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Studies on the Specificity of Anthrax Precipitin Serum

(17) Further Researches for the Antigenic Relation between *Bacillus megatherium* and *Bacillus anthracis*

By

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In a previous report,[1] it was described that a very analogous antigenic relation was recognized between *B. megatherium* and *B. anthracis*, and antigens derived from the latter strongly precipitated anti-megatherium serum. In the present study, further researches for antigenic analogy between the two organisms were performed by examining the effect of preparing procedures of megatherium immunogens upon reactions between *B. anthracis* and anti-megatherium serum prepared. Results of agar-diffusion and absorption tests are also included in the present paper.

Experiments and Results

I. Preparation of megatherium immunogens and production of antibodies which react with *B. anthracis*.

1. *Immunization with agar cultures of B. megatherium.* Immunization of rabbits with unwashed cells of agar-grown megatherium which had been killed by suspending in saline containing formalin to a concentration of 0.5 per cent caused production of antisera which fairly reacted with anthrax antigens. While immunization with cells of agar-grown megatherium which had been washed three times with saline caused serious decrease in production of antibodies against *B. anthracis*, although production of homologous antibodies did not decrease. These washed cells, when injected together with washings of the cells, recovered the production of anti-anthrax antibodies. Of course, the washings alone were incapable of producing antibodies against both organisms (Table 1).

From the results written above, it is presumable that a substance is present in washings of agar-grown megatherium and stimulates production of anti-anthrax antibodies in cooperation with homologous cells. A problem, then, arises about the properties of the substance. In order to solve this, experiments were performed under the following presumption: The reason why production of anti-anthrax antibody is increased, when washings are added to the original megatherium cells, is probably because addition of the washings results in addition of an antigenic substance which concerns with production of anti-anthrax antibodies, but which is originally lacking in megatherium cells. If this presumption had been valid, antibodies against *B. megatherium*, in the case of immunization with unwashed agar-grown megatherium cells, would have been produced earlier than those against *B. anthracis*, i.e. in the early stages of immunization. The results exhibit the concurrence of both antibodies (Fig. 1).

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Table 1. Antigenicity of B. anthracis to anti-megatherium serum.

Antiserum		Antigen							
Immunogen	No.	Preparation	1:100	1:400	1:1600	1:6400	1:12800	1:25600	1:51200
Meg. Ag. C. (unwashed)	135	Meg. Ext.	++++	++++	+++	+	+	−	−
		Anth. "	++++	++++	++	+	−		
	146	Meg. Ext.	++++	+++	+++	++	++	+	−
		Anth. "	++++	+++	+++	++	+	+	−
Meg. Ag. C. (washed 3 times)	142	Meg. Ext.	++++	+++	++	+	−	−	−
		Anth. "	+	−					
	162	Meg. Ext.	++++	++++	++	++	+	±	−
		Anth. "	+	±	−				
Meg. Ag. C. (washed 3 times)+Meg. Ag. C. washings	155	Meg. Ext.	++++	++++	+++	++	+	+	±
		Anth. "	++++	++++	+++	++	+	+	±
Meg. Ag. C. washings	145	Meg. Ext.	−						
		Anth. "	−						

Meg. Ag. C. ··· B. megatherium grown on agar.
Ext. ··· Extract.

Table 2. Antigenicity of B. anthracis to anti-megatherium serum.

Antiserum		Antigen						
Immunogen	No.	Preparation	1:100	1:400	1:1600	1:6400	1:12800	1:25600
Meg. Ag. C. (washed 3 times)+Etahnol precipitate of Meg. washings	166	Meg. Ext.	++++	++++	++	+	+	+
		Anth. "	−					
Ethalol precipitate of Meg. washings	67	Meg. Ext.	±					
		Anth. "	−					

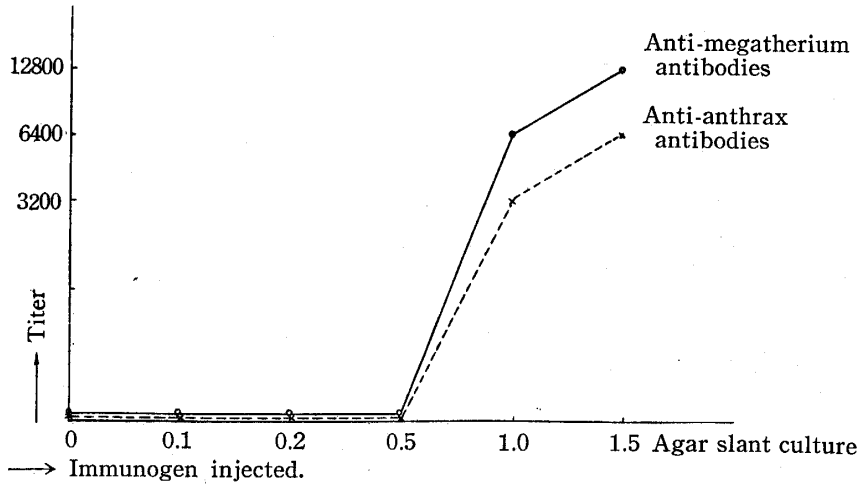

Figure 1. Antigenicity of B. anthracis to anti-megatherium serum (The relation between the frequency of injection of megatherium immunogen and anti-anthrax antibodies produced)

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Immunization with washed cells of *B. megatherium* to which was added a polysaccharide antigen* isolated from washings by fractionation with 70% (v/v) ethanol was also ineffective to produce anti-anthrax antibodies (Table 2).

From the above results, it may be presumed that the substance which is contained in washings and improves production of anti-anthrax antibodies in cooperation with megatherium cells is not such a simple polysaccharide antigen, but another one participating.

2. *Immunization with broth cultures of B. megatherium.* In the case of immunization with megatherium cells grown in nutrient broth, the results were somewhat different from those obtained in the case of agar-grown cells, i.e. injection with washed cells and with whole cultures (bacterial cells and culture fluids) and moreover, with washed cells grown in broth to which were added washings of agar-grown cells were all ineffective to produce anti-anthrax antibodies, although homologous antibodies were produced in all cases (Table 3).

Table 3. Antigenicity of B. anthracis to anti-megatherium serum (prepared against broth cultures).

Antiserum		Antigen						
Immunogen	No.	Preparation	1:100	1:400	1:1600	1:6400	1:12800	1:25600
Meg. B. C. (unwashed)	163	Meg. Ext.	++++	++++	++	+	±	—
		Anth. "	—					
Meg. B. C. (washed 3 times)	141	Meg. Ext.	++++	+++	++	+	—	
		Anth. "	±	—				
Meg. B. C. (washed 3 times)+ Washings of Meg. Ag. C.	143	Meg. Ext.	++++	++++	++	+	+	—
		Anth. "	—					

Meg. B. C. ··· B. megatherium grown in nutrient both.

II. Comparative examinations for megatherium and anthrax antigens by agar-diffusion and absorption techniques.

1. *Agar-diffusion tests.* Antigens of both organisms were examined by the agar-diffusion technique (in 1.5 per cent agar) after Wilson and Pringle.[3] As seen in figures 2 and 3, antigens (cell extracts and washings) of *B. megatherium* and *B. anthracis* have formed three to four dense conjugate precipitate zones with antiserum, and these exhibit the presence of group-antigens. Specific precipitate zones are also seen between homologous antigens and antisera. These results may show the presence of specific antigens in each organism. However, the zone of *B. anthracis* which is presumable to be of a specific antigen is very faint as compared with the nonspecific ones.

Between extracts of each organism and its washings conjugate precipitate zones are seen and in the former, other faint zones.

2. *Absorption tests.* As seen in tables 4 to 6, absorption tests exhibit the existence of a specific antigen in *B. megatherium* which is clearly different from that of *B. anthracis*, and the results are in agreement with those of agar-diffusion tests. However, in the case of *B. antharcis*, the results are not agreement with those of agar-diffusion tests, showing only the presence of antigens common to *B. megatherium*, but no specific ones.

In the extract and washings of each organism only common antigens were seen and no such specific ones as seen in the extract in agar-gel.

* Details for the isolation and properties of the polysaccharide antigen are in a previous report[2].

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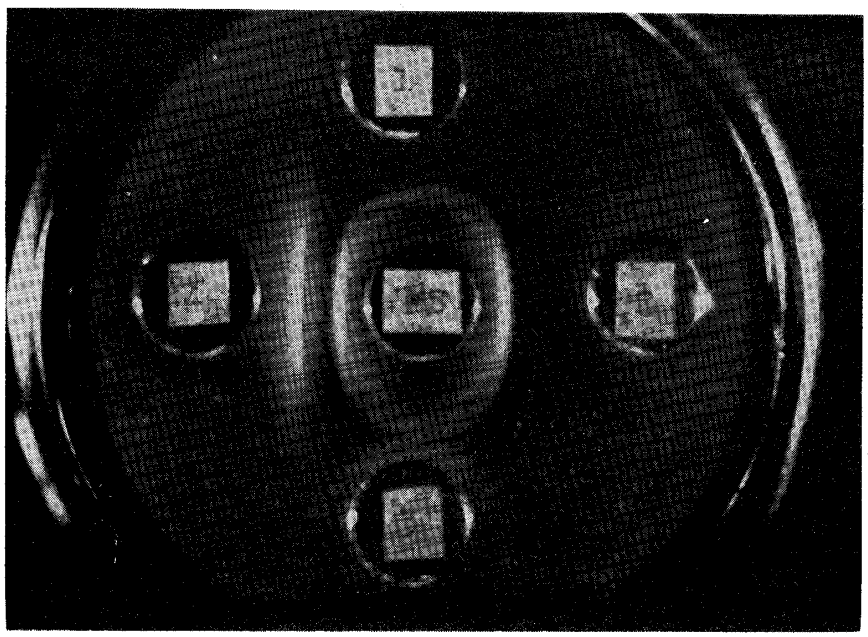

Figure 2. MS...Anti-megatherium serum,
1...Anthrax washings, 2...Anthrax extract, 3...Megatherium washings, 4...Megatherium extract.

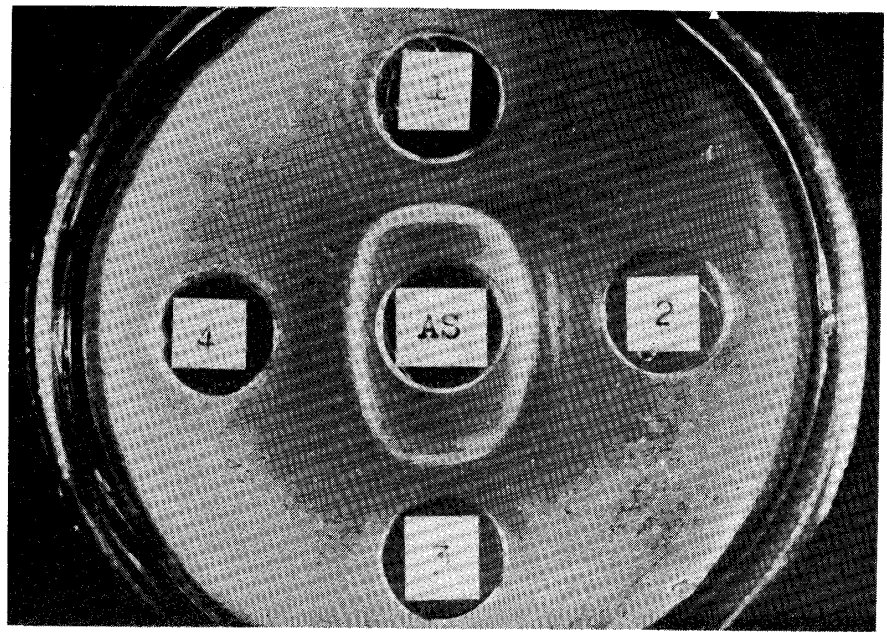

Figure 3. AS...Anti-anthrax serum,
1,2,3, and 4...Same as in figure 2.

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Table 4. Antigenic relation between B. megatherium and B. anthracis tested by absorption (1).
(Antigenic relation between extracts)

Antiserum	Antigen \ Dilution	1:100	1:400	1:1600	1:2300	1:6400	1:12800
Anti-megatherium serum	Meg. Ext.	++++	+++	++	+	+	−
	Anth. "	++++	+++	+	+	±	−
Anti-anthrax serum	Meg. Ext.	++++	++	+	+	−	−
	Anth. "	++++	+++	++	+	+	−
Anti-megatherium serum absorbed with Anthrax extract	Meg. Ext.	++++					
	Anth. "	−					
Anti-anthrax serum absorbed with Megatherium extract	Meg. Ext.	−					
	Anth. "	−					

Table 5. Antigenic relation between B. megatherium and B. anthracis tested by absorption (2).
(Antigenic relation between washings)

Antiserum	Antigen \ Dilution	1:1	1:2	1:4	1:8	1:16	1:32
Anti-megatherium serum	Meg. Wash.	++++	++	++	+	+	−
	Anth. "	+++	+	+	+	−	
Anti-anthrax serum	Meg. Wash.	++	+	+	−		
	Anth. "	++++	++	+	+	−	
Anti-megatherium serum absorbed with Anthrax washings	Meg. Wash.	++++					
	Anth. "	−					
Anti-anthrax serum absorbed with Megatherium washings	Meg. Wash.	−					
	Anth. "	−					

Wash. … Washings.

Table 6. Antigenic relation between extracts and washings of B. megatherium and B. anthracis.

Absorhed serum	Antigen	Reaction
Anti-megatherium serum absorbed with Meg. ext.	Meg. Wash.	−
	" Ext.	−
Anti-megatherium serum absorbed with Meg. wash.	Meg. Ext.	−
	" Wash.	−
Anti-anthrax serum absorbed with Anthrax ext.	Anth. Wash.	−
	" Ext.	−
Anti-anthrax serum absorbed with Anthrax wash.	Anth. Ext.	−
	" Wash.	−

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Summary and Conclusion

The results of the present experiment for antigenic relationships between *B. megatherium* and *B. anthracis* are:

1. In the case of immunization of rabbits with *B. megatherium*, significant differences were caused in the conditions of producing antibodies against *B. anthracis* by the preparing method of immunogens.

a. In the case of immunization with unwashed agar-grown cells of *B. megatherium*, production of antibodies against *B. anthracis* was in good yield, while with washed cells, though production of homologous antibodies did not decreased, yet that of antibodies which react with *B. anthracis* decreased significantly. These results may exhibit that antigens in *B. megatherium* which are common with *B. anthracis* are of a haptenic nature and in the washings of agar-grown megatherium may exist a substance which stimulates antibody production with these haptens. The properties of the stimulating substance have not yet been clarified in the present study.

b. In the case of immunization with *B. megatherium* grown in broth, washed cells, unwashed cells, and the washed cells to which were added the washings of agar-grown cells were all incapable of probucing antibodies which react with *B. anthracis*, although homologous antibody production was recognized. From these results, there seems to be a significant antigenic difference between agar-grown megatherium and its broth cultures. As it was reported[4], similar results were obtained in the case of immunogenicity of anthrax immunogens. These are interesting results and further comparative examinations on the problem are to be forewarded.

2. The results of the comparative examinations for antigenic relationships between extracts and washings of *B. megatherium* and *B. anthracis* by agar-diffusion and absorption tests are:

a. By the agar-diffusion tests, not only specific antigens to each organism but also common antigens were recognized between *B. megatherium* and *B. anthracis*. In the extract of each organism, antigens which differed from those in washings of the same organism were recognized.

b. By the absorption tests, the presence of some antigens in *B. megatherium* which differed from those of *B. anthracis* was recognized, but among the antigens which were derived from *B. anthracis* were no specific ones. In extracts and washings of each organism only common antigens were recognizable. As is stated above, the results of the agar-diffusion tests differ from those of the absorption tests, but the former results may be more valid. These differences in results may be due to the common active groups present in antigens, although they are heterologous, i.e. if common antigens are removed by absorption, the specific one may be removed at the same time by adsorption, or they may be due to the dilution of absorbed sera which may be incapable of precipitating specific antigens.

References

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