AN ELECTROPHORETIC AND IMMUNOCHEMICAL STUDY OF SERUM PROTEINS IN GUINEA PIGS DURING DEVELOPMENT OF BRUCELLOSIS

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The prolonged complement-fixation reaction (PCFR) in the cold is one of the most discriminative and sensitive methods of investigation. With the help of this reaction it is possible to demonstrate both antibodies and antigens in material under study.

E. S. Orlov and S. D. Skul'skaya [5], M. L. Feder [8], P. A. Trilenko [6], and T. M. Torosov [7] have used the PCFR in diagnosing brucellosis, and have given it a high rating. B. I. Ioffe [3] suggested that in the demonstration of specific antigen in blood sera of guinea pigs, the antigen should be concentrated by passing streams of carbon dioxide through the serum. Our observations on experimental brucellosis in guinea pigs, with the use of this method, indicate that specific antigen can be demonstrated in serum even at early stages in the development of infection. Specificity of the precipitate obtained has also been demonstrated by allergy tests on sensitized guinea pigs, and by immunizing rabbits with this precipitate and subsequently determining specific antibodies in them.

The purpose of the present study was to conduct an immunochemical and electrophoretic investigation of blood sera of guinea pigs throughout the development and the entire course of the infectious process, before and after passing CO_2 through the sera. Preliminary experiments of ours showed that as a result of the passage of CO_2, considerable depletion of serum proteins occurs; these are evidently precipitated when the pH of the solution is altered toward the acid side. When CO_2 is passed through serum from healthy animals, a precipitate is likewise formed in some cases, but considerably less than in the case of infected guinea pigs, and it is practically impossible to analyze it electrophoretically.

METHODS

Guinea pigs were inoculated with a live culture of Brucella melitensis, in a dose of 1000 microbial cells. Prior to inoculation, and at a definite time thereafter, blood was drawn from each animal to obtain the serum. Serum was diluted 1:4 with distilled water, and carbon dioxide was passed through it for 10 – 15 minutes.

The passage of CO_2, which shifted the reation of the solution toward the acid side, resulted in precipitation of certain protein components from the serum, which were subjected to electrophoretic analysis after centrifugation and two rinsings with further centrifugations – along with the supernatant fluid, which was arbitrarily designated the "non-precipitable fraction" of the serum. Simultaneously, after the same time intervals, sera from healthy animals were subjected to electrophoretic analysis before and after CO_2 was passed through them.

In the whole serum and in the precipitate formed after the passage of CO_2, the protein content was determined by Lowry's method [10]. For electrophoretic analysis, an amount of serum was used which contained 300 – 500 γ of protein. After electrophoresis the individual protein fractions were determined by densitometry. The precipitate formed after the passage of CO_2 was rinsed twice with distilled water, for the purpose of ridding it of other serum proteins, and then dissolved in buffer, yielding a faintly opalescent solution, which was poured onto paper for electrophoresis. Electrophoresis was carried out in a barbital – barbital sodium buffer at pH 8.6, ionic strength 0.1, for 17 hours, with a potential of 175 volts and a current of 6 ma, on fast-absorbing chromatographic paper measuring 40 cm x 4 cm; the paper was subsequently stained for proteins, carbohydrates, and lipids. For immunochemical study of the precipitate, we used the methods of immunoelectrophoresis in gels, with the modifications of Kohn [9] and Gendon [1], with microprecipitation in a gel and precipitation on paper [2]. In addition, qualitative reactions were carried out for the detection of lipids (staining with Sudan black) and polysaccharides (staining by Hotchkiss's method, with basic fuchsin) [3] in lyophilized precipitate.

For a control in staining for lipids and polysaccarides, crystalline human albumin was used, which did not stain with Sudan black or with basic fuchsin, and lipids and

polysaccharides isolated from brucella and typhoid bacteria, which stained well with these dyes. Simultaneously, the possible presence of DNA was determined on the basis of absorption by the precipitate in the ultraviolet at a wave length of 270 – 280 Å, with absorption by a known nucleoprotein isolated from typhoid bacteria, and rabbit serum, which does not contain DNA, as controls.

At the same time as the chemical and immunochemical studies, whole sera and individual fractions (nonprecipitable fraction and precipitate) were subjected to immunological analysis for the demonstration of specific antigenic substance. For this purpose, the PCFR in the cold and an allergy test on sensitized guinea pigs were employed. In conducting the PCFR, and in all experiments of the chemical and immunochemical analysis, we used specific brucellosis serum with a titer of 1:6400; in experiments with diffusion microprecipitation in gels, the serum was diluted 10 – 20 times.

RESULTS

Table 1 shows the quantitative determination of protein fractions of blood serum at various periods after inoculation. The control was serum from a healthy guinea pig. In all cases, 300γ of protein was taken for electrophoresis. As is evident from this table, during the development of brucellosis infection in guinea pigs a considerable reduction takes place in the content of albumins (from 63.7% to 35.4%), along with an increase in the α- and γ-globulins, with irregular fluctuations in the β-globulins.

In Table 2, quantitative data are presented on the content of various protein components in the "nonprecipitable fraction" of blood serum of guinea pigs at various times after inoculation.

It is clear from this table that the principal regularities in the changes of serum proteins during the development of infection remain the same: The albumin content is reduced and the γ-globulin content increases. The content of α-globulins in the "nonprecipitable fraction" was significantly reduced after the passage of CO_2.

On the basis of these data we may assume that α-globulins are the most unstable to changes in the pH of the solution, and are precipitated when the serum is acidified with CO_2. We were able to demonstrate the correctness of this assumption after painstaking electrophoretic analysis of twice-washed and lyophilized precipitates obtained from the sera of animals at various intervals after the moment of inoculation. Since we had extremely limited quantities of precipitate at our disposal in this work, we could not approach the estimation of it in a strictly quantitative manner, and were forced to limit ourselves to a qualitative characterization. Figure 1 shows the electrophoretic chromatogram of a precipitate obtained from blood serum taken from a guinea pig 24 hours after inoculation with a brucella culture. By this time, significant quantitative changes have occurred in the protein composition of the serum. The same figure shows a curve of the quantitative relationships of the proteins detected, and the electrophoretic chromatogram of normal guinea pig serum. As can be seen, the principal protein component of the precipitate corresponds in position to the α-globulin fraction.

The least mobile component of the precipitate is distributed between the β- and γ-globulins. This demonstrates the correctness of our belief that when CO_2 is passed through guinea pig serum the α-globulins are precipitated from it. Although a reduction in albumin content occurs when infection develops, albumin was not detected in the precipitate*

As mentioned earlier, precipitates formed from the sera of infected guinea pigs show up in the prolonged complement-fixation reaction (PCFR) as antigens. It might be assumed that proteins precipitated from serum upon acidification with CO_2 adsorb antigens, or products of microbial metabolism having an antigenic action, on their surface. For this reason, it was of great interest to test the antigenic properties of precipitates by the latest immunochemical methods. Despite repeated efforts, using the methods of immunoelectrophoresis, microprecipitation on a microscope slide, and precipitation on paper, we were unable to demonstrate any antigenic properties of the precipitates. From this we concluded that either

* We wish to thank A. Ya. Fridenshtal' and M. Ya. Kern for assistance in analyzing precipitates for lipids, polysaccharides, and DNA.

TABLE 1. Electrophoretic Components of Whole Blood Sera of Guinea Pigs at Various Times After Inoculation

Time after inoculation	Amount present (%)			
	albumin	globulins		
		α-	β-	γ-
Normal	63,7	17,2	8,7	10,4
9 hours	66,1	22,0	2,6	10,3
12 »	50,1	21,7	12,2	16,6
48 »	42,2	16,9	7,0	33,9
120 »	43,1	31,3	5,9	19,6
240 »	37,8	32,8	7,0	22,4
360 »	35,4	36,2	6,8	24,7

TABLE 2. Electrophoretic Components of "Nonprecipitable Fraction" of Blood Sera of Guinea Pigs at Various Times After Inoculation

Time after inoculation	Amount present (%)			
	albumin	globulins		
		α-	β-	γ-
Normal	51,2	27,3	7,2	14,3
3 hours	50,0	31,0	6,0	14,0
12 hours	48,3	24,2	8,0	19,5
24 hours	50,5	29,0	7,0	14,6
48 hours	48,3	24,6	10,6	18,5
72 hours	43,0	18,0	11,0	28,0
120 hours	46,0	17,47	10,5	26,9

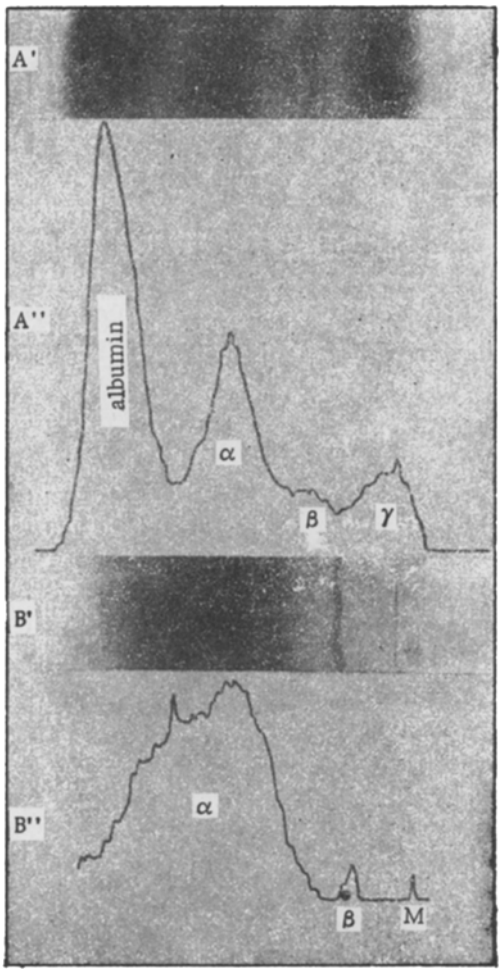

Fig. 1. Electrophoretic distribution of protein fractions of precipitate formed from blood serum of guinea pigs 24 hours after inoculation. A' – electrophoretic chromatogram of normal serum; A'' – densitometric curve; B' – electrophoretic chromatogram of precipitate; B'' – densitometric curve; M – spot marking infection.

all these methods were insufficiently precise and limited in resolving power, or else substances that have an antigenic action in the PCFR are in reality haptens and can not give typical antigenic reactions in immunochemical analyses.

The latter explanation seems probable, since experiments with allergy tests on sensitized guinea pigs, like the positive complement-fixation reaction, confirmed the presence of antigenic properties in precipitates formed upon the passage of CO_2, even at early stages after the beginning of infection.

Qualitative chemical analysis of the precipitates showed that these contain lipids and polysaccharides, but not DNA. The presence of lipids and polysaccharides may be connected with precipitation of microbial antigen as well as serum components. Experiments with differential staining of electrophoretic chromatograms for lipids and polysaccharides (Wunderly and Piller [12]) have shown that both classes of substances are present in all electrophoretic fractions of the sera studied.

On the basis of studies of ultraviolet absorption by precipitates from sera of infected guinea pigs, it was established that DNA is not contained in the precipitate.

To summarize the results of our investigations, therefore, we may conclude that in the process of development of brucellosis an increase in α-globulins and γ-globulins occurs in guinea pig serum, with a simultaneous reduction in albumin content; when CO_2 is passed through the serum at various times after the moment of inoculation, a certain fraction of the proteins is precipitated, which is similar in mobility to serum α- and β-globulins. A certain amount of lipid and polysaccharide material is also precipitated.

Biological methods (PCFR and allergy tests on sensitized guinea pigs) show that the precipitate has antigenic properties, but these could not be demonstrated by any of the physicochemical methods tried.

SUMMARY

It was shown by electrophoretic methods that in the early stages of brucellosis in guinea pigs, there is an increase in the α- and γ-globulin fractions of blood serum, and a reduction in the albumin fraction.

The α-globulin content is decreased in the "nonprecipitable fraction" left in solution after CO_2 is passed through the serum; electrophoretic analysis shows that the

γ-globulins are precipitated when the medium is acidified. The prolonged complement-fixation reaction, and allergy tests on sensitized guinea pigs, have demonstrated that the precipitate possesses antigenic properties undetectable by any of the physicochemical methods used The precipitate contained two groups of proteins whose mobility approached that of the α- and β-globulins, as well as lipid and polysaccharide material. The possibility is discussed that antigenic substances from brucella, or products of brucella metabolism, are adsorbed by serum protein components of high mobility.

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