CHANGES IN BACTERIAL VOLUME AS THE RESULT OF SPECIFIC AGGLUTINATION

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The fact that during specific bacterial agglutination globulin from immune serum is deposited upon the bacterial surface, is well shown by the experiments of Northrop and De Kruif (1), Shibley (2), and Eagle (3). The more recent work of Heidelberger and Kendall (4) indicates that the precipitate resulting from the mixture of polysaccharide of the pneumococcus and a purified homologous antibody, varies in its composition over a definite range. At the optimum reaction the precipitate is composed of 1 part antigen to 120 parts serum globulin. In both agglutination and precipitation, the optical effect (flocculation) may be said to represent deposition of globulin. In the case of agglutination, particles (bacteria) of microscopic size become coated with globulin, while in precipitation, molecules, or aggregates of molecules, are coated. That the two substances, agglutinin and precipitin, are of similar nature has been indicated by experiments of a different character. Arkwright (5) found that *B. coli*, mixed with an extract of another organism, promptly agglutinated when immune serum specific for the extracted organism was added. Jones (6), and Mudd and his coworkers (7) showed that collodion particles agglutinated if first coated with antigen and later mixed with precipitin specific for the antigen.

If it be true that bacteria in the presence of immune serum are coated with globulin, and the ratio of deposition of globulin is comparable to that found by Heidelberger and Kendall in precipitation, bacteria should increase in size as the result of agglutination. It might be argued further that the increase in size would be within certain limits proportional to the quantity and titre of the immune serum employed in the reactions.

It was with this idea that a series of experiments was undertaken. We have been able to show that the variations in antigenic volume, as the result of agglutination, can be demonstrated by the procedures outlined. In the present paper we note the procedure and the results; discussion of the findings and interpretation of the facts will be left to another paper.

EXPERIMENTAL

Most of the experiments were of a similar nature and the same procedure was rigidly followed. Bacterial antigens were prepared from young cultures grown on plain agar, and the organisms were suspended in 0.9 per cent sodium chloride. The number of organisms was always sufficient to give a volume of 5 c.mm. or more per unit after centrifugation at 2900–3000 R.P.M. for 1 hour in the maxiforce centrifuge. Antigens of such concentrations contained two to three times as many organisms as those employed in the usual agglutination tests. Special pains were taken thoroughly to mix and to distribute the antigen. The same pipette was used throughout the series and where the entire contents of the pipette were not used the measurement was always between the same points.

The immune sera were not specially prepared for these experiments. Those for the paratyphoid group of organisms were obtained by immunization of rabbits; for *B. abortus* the serum of cows naturally infected was used.

The method of determining differences in volume was as follows: Various dilutions of immune serum were added to capillary centrifuge tubes which had been previously calibrated.[1] An additional tube containing only salt solution and antigen was carried as a control. One unit of antigen was added to each tube and its contents mixed. All tubes were incubated 2 or 2½ hours, centrifuged in the maxiforce for 1 hour at a speed of 2900–3000 R.P.M., and, by means of a divided ocular and a stage micrometer, the length of column under a magnification of 16 diameters was determined. From this the volume was calculated.

It might be mentioned that neither the period of incubation, provided it was an hour or more, nor subsequent storage in the refrigerator affected the results.

Experiments 1 and 2 record the volumetric change of antigens of motile and non-motile organisms when subjected to the action of specific serum.

Experiment 1.—The growth from three agar slants of 24 hour cultures of *B. aertrycke* was suspended in 35 cc. of 0.9 per cent sodium chloride. Various concentrations of diluted antiserum in quantities of 2.5 cc. were placed in the capil-

[1] We are indebted to Dr. M. Kunitz of The Rockefeller Institute for calibration of the capillary centrifuge tubes.

lary tubes and 2.5 cc. of antigen added. The tubes were incubated for 2 hours, refrigerated 4 hours, and centrifuged for 1 hour at 2900–3000 R.P.M. A tube containing one unit of antigen and an equal volume of sodium chloride served as a control. As a further control smaller quantities of antigen were tested with the

TABLE I

Volumetric Change in B. aertrycke as the Result of Agglutination

Serum dilution	Volume	Result of regular agglutination tests
	c.mm.	
1:80	9.86	C
1:160	9.33	C
1:320	8.8	++
		++
1:640	8.1	+++
1:1280	7.92	++
1:2560	7.57	+
1:5120	7.74	+−
Control, 0 serum	7.57	−

TABLE II

Volumetric Change in B. abortus as the Result of Agglutination

Dilution of serum	Volume	Result of regular agglutination test
	c.mm.	
1:5	7.39	C
1:10	7.57	C
1:20	7.39	C
1:40	6.86	C
1:80	6.68	C
1:160	6.68	C
1:320	6.51	C
1:640	6.16	C
1:1280	6.16	++
		++
1:2560	5.98	++
1:5120	5.46	+−
Control, 0 serum	5.28	−

immune serum in the regular manner and the results recorded after 2 hours' incubation and 20 hours' refrigeration. The results are given in Table I.

Experiment 2.—The experiment was similar to Experiment 1 except that a heavy suspension of *B. abortus* was employed as antigen and the immune serum

was that of a cow naturally infected. 5 cc. of antigen and 5 cc. of diluted serum were used in this experiment. The results are given in Table II.

The measurements recorded in Tables I and II indicate that there is an increase in the volume of the antigen in the presence of immune serum. There is in general a correlation between volume and concentration of antibody.

It might be inferred that during agglutination various substances were precipitated, thus adding to the volume. The actual specificity of the change in bulk is open to question since it could be said that normal serum or immune serum freed from antibody by specific absorption might produce volumetric increase. The next series of

TABLE III

Volumetric Change in B. aertrycke in Normal and Immune Serum

	Normal serum		Immune serum	
Dilution	Volume	Agglutination	Volume	Agglutination
	c.mm.		*c.mm.*	
1:20	5.28	+−	8.97	C
1:40	5.28	+−	8.09	C
1:80	5.28	−	7.39	C
1:160	5.28	−	7.21	C
1:320	5.28	−	6.86	C
Control, 0 serum	5.28	−		

experiments may be regarded as bearing on such points. It is typical of many in which volumetric changes in antigen in the presence of normal and immune serum are recorded.

Experiment 3.—Antigen from cultures of *B. aertrycke* was prepared in the usual manner and equal quantities were added to diluted normal rabbit serum and immune rabbit serum. Adequate controls were maintained. All tubes were incubated for 2½ hours and then centrifuged at the usual speed and the volume of the antigen determined. In Table III the results are recorded.

Experiments in which non-motile hog cholera bacilli were employed with both immune and normal serum produced similar results. The same was true with *B. abortus* except that the normal cow serum agglutinated the bacilli at low dilutions and had much the same effect

as a weak agglutinin with the result that there was an increase in volume at the lowest dilutions (1:5 and 1:10) in which the organisms were agglutinated.

The question of whether the phenomenon of volume increase might be caused by an addition of matter extraneous to antigen-antibody union was considered in Experiment 4.

Experiment 4.—Equal quantities of *B. abortus* suspension were mixed with diluted immune serum (cow). All tubes were incubated and centrifuged. The volumes were determined and all the supernatant was withdrawn and replaced

TABLE IV

Volume Change in B. abortus Exposed to Immune (Cow) Serum and Subsequently Washed Twice

Dilution	Volume in original mixture	After first washing in NaCl	After second washing in NaCl	Agglutination
	c.mm.			
1:5	7.39	7.39	7.39	C
1:10	7.57	7.39	7.39	C
1:20	7.39	7.57	7.39	C
1:40	6.86	7.21	6.86	C
1:80	6.68	7.21	6.34	C
1:160	6.68	7.04	6.16	C
1:320	6.52	6.69	6.69	C
1:640	6.16	6.33	5.46	C
1:1280	6.16	5.80	5.28	++
				++
1:2560	5.98	5.28	4.93	++
1:5120	5.46	5.01	4.75	+−
Control, 0 serum	5.28	5.01	4.75	−

with 10 cc. of 0.9 per cent sodium chloride solution. After thorough mixing the tubes were again centrifuged and the volumes redetermined. The washing and centrifugation were again repeated and the results recorded as indicated in Table IV.

The same results were obtained in a similar experiment when *B. aertrycke* and its immune serum were mixed and the agglutinated bacteria washed in sodium chloride solution. Washing and repeated centrifugation only served to accentuate more sharply the volumetric differences between agglutinated and unagglutinated organisms.

There is then no readily soluble substance of an extraneous nature that might be considered the cause of the increase in antigenic volume.

Experiment 5.—B. aertrycke immune serum diluted 1:5 was twice absorbed with massive numbers of the specific organism. After the final centrifugation the effect of the clear supernatant on the volume of antigen was determined. Another portion of the same serum, not subjected to absorption, was used for control. The volumes under the given conditions were determined after incubation and centrifugation and are recorded in Table V.

While it is true that some increase in the volume of the antigen occurred in the lower dilution of the absorbed serum, nevertheless the differences between the measurements in this series and those in the

TABLE V

The Effect of Specific Agglutinin Absorption on Volumetric Change in B. aertrycke

Absorbed serum			Unabsorbed serum	
Dilution	Volume	Agglutination	Volume	Agglutination
	c.mm.		*c.mm.*	
1:20	6.86	+++		
1:40	6.86	++	9.68	C
1:80	6.51	+	8.62	C
1:160	5.63	+−	8.10	++
1:320	5.63	−	7.57	++
				++
1:640	5.80	−	7.39	+++
Control, 0 serum	5.80	−	5.80	−

other are sharp. The absorbed serum behaved like a weak agglutinin, and in fact can be so regarded, since all the agglutinin was not removed by absorption. The same effects were noted with *B. abortus* in a specifically absorbed serum.

DISCUSSION

We have already stated that the aim of the experiments was to determine whether or not there was a detectable increase in the volume of antigen as the result of agglutination. The experiments indicate that by the procedure described, differences in antigenic volume can be measured. As a general proposition it is true that increase in

volume appears to follow concentration of immune serum. The effect of immune serum is specific since antigenic volume is not appreciably affected in normal serum nor in specific serum from which agglutinins have been removed by previous adsorption with antigen. Experiments in which antigenic volume was measured after agglutination and the packed organisms subsequently twice washed further indicate that the increase is a persistent one and cannot be attributed to the precipitation of matter extraneous to the antigen-antibody union.

Further discussion of the fact that there occurs, as the result of specific agglutination, an increase in antigenic volume will be left for a later paper.

SUMMARY

Measurements indicate that bacterial antigens increase in volume as the result of specific agglutination. There is a general parallelism between the increase in antigenic volume and the concentration of the immune serum. The phenomenon is specific. There is no increase with normal serum; with absorbed serum the increase is slight and it can be correlated with the presence of unabsorbed antibody. The effect is enduring as shown by volumetric determinations upon repeatedly washed, agglutinated bacteria.

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