

## THE STABILITY OF BACTERIAL SUSPENSIONS.

### V. THE REMOVAL OF ANTIBODY FROM SENSITIZED ORGANISMS.

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The experiments described in the preceding paper (1) show that the combination of antibody and organism in the case of *Bacillus typhosus* is less on the acid side of pH 4.0 than on the alkaline side. It seemed possible, therefore, that antibody could be more completely removed by washing at pH 3.0. Experiment showed, however, that this was not the case. It was further found that much more antibody could be recovered by washing with distilled water than by washing with salt solution. This is in confirmation of the results of Huntoon (2) and other workers. The result of such an experiment is given in Table I. The technique was the same as that described in the preceding paper. It is evident that some of the immune body is easily removed but that the equivalent of about twelve agglutinating doses remains on the organisms. The suspension which had originally less than this number did not give up any measurable immune body. If it be supposed that the immune body forms a film on the surface of the organism, this is the result that would be expected. It has been shown by Langmuir (3) in the case of gaseous films that the first layer of molecules can be removed only with the greatest difficulty, whereas the succeeding ones are relatively easily removed. It was shown in a former paper that with optimum salt concentration agglutination occurred with one-eighth to one-sixteenth of the amount of serum required to cause agglutination in the most dilute salt. This result agrees with the present experiment in indicating that agglutination will occur when the surface is about one-eighth covered and therefore that a complete surface film represents about eight agglutinating doses.

TABLE I.

*Recovery of Immune Body from Bacterial Sediments.*

Supernatant fluid.	pH at which sediment had absorbed immune body.	Supernatant fluid diluted with buffer pH 6.3, 43°C., 14 hrs.						Units in sediment before washing.	Recov-ered.	Anti-body remain- ing on or- ganism.
		0.5	0.25	0.125	0.06	0.03	0.015			
Supernatant fluid No. 1 from buffer pH 7.5 treatment.	7.5	++	Tr.	-	-	-	-	16	-	16
	5.2	+	-	-	-	-	-	16	-	16
	4.7	+	Tr.	-	-	-	-	16	-	16
	3.9	+	Tr.	-	-	-	-	14.7	-	15
	3.3	++	Tr.	-	-	-	-	14	-	14
	3.0	++	Tr.	-	-	-	-	7.8	-	7.8
Supernatant fluid No. 2 from distilled water treatment.	7.5	C.	C.	C.	C.	++	Tr.	16	4.1	12
	5.2	C.	C.	C.	C.	++	+	16	4.1	12
	4.7	C.	C.	C.	C.	++	Tr.	16	4.1	12
	3.9	C.	C.	C.	++	++	Tr.	16	4.1	12
	3.3	C.	C.	++	+	-	-	14	1.7	12.3
	3.0	Tr.	-	-	-	-	-	8.2	<1	7.8

TABLE II.

*Effect of Repeated Distilled Water Washing on the Acid Agglutination Optimum of B. typhosus Laden with Immune Body.*

Series.	No. of washings of sediment + immune body.	43°C. 14 hrs.								3.3 = pH of buffer.
		7.9	7.5	6.6	6.0	5.5	5.0	4.4	4.0	
1	Not washed.	++	++	C.	C.	C.	C.	C.	C.	C.
2	1	+	+	+	++	++	C.	C.	C.	C.
3	2	Tr.	+	+	++	++	C.	C.	C.	C.
4	3	Tr.	Tr.	Tr.	+	+	++	C.	C.	C.
5	4	-	Tr.	Tr.	+	+	++	C.	C.	C.
6	5	-	Tr.	Tr.	+	+	++	C.	C.	C.
7	6	-	-	Tr.	+	+	++	C.	C.	C.
8	7	-	-	Tr.	Tr.	+	+	C.	C.	C.
9	8*	-	-	Tr.	Tr.	Tr.	+	++	C.	C.
10	Control suspension (no immune body).	-	-	Tr.	Tr.	Tr.	Tr.	+	C.	C.

\* Stood over night at room temperature in distilled water, 3.0 cc.

The question arises whether all the immune body can be removed if washing is sufficiently prolonged. This cannot be determined by testing for the recovered antibody, since the solution is so dilute that no reaction is obtained.

It was shown in a preceding paper (4) that the immune serum widened the acid agglutination zone and moved it to the alkaline side. The limit of acid agglutination, therefore, serves as a sensitive test for the presence of immune body. The following experiment shows that sufficient washing removes all the immune body.

6 cc. serum anti-*typhosus* (1:50 in buffer pH 6.3) + 6 cc. of 10 times standard *typhosus* suspension, 18°C., 5 minutes.

The mixture was centrifuged, the supernatant fluid pipetted off, and the sediment resuspended in 10 cc. of distilled water.

1 cc. of the resulting heavy suspension was diluted to 10 cc. with distilled water, and of this second suspension 1 cc. amounts were tested against buffer series pH 7.9 to 3.3.

The balance of the heavy suspension (9 cc.) was centrifuged, resuspended in 9 cc. of distilled water, 1 cc. removed, diluted to 9.0 in distilled water, as before, and the resulting suspension tested against the same buffer series.

This process was repeated 8 times, the distilled water for washing being reduced in amount by 1 cc. each time. This was done to keep the concentration of the original suspension of organisms constant, since 1 cc. was removed at every washing for the preparation of the dilute suspension to be tested against the buffer series.

Table II shows that the acid agglutination zone of organisms laden with immune body is greatly broadened. Thus in Series 1, Table II, the unwashed sediment is agglutinated completely at pH 6.6, and ++ agglutination occurs at pH 7.5 and 7.9. As washing proceeds, and the immune body is removed, progressively higher  $C_H$  is necessary to cause complete flocculation. At the eighth washing (Series 9) the agglutinability by acid has returned practically to that of organisms to which no immune body has been added—Series 10. The immune body can therefore be completely removed by sufficient washing.

This experiment suggests the use of this method as a test for the presence of agglutinins in bacterial suspensions. A modification of the method would be to determine the salt concentration required for agglutination, since this is also a sensitive test for immune body.

## SUMMARY.

1. The removal of antibody from *Bacillus typhosus* is no more complete at pH 3 than at pH 7.

2. Approximately twelve agglutinating doses are firmly combined with the organisms. Immune body in excess of this amount is easily removable by distilled water.

3. A method of testing for the presence of immune body on the organism is described which depends on the difference in the acid agglutination of sensitized and unsensitized organisms.

4. Repeated washing in distilled water will serve to remove all the immune body from sensitized bacteria.

## BIBLIOGRAPHY.

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