

STUDY OF THE TOXIC, ANTIGENIC,  
AND SENSITIZING PROPERTIES OF PREPARATIONS  
ISOLATED FROM THE BRUCELLA CELL WALL

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UDC 576.851.420.97

A study of the toxic, antigenic, and sensitizing properties of the Boivin antigen, protein-polysaccharide complex, and polysaccharide isolated from a culture of Brucella melitensis showed that the polysaccharide was the most toxic. All antigens induced the formation of antibodies in the blood serum of immunized animals and an allergic response to the homologous antigen. The immunogenic properties of the antigens were slight.

The search for chemical vaccines with high immunogenic properties, yet not inducing permanent postvaccinal reactions, is under active consideration at the present time. Data in the literature show that the immunogenic fraction of brucellas is evidently located in the cell wall or on its surface [3-6].

The object of the present investigation was to compare the toxic, antigenic, and sensitizing properties of various antigens from the brucella cell wall.

EXPERIMENTAL METHOD

The following preparations were isolated from the cell wall of Brucella melitensis 565 in the S-form: Boivin antigen, protein-polysaccharide complex by White's method, and a polysaccharide.

The toxicity of the antigens was tested in albino mice weighing 18-20 g by intraperitoneal injection. The antigenic, sensitizing, and immunogenic properties of the specimens were studied in guinea pigs, into which the antigens were injected in doses of 3, 1.5, and 0.75 mg/ml (first injection), followed after 10 days by doses of 3 mg in 1 ml (second injection). Antibodies were detected in the blood serum of the immunized animals by the agglutination test (AT) and the passive hemagglutination test (PHAT) [2], and the preventive properties of the sera also were studied [1].† Altogether 45 guinea pigs weighing 300-350 g and 270 albino mice weighing 14-20 g were used in the experiments.

EXPERIMENTAL RESULTS

A study of the toxic properties of the antigens showed that the protein-polysaccharide complex possessed the least toxicity ( $LD_{50} = 4$  mg). The polysaccharide isolated from Boivin antigen by mild alkaline hydrolysis proved to be more toxic ( $LD_{50} = 0.77$  mg) than the original endotoxin ( $LD_{50}$  of Boivin antigen = 1.32 mg).

Investigation of the antigenic properties of the 3 preparations revealed no difference in antibody production in the guinea pigs depending on the primary dose of antigen injected. The results of investigation

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† The preventive dose of the test serum ( $PD_{50}$ ) was established by the Reed-Muench method.

Laboratory of Brucellosis, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 71, No. 6, pp. 62-64, June, 1971. Original article submitted October 1, 1970.

TABLE 1. Results of Investigation of the Blood Sera of Guinea Pigs Immunized by Different Antigens

Antigen	Mean titer in AT					Mean titer in PHAT				
	after first injection		after second injection			after first injection		after second injection		
	5th	10th	5th	10th	15th	5th	10th	5th	10th	15th
	days									
Boivin's . . . . .	1:27	1:46	1:178	1:256	1:168	1:42	1:10	1:24	1:45	1:60
White's . . . . .	12/0	6/0	11/0	10/0	10/0	8/6	2/4	7/4	9/0	2/8
	1:42	1:113	1:123	1:178	1:204	1:82	1:320	1:77	1:140	1:380
Polysaccharide . . .	15/0	6/0	11/0	10/0	9/1	12/3	6/0	10/0	9/0	8/0
	1:12	1:50	1:85	1:180	1:200	1:20	1:20	1:54	1:30	1:60
	10/4	10/0	13/0	9/0	8/0	9/6	1/5	5/8	4/5	5/4

Note. Numerator shows number of guinea pigs with positive reaction; denominator, number with negative reaction.

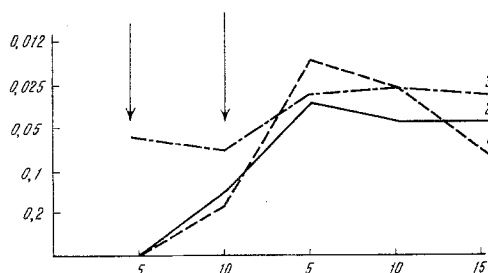


Fig. 1. Preventive properties of blood serum of guinea pigs immunized by different antigens. 1) Boivin's antigen; 2) polysaccharides; 3) White's antigen. Arrows indicate times of first and second injections of antigens, respectively. Abscissa, time of investigation (in days); ordinate,  $PD_{50}$  (in ml).

of the sera of guinea pigs immunized by the different brucella antigens are given in Table 1. They show that a single injection of the preparations induced agglutinin formation, the highest titers of the AT being found in response to injection of White's antigen. After the second injection of antigens (3 mg/ml), an increase was observed in the agglutinin titer, especially in guinea pigs immunized by Boivin antigen (by 4 times). So far as hemagglutinin formation is concerned, the highest titers were found in animals receiving White's antigen (Table 1).

A study of the protective properties of the serum of guinea pigs immunized by Boivin's and White's antigens and by polysaccharide showed that the serum was the most active after a single injection of White's antigen (Fig. 1). After the second cycle of immunization, no significant difference could be found between the antigens. However, a second injection of antigens increased the preventive activity of the serum of

all the animals, but only for a short time (Fig. 1). The minimal dose of these sera ( $PD_{50}$ ) was 0.018-0.035 ml. A correlation was found between the height of the agglutinin titer and the preventive activity of the serum.

To detect the sensitizing properties of the antigen, an allergic skin test was carried out on the immunized guinea pigs 30 days after the second injection of the antigens. Homologous preparations and brucellin were used as the allergens. The experiment showed that an allergic response developed in the guinea pigs mainly to the homologous antigen. The allergic skin test to brucellin was negative in most animals.

For a preliminary investigation of the immunogenicity of antigens isolated from the brucella cell wall, immunized guinea pigs were infected 30 days after the injection with a virulent strain of *Br. melitensis* in a dose of 40 bacterial cells. The guinea pigs were sacrificed 1 month after injection for bacteriological tests. The results of these tests are given in Table 2.

TABLE 2. Results of Tests of Immunogenic Properties of Brucella Cell Wall Antigens

Antigen	No. of guinea pigs	Mean titer after infection		Immun-ity	Infection		Index of infectivity (in %)
		AT	PHAT		generalized	regional	
Boivin's . . . . .	15	1/2930	1/420	2	13	None	76
White's . . . . .	14	1/380	1/1090	1	10	3	51.6
Polysaccharide.	11	1/2600	1/370	2	9	None	80

It is clear from Table 2 that after 2 injections of antigens into guinea pigs immunity was not produced in sufficient intensity to prevent infection by the virulent strain.

The results thus indicate that specific antibodies are formed and an allergic response develops in guinea pigs immunized with antigens isolated from the brucella cell wall, but the immunogenic properties of these antigens are weak. This result correlates with the low protective properties of the serum at the time of testing the immunity. Investigations to test the immunogenic properties of the preparations, with the use of different schemes of immunization, will be continued.

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