

A STUDY OF THE PROPERTIES OF SERA OBTAINED
BY IMMUNIZATION OF RABBITS WITH FRACTIONS
OF THE PLAGUE BACILLUS

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The object of the present work has been to study the sera of rabbits immunized with various preparations obtained from plague bacteria by the method of Baker and his co-workers [2]. The immunogenic properties of these fractions have been described previously [1].

EXPERIMENTAL METHODS

For the experiment we took fractions IB, II, and the residue which was insoluble in water. The rabbits were killed in groups according to the preparation used for their immunization. All the animals were immunized by increasing amounts of antigen injected subcutaneously.

An immunization cycle consisted of seven injections, of which four contained a depot substance, and three did not. The depot substance used was apricot oil. Antigens with this substance were prepared by careful suspension of the dry preparation in the apricot oil. The antigens not in the apricot oil were diluted in physiological saline.

The first group of rabbits was immunized with fraction IB, the second with fraction II, and the third with the insoluble residue.

For comparison we took two groups of rabbits, one of which was immunized with freeze-dried cells of strain EV, by the method described, and the second by cells of this strain killed with acetone. There were eight animals in each group.

On the eighth day after the last injection blood was taken from the rabbits, and the sera from the group containing a particular antigen were combined.

EXPERIMENTAL RESULTS

We began the study of these sera with an investigation into their preventive properties.

White mice were infected with 10 Dcl of a virulent strain of plague bacillus No. 1435. Two subcutaneous injections of the sera were given to white mice; 0.3 ml were given together with the infective dose, and 0.2 ml were given 24 h later. The preventive properties of the serum were evaluated from the number of survivals.

From the results shown in Table 1 it can be seen that in the case of plague, only the serum obtained from rabbits immunized with the insoluble residue exerted a protective action ($P > 0.99$). In the group of mice which received this serum prophylactically, 65% survived, and the mean length of life of those which succumbed was 8.4 days.

We could find no difference between the protective properties of normal serum and of sera obtained from rabbits immunized with the other antigens (confidence limit less than 0.95).

In the next experiment the therapeutic action of the serum was tested.

Some white mice weighing 18-20 g were divided into seven groups and infected with the same dose used to determine the protective properties of our preparations (10 Dcl). One day later, and subsequently after 2, 5, and 7 days from the time of infection all groups except the controls received treatment. In the treatment, each animal received subcutaneously 0.8 ml of the serum to be tested.

TABLE 1. Results of a Test of the Protective Properties of Sera Obtained by Immunizing Rabbits with Different Fractions of the Plague Bacillus

Material used for immunization	Number of white mice				Mean survival time of animals which died (in days)
	total	died	survived		
			absolute	%	
Fraction 1B	20	15	5	25	8.6
Fraction II	20	18	2	10	6.3
Residue insoluble in water	20	7	13	65	8.4
Freeze-dried cells	20	13	7	35	7.3
Cells treated with acetone	20	14	6	30	7
Normal serum	20	18	2	10	5.8
No serum injected (control)	20	19	1	5	4.8

TABLE 2. Results of Tests of the Therapeutic Properties of Sera Obtained by Immunization of Rabbits with Various Fractions of Plague Bacillus

Immunizing substance used	Number of white mice				Mean survival time of animals which died (in days)
	total	died	survived		
			absolute	%	
Fraction 1B	20	17	3	15	7.8
Fraction II	20	18	2	10	6.3
Residue insoluble in water	20	6	14	70	12.6
Freeze-dried cells	20	19	1	5	7.7
Cells treated with acetone	20	11	9	45	8.5
Normal serum	20	20	—	0	4.6
No serum injected (control)	20	19	1	5	4.8

From the results given in Table 2 it can be seen that of the sera investigated the most effective was that obtained from rabbits immunized with the insoluble residue from plague bacillus ($P > 0.99$). In the group of mice treated with this serum, 70% survived, and the average survival time of those which died was 12.6 days. The therapeutic properties were also demonstrated in the serum of rabbits immunized with cells of strain EV treated with acetone ($P > 0.95$). No therapeutic property of the other sera could be demonstrated ($P < 0.95$).

The next step in the work was to determine the power of the sera to neutralize toxin. For this purpose we took sera from rabbits immunized with fractions 1B and II.

Experiments on neutralization of the toxin were carried out as follows: 0.4 ml of the serum to be tested was mixed with 8 LD₅₀ of fractions 1B and II plague bacillus diluted with 0.1 ml of physiological saline. The mixture was then incubated for one hour in darkness at 37°, and then injected into the white mice subcutaneously. The mice of the control group received the same amount of the corresponding fraction, but no serum.

A post mortem examination was made of the animals which died. In the control mice a hyperemia and edema of the subcutaneous tissue was found at the site of the injection; there were also hemorrhages into the serous coat of the intestine, a pulmonary hyperemia, an increase in size and engorgement of the liver with dystrophic signs in the parenchyma, and an enlarged spleen which sometimes contained small necrotic zones.

In the animals which received the preparations described mixed with the serum and which died long afterwards, post mortem investigations showed a general emaciation, sometimes with atrophy of the viscera, but without signs of toxemia.

The degree of protective effect of the serum was judged from the number of animals which survived.

A statistical analysis of the results of Table 3 shows that there is no essential difference in the power of the different types of serum to neutralize toxin. Despite the 100% effect of the serum from rabbits immunized with fractions 1B and II, in the repeated experiments which gave a probability of 0.95 it would not have been unexpected if 25% of the white mice would have died. Approximately this percentage of deaths was observed when the sera from rabbits treated with a culture of strain EV was used.

TABLE 3. Neutralizing Properties of Sera Obtained by Immunizing Rabbits with Various Fractions of Plague Bacillus

Substance used for immunization	dose	Number of white mice				Mean length of life of animals which died (in days)
		total	died	survived		
				absolute	%	
Fraction LB	8 LD ₅₀	10	—	10	100	—
Freeze-dried cells	"	10	3	7	70	9.3
No injection (control)	—	10	10	—	0	1
Fraction II	8 LD ₅₀	10	—	10	100	—
Freeze-dried cells	"	10	2	8	80	7.5
No injection (control)	—	10	10	—	0	1

A similar result was obtained from experiments in which the preparations were injected intraperitoneally.

We have also made an electrophoretic study of the protein constitution of the sera. When animals were immunized with fraction 1B or with freeze-dried plague cells it was shown that in every case there was some reduction of total protein and of the albumin fraction (except for the sera of rabbit immunized with fraction II), but an increase of the alpha- and gamma-globulins and a small increase in the beta-globulin fraction; when immunization was made with fraction II and plague cells treated with acetone, the beta-globulins were somewhat increased.

The results obtained are of comparative interest only, because for the electrophoretic study we used only one sample of each serum. In further work on the protein composition of the sera, we intend to carry out repeated observations, and to relate them to a study of the biological properties of the sera.

As experiments on white mice have shown, sera of rabbits immunized with the insoluble residue of strain EV possess therapeutic and protective properties; for the properties whose investigation we have described we were not able to demonstrate any difference between normal serum and the sera of rabbits immunized with fractions 1B and II of plague cells.

The sera of rabbits immunized with fractions 1B and II of plague cells did not differ appreciably in their toxin neutralizing power from sera of rabbits treated with strain EV.

SUMMARY

Experiments were carried out on white mice. Sera were obtained from rabbits immunized with 1B and II fractions of *P. pestis*, and with the water-insoluble residue of the EV strain; only the latter serum possessed therapeutic and protective properties. Normal serum, and sera from rabbits immunized with the two other antigens showed no differences with respect to these properties. The sera of rabbits treated with the EV strain did not differ significantly from the sera of others immunized with fractions 1B and II of *P. pestis* with respect to the power to neutralize toxin.

LITERATURE CITED

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