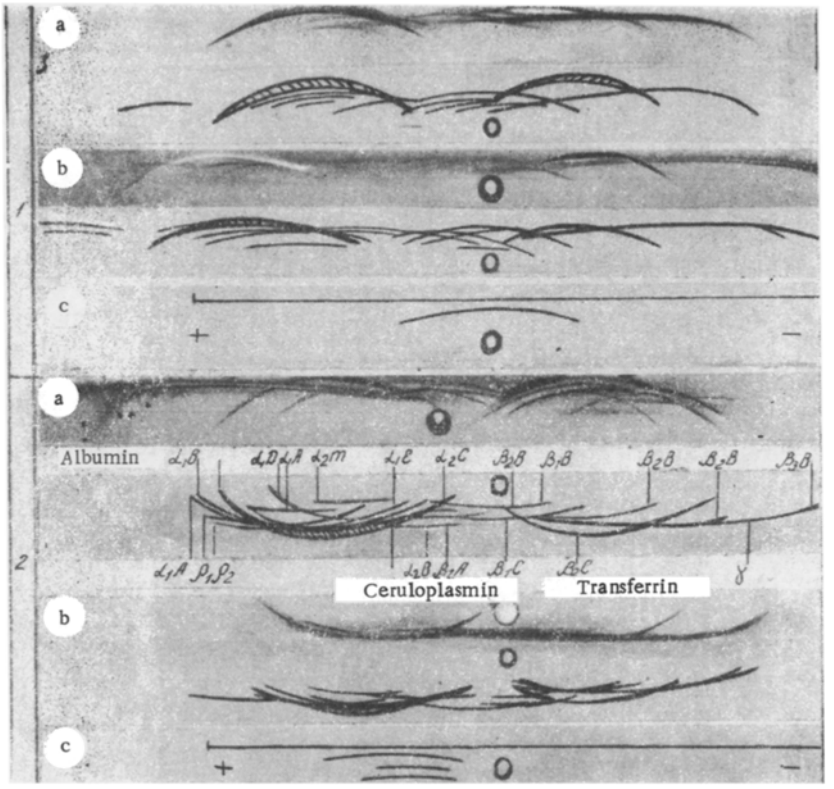

Fig. 3. Immunoelectrophoresis of proteins from thoracic duct lymph and blood serum of rats. Lymph proteins (1) and blood serum proteins (2) used as antigens. The following were used as antiserum: rabbit antiserum against proteins of rat thoracic duct lymph, either unexhausted (1a, b) or exhausted by blood serum proteins (1c); rabbit antiserum against rat blood serum proteins, either unexhausted (2a, b) or exhausted (2c) with thoracic duct lymph proteins.

During enzyme-electrophoresis, one fraction of alkaline phosphatase migrating with the mobility of serum β globulins and two fractions of nonspecific esterases, located in the zone of serum prealbumins and α_1 globulins, were discovered in the lymph. Immuno-enzyme-electrophoresis revealed up to 4 components among these esterases (Fig. 2). In corresponding experiments, two isoenzymes of alkaline phosphatase (zone of α_2 and β globulins) and five fractions with esterase activity, the anodic components of which, just as the lymph, were located in the zone of prealbumins and α globulins, were detected in the rat blood serum. The content of one of the serum esterases in the lymph was very low, so that this antigen could not be detected by immunoelectrophoresis of antilymph serum, and the antiserum distinguished it in the lymph only as a very weak precipitation arc. "Fast" esterases of lymph and serum (A and B) were not distinguished immunologically, but during electrophoresis of the lymph these enzymes migrated more rapidly than when the rat blood serum was tested.

During immunoelectrophoresis (Fig. 3), the homologous serum distinguished up to 20 precipitation arcs in lymph, 19 of them also detectable by antiserum, whereas one of the lymph β globulins could be detected only by means of the antilymph serum. In immunoelectrophoresis of lymph and blood serum, an equal number of antigens (three components) was found in the zone of α_2 globulins, but the corresponding sera evidently detected different antigens there. Cross-exhausted antisera against rat lymph and serum distinguished one antigen ("its own") in the corresponding fluids, migrating with the mobility of α_2 globulins. Antiserum, exhausted with lymph, detected a further two weak precipitation lines in the zone of α_2 globulin, formed by serum lipoproteins and staining with Sudan black. The presence of these "individual" antigens in the blood serum of animals may be connected with the arrival of certain serum proteins directly from their place of synthesis into the blood stream. Comparison of the results of electrophoresis and immunoelectrophoresis of the lymph and blood serum proteins showed that albumin and prealbumins of lymph migrate during electrophoresis later than those of blood serum. The precipitation lines of these antigens in lymph preparation lie much closer to the anode than in preparations of blood serum (Fig. 3). Albumin, of course, readily froms complexes with various compounds and participates in their transport, so that

differences in the rate of migration of this protein in lymph and serum may be connected with differences in the degree of its "loading" with transportable compounds.

LITERATURE CITED

1. K. P. Kashkin, P. N. Grabar, and D. Courcon, Biokhimiya, No. 1, 89 (1963).
2. K. P. Kashkin, A. N. Sharetskii, B. P. Surnov, et al., Vopr. Med. Khimii, No. 3, 235 (1969).
3. I. Rusznyak, M. Földi, and D. Szabo, Physiology and Pathology of the Lymphatic Circulation [in Russian], Budapest (1957).
4. H. R. Bierman, R. Z. Byron, and K. H. Kelly, J. Clin. Invest., 32, 637 (1953).
5. J. L. Bollman, J. C. Cain, and J. H. Grindley, J. Lab. Clin. Med., 33, 1349 (1948).
6. J. L. Bollman, J. Lab. Clin. Med., 33, 1348 (1948).
7. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
8. J. Uriel, in: P. Grabar and P. Burtin: Analyse Immuno-Electrophorétique, Ses Applications aux Liquides Biologiques Humanis, Paris (1960), p. 33.