

The points of the chitinous ribs which strengthen the segments of the ovipositor project above the upper border of the segment, and to them are attached the muscles of the ovipositor. The narrowed terminal portion of the rectum enters the ovipositor on the dorsal surface of the uterus and runs down to the anal opening between the external plate and the last segment.

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*The Specificity of the Opsonic Substances in the Blood Serum.*

By WILLIAM BULLOCH, M.D., and G. T. WESTERN, M.A., M.B.

(Communicated by Leonard Hill, F.R.S. Received February 15,—Read March 1, 1906.)

(From the Bacteriological Laboratory, London Hospital, E.)

A relatively high degree of specificity has been demonstrated for most of the antibodies which exist in immune sera, *e.g.*, in the case of agglutinins, lysins, præcipitins, antitoxins. With normal sera the proof of specificity is often difficult on account of the fact that the antibodies are present in the majority of cases only in small quantities.

The following experiments are concerned with the specificity of the opsonic substances of normal and immune sera. As is well known, these opsonic substances, discovered by Wright and Douglas, act on bacteria in such a way that the latter become an easy prey to the phagocytic leucocytes.

If a given serum be tested it will be found to exert an opsonic action on more than one kind of bacterium, and the question we have sought to answer is whether there is one or more than one opsonic substance; in other words, whether the opsonins are specific for the different bacteria on which they exert their opsonic action.

In a previous communication\* one of us (B.) has shown that when a microbe, *e.g.*, staphylococcus, is digested with normal serum at 37° C. for 15 minutes, and the cocci are then brought down by the aid of a centrifuge, the supernatant liquid is found to be devoid of opsonic action for staphylococci. Where the contact of the microbe with serum has been sufficiently long, and the centrifugalisation has been complete, the opsonin for the particular microbe is totally removed.

\* 'Roy. Soc. Proc.,' vol. 74.

We have attempted to determine whether the opsonins are specific by experiments of two kinds:—

1. The first method consisted in estimating the opsonic content of a given serum towards two different bacteria. A suspension of one of these bacteria was digested with the serum, and the mixture was thereafter centrifugalised, the resulting supernatant liquid being tested on both kinds of bacteria. To a quantity of the supernatant liquid the second bacterial suspension was added, and after the lapse of a certain time the centrifuge was again applied, and the resulting liquid was again tested.

2. The second method consisted in estimating from day to day the opsonic content of the serum of human beings suffering from lupus. At certain periods tubercle or staphylococcus vaccines were inoculated, and the effect on the two opsonic curves was determined.

1. Experiment on the opsonic action of normal human serum towards *Staphylococcus aureus* and *Bacterium pyocyaneum* respectively.

Normal human serum was mixed with an equal volume of a suspension of *Staphylococcus aureus*, and the mixture was placed in the incubator for 1 hour at 37° C. At the end of this time the mixture was centrifugalised, the supernatant liquid "A" being removed from the deposit of cocci by means of a pipette. The supernatant liquid was in part retained, the remainder being digested for 1 hour at 37° C. with a suspension of *Bacterium pyocyaneum*, the latter being finally brought down as a deposit in the centrifuge, leaving a supernatant liquid "B," which was pipetted off.

#### Result.

1. Normal serum (1 in 2 dilution) + staphylococci	+ leucocytes	= 22.9	} Bacteria per leucocyte.
2. " " (1 in 2 " ) + <i>B. pyocyaneum</i>	+ "	= 4.7	
3. " " (1 in 4 " ) + "	+ "	= 3.0	
4. Fluid "A"	+ staphylococcus	= 0.5	
5. " "A"	+ <i>B. pyocyaneum</i>	= 4.0	
6. " "B"	+ "	= 0.4	

The contact of the serum with staphylococcus leaves the opsonic action of the serum for *Bacterium pyocyaneum* practically unchanged, the pyocyanic opsonin being finally removed by contact of the serum with this microbe.

A similar result was obtained when the serum was brought to act on staphylococcus and tubercle bacillus, as can be seen in the following experiment.

1. Normal human serum was mixed with an equal quantity of an emulsion of tubercle bacilli in 0.85 per cent. NaCl solution. The mixture was digested for 30' at 37° C. and then centrifuged. In this way a deposit and a supernatant liquid "A" was obtained.

No. of Microbes per Leucocyte.

	Expt. I.			Expt. II.
	B.	W.	Mean.	
1. Normal serum + saline <i>a.a.</i> (3 parts) + T.B. (1 part) + leucocytes (3 parts) .....	3.03	3.0	3.015	1.61
2. Normal serum + saline <i>a.a.</i> ( " ) + staphylococcus ( " ) + " ( " ) .....	12.6	12.3	12.45	7.00
3. Normal serum 1 + saline 3 ( " ) + T.B. ( " ) + " ( " ) .....	1.4	1.4	1.4	1.40
4. Normal serum 1 + saline 3 ( " ) + staphylococcus ( " ) + " ( " ) .....	11.0	11.4	11.2	5.20
5. Fluid "A" ( " ) + T.B. ( " ) + " ( " ) .....	0.4	0.5	0.45	0.13
6. " "A" ( " ) + staphylococcus ( " ) + " ( " ) .....	9.0	10.23	9.96	5.00
7. " "B" ( " ) + T.B. ( " ) + " ( " ) .....	2.7	—	2.7	1.20
8. " "B" ( " ) + staphylococcus ( " ) + " ( " ) .....	0.43	0.26	0.34	0.80
9. " "C" ( " ) + " ( " ) + " ( " ) .....	0.13	0.26	0.19	0.40
10. " "D" ( " ) + T.B. ( " ) + " ( " ) .....	0.16	0.76	0.51	0.32
11. " "A" (3 parts) + saline (1 part) + leucocytes (3 parts) (stained for T.B.) .....	0.09	0.0	0.09	0.10
12. " "B" ( " ) + " ( " ) + " ( " ) ( " ) staphylococcus	0.0	0.0	0.0	0.00
13. " "C" ( " ) + " ( " ) + " ( " ) ( " ) "	0.0	0.0	0.0	0.00
14. " "D" ( " ) + " ( " ) + " ( " ) ( " ) T.B.) .....	0.05	0.0	0.05	0.00
15. Saline, 0.85 per cent. (3 parts) + T.B. (1 part) + leucocytes (3 parts) .....	0.0	0.08	0.08	0.06
16. " "0.85 " ( " ) + staphylococcus ( " ) + " ( " ) .....	0.13	—	0.13	0.08

2. Normal human serum was mixed with an equal quantity of an emulsion of *Staphylococcus aureus* in 0·85 per cent. NaCl solution. The mixture was digested for 30' at 37° C. and then centrifuged, a supernatant liquid "B" being obtained.

3. The fluid "A" was mixed with an equal quantity of an emulsion of *Staphylococcus aureus*. The mixture was digested for 30' at 37° C. and a deposit separated from a fluid "C" by the centrifuge.

4. The fluid "B" was mixed with an equal quantity of an emulsion of tubercle bacilli. The mixture was digested for 30' at 37° C., and a deposit separated from a fluid "D" by the centrifuge.

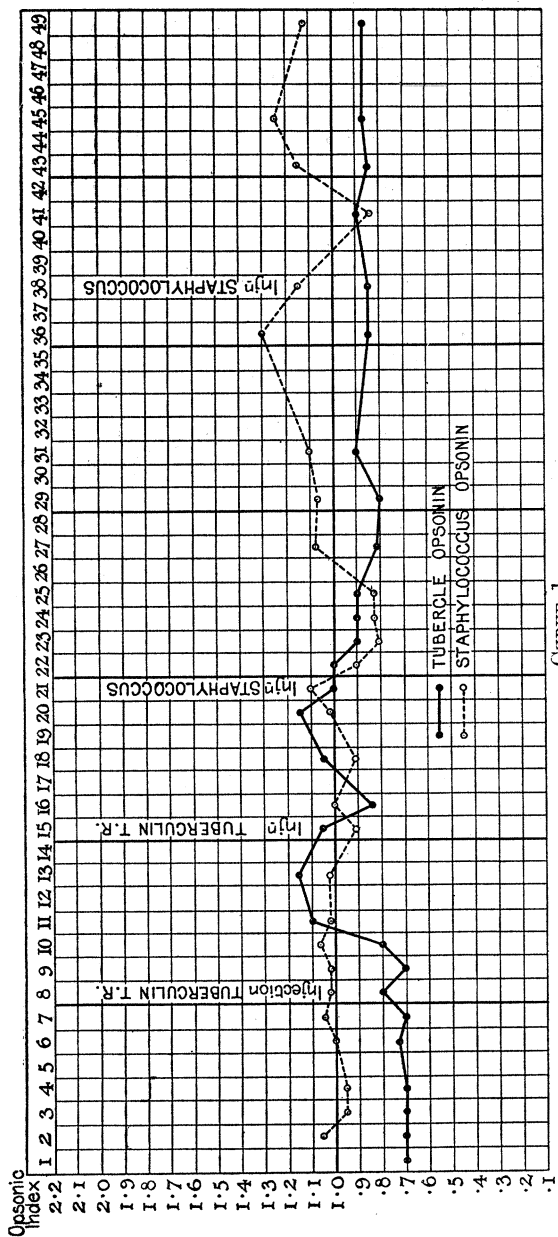
The opsonic content of the serum and of the fluids "A," "B," "C," and "D" was then determined both for *Staphylococcus aureus* and tubercle bacillus in the usual way, the necessary controls being added. In the first experiment the determinations made by each of us separately in a series of different films are given under the designation B. and W., and the mean of these determinations. In the second experiment the result was obtained by one of us (W.) alone.

It will be seen that a considerable degree of specificity exists in so far that staphylococci remove almost the whole of the opsonin for this microbe, while the opsonic substance for tubercle bacilli is in large part left unaltered. In almost all cases we have observed a slight diminution in the quantity of the opsonin left behind. Thus while the contact of a serum with tubercle bacilli lowered the opsonic content for this bacillus from 3·03 to 0·4, it also produced a slight lowering of the staphylococcus opsonin from 11·2 to 9·96. Similarly, contact of a staphylococcus with serum reduced the staphylococcus opsonin from 12·45 to 0·34, and at the same time it lowered the tubercular opsonin from 3·015 to 2·7.

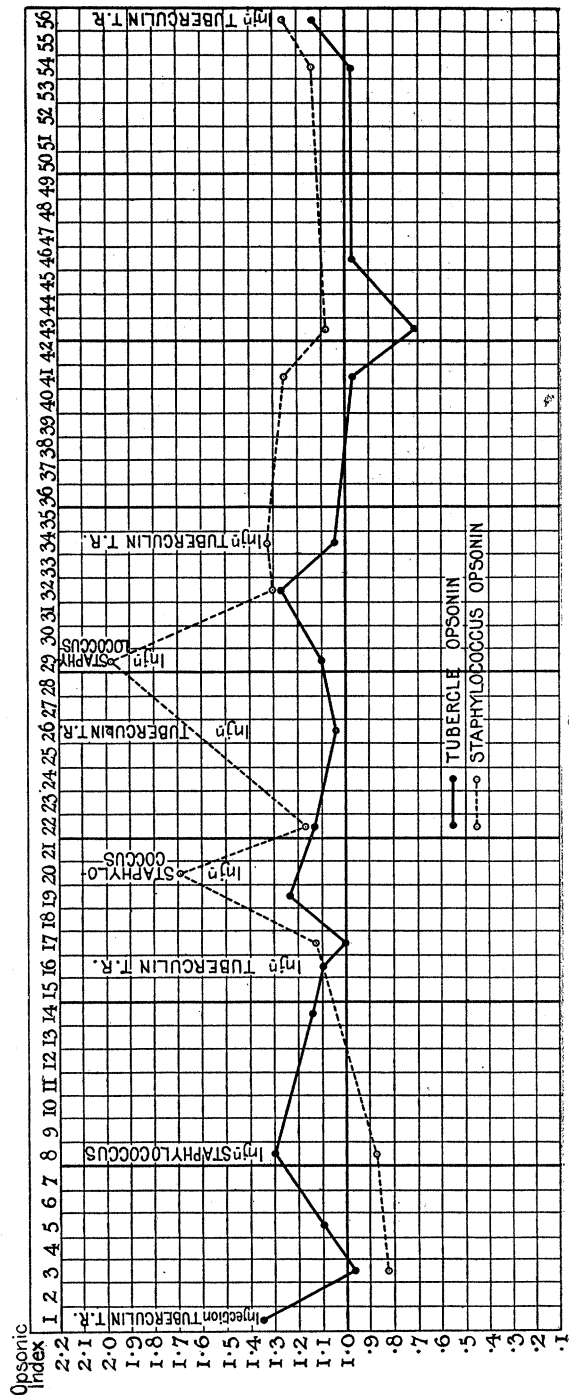
## 2. Experiment:—

The opsonic content of the serum of a patient suffering from lupus was repeatedly determined on tubercle and staphylococcus suspensions. Two inoculations of tuberculin and two of staphylococcic vaccine were injected, and the influence of the inoculations is set forth in the following opsonic curve, which shows that there is no correspondence in the quantities of tuberculo-opsonins and staphylococcus opsonins when one or other of the corresponding vaccines is inoculated (Case I).

In a second experiment (Case II) opsonic determinations were made in a similar case, with the exception that the patient was not only suffering from lupus, but septic infection of the tuberculous lesions at the same time (Curve 2).



CURVE 1.



CURVE 2.

*Conclusions.*

1. When staphylococci are brought into contact with normal human serum and are subsequently removed by centrifugalisation, the serum loses its opsonic power for staphylococcus, although the opsonic power of *Bacterium pyocyaneum* is preserved.

2. Contact of normal human serum with tubercle bacilli leaves the opsonic power of that serum for staphylococcus almost intact, while the opsonic power for tubercle bacillus is completely removed.

3. Contact of normal human serum with staphylococcus leaves the opsonic power of that serum for tubercle bacillus almost intact, while the opsonic power for staphylococcus is completely removed.

4. Inoculation of a human being with tuberculin causes quantitative increase in the tuberculo-opsonin, whereas the quantity of staphylococcus opsonin is unaltered.

5. Inoculation of a human being with staphylococcus vaccine causes a quantitative increase in the staphylococcus opsonin, whereas the quantity of tuberculo-opsonin is unaltered.

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