

## ON THE IMMUNOLOGICAL CHARACTERISTICS OF SERUM PROTEINS

### COMMUNICATION II. DETERMINATION OF THE CONTENT OF PROTEIN FRACTIONS IN THE GLOBULIN COMPOSITION IMMUNOLOGICALLY SIMILAR TO ALBUMINS

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In a previous report [6] we showed that when the  $\alpha$ -globulins were isolated by the use of starch block electrophoresis, protein fractions migrated with them which were similar to the albumins in their anaphylactogenic properties. S. Ya. Kaplanskii and co-workers [4] have pointed out the impossibility of obtaining pure antisera to the  $\alpha$ -globulins because such antisera yield cross-over serological reactions with the albumins. Using the method of immunoelectrophoresis, traces of albumins were even demonstrated in the composition of purified  $\gamma$ -globulin preparations [3]. These data support the theory of immunological non-uniformity in the individual electrophoretically pure fractions of the serum proteins.

We considered it of interest to determine whether the cross-over serological reactions were a result of inadequate homogeneity of the  $\alpha$ -globulins migrating along the path of the albumins in electrophoresis. It is known that the inert carriers used for preparative electrophoresis possess, to a varying degree, a non-specific protein adsorption pattern. However, the possibility is not excluded that a given percent of the  $\alpha$ -globulins, possessing a certain similarity to the albumins in their physico-chemical properties [8], are also similar to the latter in their antigenic properties.

#### METHOD

One group of rabbits was immunized with electrophoretically pure fractions of human serum albumins, isolated by starch block electrophoresis. For the second group we used whole horse serum. The usual scheme of immunization was used on the animals [7]. With the antialbumin sera obtained we carried out a subsequent series of precipitation reactions, adding increasing concentrations of isolated protein fractions from human serum. The degree to which the isolated fractions of the globulins were immunologically similar to the albumins was determined in the ascending portion and in the optimal zone of the precipitation curve constructed for the albumins [10]. The content of antibodies against each of the isolated protein fractions from the horse sera was established in the equivalent zone of the precipitation curve, since in that zone the precipitate contains approximately 10% of the antigen [2].

The concentration of protein in the solutions and in the precipitate was determined by the method of Lowry [9] in the records of A. E. Gurvich and R. B. Kapner [2]. Calibrated curves were separately constructed for crystalline albumin and the  $\gamma$ -globulins. Micro-electrophoresis of the serum was carried out in a Flynn and De Maio type chamber modified by A. E. Gurvich [1].

#### RESULTS

The antialbumin sera obtained from the rabbits, both after initial immunization and after numerous reimmunizations, invariably yielded cross precipitation reactions with the isolated  $\alpha$ -globulin fractions. As can be seen from Table 1, from 10 to 30% of the proteins similar to the albumins in immunological properties migrated in the composition of the  $\alpha$ -globulins. It should be noted that in these experiments we used only those fractions of the globulins which did not show observable contamination with proteins of albumin mobility in paper electrophoresis. As a control we used concentrated solutions of globulins (7-10 mg/ml), so as to disclose possible albumin contami-

TABLE 1. Concentration of Protein Fractions Immunologically Similar to Albumins in the Globulins (Average of 3 analyses, performed May 4-10th, 1960, rabbit No. 30)

Rabbit antiserum to human albumin		Antigen			Precipitate	Concentration of protein immunologically similar to albumins (in percent of the total amount of protein in the given fraction)
Concentration of antibodies (in mg/ml)	Volume (in ml)	Globulin fraction of human serum	Concentration (in mg/ml)	Volume (in ml)	Protein concentration (in mg/ml)	
3.10	0.15	$\alpha_1$	0.45	0.15	1.05	33.3
3.10	0.15	$\alpha_2$	0.75	0.15	1.39	24.0
3.10	0.15	$\alpha_3$	0.45	0.15	0.58	12.2
3.10	0.15	$\beta$	0.90	0.15	0.30	2.7
3.10	0.15	$\gamma$	0.80	0.15	0.12	1.2

TABLE 2. Concentration of Precipitins to the Individual Protein Fractions Subsequent to Immunization of the Rabbits with Whole Serum (Rabbit No. 13, performed October 14-15th, 1959, average of 3 analyses)

	Horse serum-antigen			Antiserum	Precipitate	
Serum fractions	Concentra- tion (in %)	For precipitation				
		Protein con- centration (in mg/ml)	Volume (in ml)	Volume (in ml)	Protein con- centration (in mg/ml)	Concentration of antibodies (in mg/ml)
Albumins	42.3	0.30	0.15	0.15	0.48	0.43
Globulins:						
$\alpha$	14.8	0.70	0.15	0.15	1.60	1.44
$\beta$	18.3	0.70	0.15	0.15	2.05	1.85
$\gamma$	24.6	0.80	0.15	0.15	1.95	1.76

nants, found by the immunochemical method.

Gamma-globulins drawn by an endosmotic current to the cathode from the site of application of the serum produced an extremely small precipitation reaction with the antialbumin sera. Various samples of the  $\gamma$ -globulins contained approximately 1% of proteins immunologically similar to the albumins. A somewhat higher percent concentration of these proteins was seen in the composition of the  $\beta$ -globulins (an average of 2.5%), although in certain experiments we set up conditions of starch electrophoresis where the  $\beta$ -globulins migrated to the cathode from the site of application of the serum. In this manner it was possible to exclude the influence of the "trailing effect" of the albumins.

The question arises as to why the antisera to the  $\beta$ - and  $\gamma$ -globulins do not yield cross precipitation reactions with the albumins, since these preparations contain minimal amounts of protein which is immunologically similar to the albumins. The purity of these antisera was ascertained several times by S. Ya. Kaplanskii and co-workers. Apparently, when the rabbits are immunized with two or more antigens, the immunological reaction of the organism is dependent upon the strength and concurrent proportion of the different antigens. As can be seen from Table 2, with immunization of the rabbits, using normal horse serum in which the concentration of the albumins and globulins is approximately the same, the production of precipitins against each protein fraction was different. In this case the amount of antibodies to the albumins was always 3-4 times lower than to each of the globulin fractions individually. Naturally, the minimal amount of protein found in the composition of the  $\beta$  and  $\gamma$ -globulins which is immunologically similar to the albumins does not cause a marked production of antibodies to the albumins.

The results obtained by us might be of interest in explaining the mechanism of the combined reactions of serum albumins and globulins in a whole series of diseases. Despite the different pathogenesis of many illnesses (excluding cirrhosis of the liver, multiple myeloma and certain other illnesses), a uniform reaction may be noted

as regards the fractional composition of the serum proteins: hypoalbuminemia with simultaneous increase in the concentration of the  $\alpha$ -globulins, and sometimes also the  $\beta$ -globulins. Wouldn't it appear that this increase in the concentration of the globulins is a result of an increase in the production of that protein fraction which is immunologically similar to the albumins? One encounters evidence in the literature that restoration of the plasma proteins following heavy blood losses in dogs is accompanied by a rapid increase in the concentration of the  $\alpha_3$ -globulins, entering into the blood circulation in proportion to the albumin deficit [5]. In this case the  $\alpha_3$ -globulins appear to be albumin substitutes [8].

#### SUMMARY

Isolation of individual fractions of serum proteins was carried out by means of starch block electrophoresis. As shown by the immunochemical quantitative method, a fraction of proteins immunologically similar to albumins is present in the globulin composition. It averaged 30% in the  $\alpha_1$ -globulins, 22% in the  $\alpha_2$ -globulins, 10% in  $\alpha_3$ -globulins, 2.5% in the  $\beta$ -globulins, and 1% in the  $\gamma$ -globulins.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. Some or all of this periodical literature may well be available in English translation. A complete list of the cover-to-cover English translations appears at the back of this issue.

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