APPLICATION OF IMMUNO-ELECTROPHORESIS AND IMMUNOFILTRATION TO THE SEPARATION OF ELABORATE ANTIGEN COMPLEXES

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Vi-antigen obtained by various methods usually contains somatic O-antigen as an impurity. Some authors have pointed out that by acid hydrolysis the pure Vi-antigen may be obtained [3, 4, 7, 8]; however hydrolysis of the antigen leads to partial degradation of the preparation. Recently electrophoresis in agar has been used to separate the different antigen fractions [6].

A new method of immunofiltration has also been proposed [1]. The preparation of Vi- and O-antigens of typhoid bacteria is important for determination of the chemical nature of the antigens and of the significance of these preparations in immunogenesis.

METHOD

From the acetone-dried mass of S. typhi (strain No. 4446) we obtained preparations of Vi-antigen by Konikov and Klyucheva's modification Webster and Landy's method as well as by the original Webster and Landy method; we used 2- and 24-h hydrolysis (subsequent preparations were kindly presented to us by D. D. Efimov).

TABLE 1. Serological Properties of Fractions in the Hemagglutination Reaction

Fraction	Sera		Characteristic of the fraction
	Vi(1:2000)	O(1:1000)	
I	–	1:2	Cathodic fractions
II	1:32	1:4	
III	1:4	1:4	
IV	1:4	1:4	
V	1:4	1:4	
VI	1:32	1:4	
Line of Zero Electrophoretic Mobility			
VII	1:512	1:4	Anodic fractions
VIII	1:2000	1:2	
IX	1:1000	–	
X	1:1000	–	
XI	1:1000	–	
XII	1:64		

To study the structure of the antigens we applied the method of specific precipitation in agar and analytic immuno-electrophoresis. To separate the different fractions we used electrophoresis and immunofiltration. These methods have been described previously [5]. To obtain antisera we immunized rabbits six times with the antigens and with the separate fractions, giving amounts from 0.1 to 1 mg/ml. The γ-globulin was separated from the antisera. Serological reactions were carried out with the γ-globulin.

RESULTS

By studying the antigens mentioned above by means of specific precipitation in agar and by analytical immuno-electrophoresis we demonstrated that the Vi-typhus antigen has quite an elaborate structure. We showed that this antigen consists of various components having different electrophoretic mobilities. The most mobile part of the antigen moving towards the anode consisted of components 1 and 2 (Fig. 1A) which gave a precipitation line only with Vi-γ-globulin. The third, fourth, and fifth components produced precipitation lines both with Vi- and with O-γ-globulins; in these cases there was a strict correspondence to the Vi- and O-lines of precipitation, in both position and

TABLE 2. Study of Hyperimmune Sera Obtained by Immunization with "Pure" Vi-typhoid Antigens

Animal	Immunizing substance	Titer before immunization		Titer after immunization	
		Vi	O	Vi	O
Rabbit No. 36	Fraction XI	–	–	1:32	1:8
Rabbit No. 11	Webster-Landy antigen (24-h hydrolysis)	–	–	1:512	1:8
Rabbit No. 24	Webster-Landy antigen (2-h hydrolysis)	–	–	1:256	1:8
Mice of group No. 1	Webster-Landy antigen (24-h hydrolysis)	*	*	1:32	1:4
Mice of group No. 2	Webster-Landy antigen (2-h hydrolysis)	*	*	1:16	1:4
Mice of group No. 3	Immunofiltration fraction	*	*	1:8	1:4

* It was not possible to establish a control for the initial sera in the case of experiments on mice.

TABLE 3. Immunogenic Properties of the Fractions and of the Antigens

Immunizing substance	Minimum immunizing dose (in mg/ml)	Characteristic of the fraction
Fraction II	0.2	Cathode fraction
Fraction III	0.04	Anodic fraction
Fraction IX	0.0016	Anodic fraction
Fraction XI	0.0016	Anodic fraction
Antigen, 2-h hydrolysis	0.000417	Antigen obtained by the Webster-Landy method
Antigen, 24-h hydrolysis	0.04	Antigen obtained by the Webster-Landy method

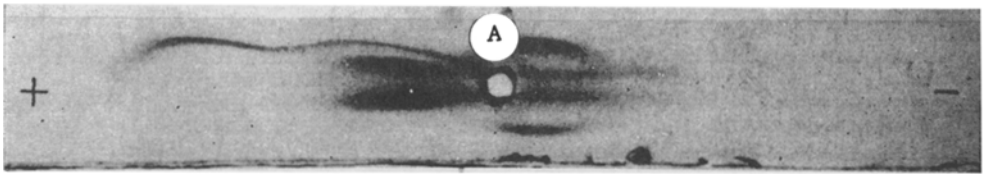
form. The components just described carried different charges. The third component moved towards the anode, and the fourth and fifth towards the cathode, although they all possessed an anodic mobility, as was shown by the fact that they moved towards the positive pole with respect to the point of zero mobility.

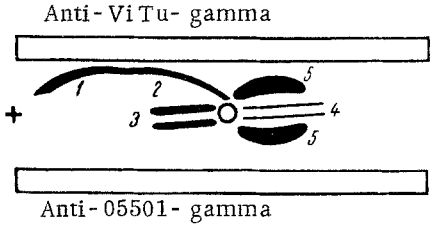
In the analytic immuno-electrophoresis procedure the antigen obtained by the original Webster-Landy method using a 2-h acid hydrolysis formed a precipitation line only with Vi-γ-globulin. This precipitation line corresponded to the rapidly moving components (first and second components). The antigen which had been subjected to a 24-h hydrolysis gave no precipitation band with immune γ-globulins (apparently because of degradation). This is the phenomenon corresponding to what has been published previously [3].

To prepare the Vi-antigen by electrophoresis we separated 12 antigen fractions. These fractions were eluted from agar, and dialyzed against distilled water, and then freeze-dried. The fractions of antigen obtained were studied by precipitation in agar. Most of the fractions reacted both with Vi- and with O-γ-globulin. Only fractions IX, X, XI, and XII, which had high electrophoretic mobilities and the Vi-antigen fraction after immunofiltration gave a precipitation band only with Vi-γ-globulin.

In tests of the serological properties of the fractions separated by hemagglutination it was shown that some of the fractions (II-VIII) gave a positive reaction with both Vi- and with O-antisera. Other fractions (IX-XII) reacted only with Vi-antisera and gave no reaction with O-antisera. By taking into account the results of the hemagglutination and precipitation we were able to select fractions which appeared to be free from contamination with O-antigen. Such fractions were IX, X, XI, and XII (Table 1).

The antigen obtained by immunofiltration did not differ in its properties from fractions IX, X, XI, and XII.





Electrophoregram of analytic immuno-electrophoresis of the Vi-typhoid antigen (A), and illustration of precipitation band (B). 1, 2, 3, 4, 5) Components of the Vi-antigen.

For a more accurate determination of the purity of the fractions we immunized rabbits and mice with the fractions listed above. From the 30 rabbits we selected only 3 which did not contain normal Vi- or O-antibodies. In studying the sera obtained from 30 mice we found no mouse having normal Vi- and O-antibodies.

The rabbits were immunized with antigen fraction XI prepared by electrophoresis, and antigens prepared by the Webster-Landy method with 2- or 24-h hydrolysis. Immunization was produced by 6 intravenous injections at 6-day intervals; the following amounts were given: 0.1 (subcutaneously), 0.1, 0.2, 0.4, 0.8, and 0.1 mg/ml (intravenously). The animals were exsanguinated 6 days after the last injection.

The mice were immunized subcutaneously by a single dose of 0.04 mg in 0.5 ml of antigens produced by the Webster-Landy method, and by the Vi-antigen fraction obtained by immunofiltration. The mice were exsanguinated on the 10th day after immunization.

Sera taken from immunized animals and sera obtained before immunization of the rabbits were studied together by hemagglutination with human erythrocytes of group I blood. From experiments in vitro it was shown that the fractions we obtained and the Webster-Landy antigen possessed only the Vi-antigen; however when the rabbits or mice were hyperimmunized both Vi- and O-antibodies were formed (Table 2).

These results indicate that in the preparations studied a small amount of O-antigen was present, and that it was detectable only when the animals were hyperimmunized. This fact throws light on the structure of the Vi-typhoid antigen. Probably this antigen is present in various forms and is bound to the somatic O-antigen. Besides loosely bound complexes which are readily degraded by acid hydrolysis, and which display various electrophoretic mobilities, there are other stronger connections between the O- and Vi-components. This conclusion is in line with data obtained by A. N. Belozerskii and V. D. Gekker [2], who attempted to obtain pure "complete antigen" from a dysenteric culture. They came to the conclusion that nucleoprotein and its separate fractions cannot be completely separated from the "complete antigen". The main mass of the specific complex is loosely bound to protein, and can be readily extracted. A part of the "complete antigen" is organically bound to elements of the protoplasm.

We have studied the immunogenic properties of fractions separated by hemagglutination and precipitation, and which contain only Vi-antigen; also fractions containing mainly O-antigen. In addition we investigated antigens obtained by the Webster-Landy method. The mice were immunized by doses from 0.00032 to 0.2 mg of the antigens listed above in 0.5 ml of physiological saline diluted 5-fold. They were infected on the 10th day after immunization with 25 million S. typhi Ty_2 [4 DCL] give intraperitoneally in 0.5 ml of physiological saline. The minimum immunizing dose was calculated by the method of Rid and Mench.

As can be seen from Table 3 the strongest immunity was conferred by the antigen obtained by the Webster-Landy method and a 2-h hydrolysis, and by fractions IX and XI. Probably the differences in the immunizing power of the fractions and of the Webster-Landy antigen are due to contamination of the former with agar (because the fractions were prepared by electrophoresis in agar). It was not possible to calculate the precise amount of antigen in the fractions.

Fractions containing O-antigen were much less immunogenic in the case of infection with a Ty_2 culture. Possibly these fractions were much more immunogenic in the event of infection with the O-strain.

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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.**
