ELECTROPHORETIC ANALYSIS OF THE COMPOSITION OF THE SPECIFIC PRECIPITATE

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In numerous investigations by Heidelberger and his co-workers, the immunological specific precipitation reactions have been studied in detail [8, 9]. At the present time, some workers [4, 5] when calculating the content of antibodies in the serum, adopt the view of Kabat and Heidelberger [9], that in an equivalent albumin–antibody zone, the precipitate is composed of 90% antibody protein and 10% antigen.

V. S. Gostev and his co-workers [1, 2] who investigated the composition of the supernatant fluid after centrifugation of a specific agglutinate, found that not only had the specific proteins disappeared, but that the nonspecific proteins also were diminished. On the basis of these experiments, these authors concluded that nonspecific proteins, as well as the antigen–antibody complex, enter into the composition of the agglutinate.

The aim of the present research was, on the one hand, to determine the fractional composition of the proteins of an immune serum before and after precipitation, and on the other hand, to determine the composition of the specific precipitate by the method of microelectrophoresis on paper.

METHOD

Rabbits were immunized with solutions of pure protein fractions of normal horse serum according to a fixed program [4]. For preliminary isolation of the protein fractions, the horse serum was fractionated in a starch block (1 × 13 × 26 cm) by cold electrophoresis. The conditions of electrophoresis were: 450 v, 10 ma, duration 48 hr, veronal medinal buffer, pH = 8.6, ionic strength 0.1 μ. The purity of the fractions obtained was checked by means of paper electrophoresis.

Paper microelectrophoresis was carried out in an apparatus of the Flynn and De Mayo type, as modified by A. E. Gurvich [3]. We used B (rapidly absorptive) chromatographic paper from the Leningrad Volodarskii factory The conditions of microelectrophoresis were: potential gradient 10 v/cm, current up to 1 ma to a strip 4 × 40 cm, duration 4-8 hrm veronal medinal buffer, pH = 8.6. The electrophoregrams were stained with mercuric chloride solution of bromphenol blue of the ordinary formula; excess stain was washed out with a 2% solution of acetic acid.

The precipitating antibodies in the individual protein fractions of the immune serum were estimated by A. E. Gurvich's electrophoresis-precipitate method [4]. The precipitation reactions were performed in an expanded series, with solutions of antigen of increasing concentration, antigen solution being added to the immune serum. This order of mixing the ingredients led to a much more abundant precipitate [7]. After incubation for 30 minutes at 37°, the tubes were kept in the refrigerator for 24 hours. Electrophoretic analysis of the supernatant fluid and precipitate was performed in the equivalent zone. The precipitate was dissolved in solutions of NaOH, HCl, and urea of different concentrations, with excess of antigen.

The concentration of protein in solution and the protein content of the precipitate were estimated by Lowry's method [10]. The relative content of the protein fractions was calculated from the results of extinction (FEK-M) by the eluted dye from the electrophoregrams, cut up into fractions.

RESULTS

The table shows the summarized results obtained in 4 experiments in which the precipitation reaction was carried out with various antigens – the separate fractions of the serum proteins.

It will be seen from the figures in the table that, after precipitation, it is not only the content of γ-globulins, which include the antibodies, in the serum that was diminished, but also the other protein fractions. During precipitation with globulin antibodies, for instance different quantities of the protein fractions of the immune serum passed into the precipitate: from 46 to 51% (of the total weight of the precipitate) of γ-globulins, from 27 to 33% of albumins, and from 18 to 20% of α- and β-globulins. After careful washing of the precipitate in physiological saline and water, however, albumins could no longer be found in it; the content of α- and β-globulins was

Fractional Composition of the Specific Precipitate Obtained with Various Antigens—the Protein Fractions of Serum

Protein antigen of the blood serum			Antirabbit serum			Precipitate				
				Content of protein (in mg/ml)		quantity of protein calculated per ml of serum	relative content of protein fractions before and after washing with physiological saline			
fraction	content (in mg/ml)	volume (in ml)	volume (in ml)	before precipitation	after precipitation		albumin	globulin		
								α	β	γ
Albumins	0,50	0,15	0,15	31,0*	28,2*	2,8 2,2**	41,1 0	13,1 8,2	13,2 10,1	32,6 81,7
Globulins										
α	0,45	0,15	0,15	34,8	30,3	4,5 3,6	33,0 0	9,3 6,2	9,7 9,1	48,0 84,7
β	0,60	0,15	0,15	37,0	31,9	5,1 4,0	27,0 0	10,4 8,2	11,5 9,2	51,1 82,6
γ	0,53	0,15	0,15	35,4	31,8	3,6 3,1	33,3 0	10,0 8,7	10,2 8,6	46,5 82,7

* Each figure given is the mean of three determinations.

** Precipitate after washing with physiological saline.

slightly decreased, and the relative content of γ-globulins was increased (to 84.7%). During precipitation of the immune serum with an albumin antigen, there was a much more pronounced fall in the albumins than in the globulins in the supernatant fluid. In the washed precipitate, no albumins could be found; the relative content of γ-globulins was also increased (to 81.7%). It should be pointed out that, even in concentrated solutions of the precipitate, the albumin antigen could not be detected electrophoretically. In this case the dissolved albumin–antibody complex had evidently acquired an electrophoretic mobility close to that of the γ-globulins (antibodies). Similar views about the dissolved antigen-antibody complexes may be found in the literature [6].

Determination of the precipitating antibodies in the various protein fractions of an immune serum by means of A. E. Gurvich's electrophoresis-precipitate method showed beyond doubt that the precipitins migrate along with the γ-globulins entirely (Fig. 1). Nevertheless, in the composition of thoroughly washed precipitate, it is possible to find not only γ-globulins, but also other protein fractions, whose electrophoretic mobility is close to that of the α_2- and β-globulins (Fig. 2). The relative contents of the latter are approximately the same (about 15%) in all the precipitates obtained with antigens of different molecular weights.

Our findings show that a comparatively large quantity of nonspecific proteins is adsorbed on the antigen-antibody complex in the process of formation of a specific precipitate with protein antigens of different molecular weights. These adsorbed proteins, however, are easily washed out with physiological saline, and the precipitate thus obtained consists mainly (about 85% of its weight) of the γ-globulins of the immune serum. A small part of the precipitate (15%) has an electrophoretic mobility similar to that of the α_2 – β-globulins. These protein fractions, firmly combined with the specific antigen-antibody complex, probably consist of nonspecific proteins.

SUMMARY

An electrophoretic analysis of the dissolved precipitate and supernatant fluid was conducted in the equivalent zone. For microelectrophoresis on paper, a Flynn and De Mayo type of chamber as modified by A. E. Gurvich was used. The specific precipitate, thoroughly washed with saline solution, largely consists of the γ-globulins of the immune serum (about 85%) and proteins, the electrophoretic mobility of which approaches that of the α_2 and β-globulins. Albumins adsorbed by the specific antigen-antibody complex are lost in washing the precipitate. A suggestion is made that about 15% of the precipitate consists of nonspecific proteins.

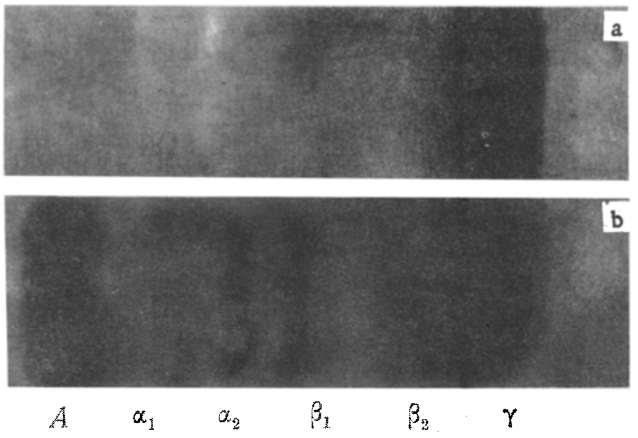

Fig. 1. Electrophoregrams of an immune rabbit serum. b) Before specific treatment by the electrophoresis-precipitate method; a) after treatment; A) albumins; $\alpha_1, \alpha_2, \beta_1, \beta_2, \gamma$) globulins.

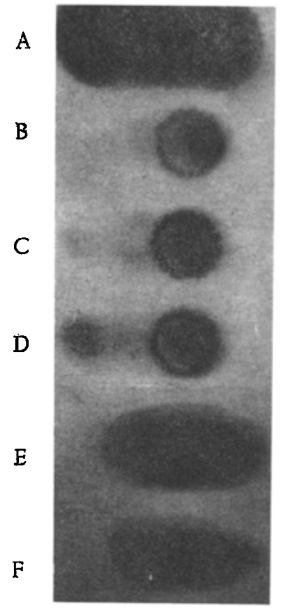

Fig. 2. Electrophoregrams of an immune serum and dissolved precipitate. A) Immune serum; B) precipitate (immediately after dissolving in N/10 alkali), washed three times; C) the same, washed twice; D) the same, washed once; E) precipitate (2 hours after dissolving), washed three times, antigen – β-globulin; F) the same, antigen – albumin from normal horse serum.

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