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THE FLOCCULAR AND GRANULAR AGGLUTININS IN
TYPHOID FEVER.

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AN outbreak of typhoid fever in the South West district of London during the latter part of 1929 supplied the opportunity of estimating the floccular ("H") and granular ("O") agglutinins in the blood of a number of cases, both during their illness and some months after their recovery. None of these patients had been inoculated. It was also possible to compare the results with the agglutinins to be found in the blood of soldiers who had been given antityphoid inoculation at some time during their service. These inoculated controls were men who for some reason or another had been sent to one of the army laboratories for blood-tests.

The interest of this investigation lies in the opportunity it affords for estimating the value of "the qualitative receptor analysis" as a means of diagnosing enteric fever, and in the production of certain observations which have a distinct bearing on immunity in general.

In the following article it will be necessary to give some value to the agglutinin content of a serum, and this is best done by simply quoting the denominator of the final dilution in which a standard agglutination has occurred; in the same way it is possible to give a value to the average amount of agglutinin in a group of sera.

I.

The method advocated by Felix (1924) for serologically differentiating between a typhoid case and an inoculated man not infected with *B. typhosus* depends for its applicability on two fundamental principles:—

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(1) That "O" agglutinins are more closely identified with the actual disease than the "H" agglutinins.

(2) That "O" agglutinins are not produced by inoculated persons to any significant extent.

The results obtained in this investigation amply confirm the first, whilst only partially agreeing with the second of these principles.

In proof of the first principle it will be seen, by referring to Tables I and II, that if the "H" and "O" agglutinins of the typhoid cases and of the inoculated men are averaged the following will be the values of each:—

		"H" agglutinins	"O" agglutinins
Average of 15 typhoid cases	710	651
Average of 54 inoculated men	515	49.

Thus the disease and the inoculation produce comparable amounts of "H" agglutinin, whereas the disease produces twelve times as much "O" agglutinin as does inoculation, proving that the "O" agglutinins are more closely identified with the disease than are the "H" agglutinins.

With regard to the second principle referred to above, it is obvious from the figures quoted that the amount of "O" agglutinin in the average typhoid case greatly exceeds the amount in the average inoculated man, but Tables I and II show that certain individual cases of typhoid fever possess no more "O" agglutinins than do certain healthy inoculated persons. This point is illustrated by Nos. 2, 5, 9, 10, 12, 14, 15, in Table I, and Nos. 19, 30, 31, 38, 39, 42, 52, 58, 61, 62, in Table II.

There are two possible reasons for these facts:—

(1) That a proportion of inoculated persons *have* produced a significant amount of "O" agglutinin.

(2) That a number of proved cases of typhoid fever, amounting to forty-four per cent of those examined, have failed to produce a significant amount.

Either of these reasons for the observed facts tends to limit the general application of Felix's differential method of diagnosis.

An attempt has been made to find out what amount of "O" agglutinins may be justifiably considered to be significant of some association with the typhoid antigen. For this purpose the blood of sixty-seven normal uninoculated persons has been examined, and the results are summarized in Table III. It will be noticed that "O" agglutination took place in a dilution of 1 in 50 twice. None of these cases gives any history of having had typhoid fever, and one must therefore assume that no significance can be attached to granular agglutination in a dilution of less than 1 in 125 with the particular "O" suspension in use during this investigation.

If, therefore, the dilution of 1 in 125 is taken to be the dilution of "significance," it will be seen that ten, that is about twenty per cent of the inoculated men, showed a significant amount of "O" agglutinin. The estimation of "O" agglutinins would appear, therefore, to have its limitations as a means of eliminating uninfected inoculated persons.

In this connexion Case No. 4 is of particular interest in that the "H" agglutinins were entirely absent during the first few weeks of illness, and the case could only have been diagnosed serologically by estimating the "O" agglutinins. Similar cases have been reported in Palestine (Felix, 1924), in South Africa (Pijper, 1930), and in England (Gardner, 1930).

II.

The lack of a reliable test for immunity against typhoid fever has continually handicapped work in connexion with prophylactic vaccination, and it appears a suitable opportunity for attempting to find some common "function" present in the blood of persons who are immune or partially immune and which is absent from the blood of the entirely unprotected.

The agglutinins are an obvious field for investigation, and of the two kinds the "O" agglutinin seems the most promising, because it has been shown to be more closely connected with the actual disease, and because it has been proved experimentally that the "O" portion of an organism is the one chiefly concerned in the production of artificial immunity. (Felix, 1924; Arkwright, 1927; Topley, 1929.)

Reference to Tables I, II and III shows:—

(1) That during the actual course of typhoid fever, "O" agglutinins are very prominent in the blood; (2) that a proportion of inoculated persons show a significant amount of "O" agglutinins; and (3) that none of the non-inoculated persons examined shows any significant amount of "O" agglutinins.

Here, then, would seem to be a satisfactory test of immunity, but if reference is again made to Table I it will be seen that out of ten cases that were tested six months after their recovery from typhoid fever only two showed a significant amount of "O" agglutinin (see Nos. 1 to 10, Table I). Their immunity, however, was presumably quite as good, if not better, at this time than during the first few weeks of the disease.

For these reasons it is obvious that the presence of "O" agglutinins in the blood cannot be accepted as the only criterion of immunity.

It has been suggested by Topley that "the ability to produce antibodies briskly and in large amounts in response to a relatively slight specific stimulus may be just as effective from the point of view of potential immunity as the possession of an adequate supply of preformed antibodies in the blood and tissue fluids."

If this surmise is correct, it should not be difficult to formulate some test by which the rate of antibody response to a minimal stimulus may be suitably measured and used as an indication of the amount of immunity possessed by an individual.

III.

The details of the tests here recorded are as follows:—

Floccular ("H") agglutinins were demonstrated by using an agglutinating suspension which had been standardized against the Oxford standard emulsion and had a suspension factor of 7.3.

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The highest dilution to show Dreyer's standard agglutination, after two hours in a water bath at 56° C., was taken as indicating the "H" agglutinin content of a serum.

Granular ("O") agglutinins were demonstrated by using an alcoholized suspension of a strain of *B. typhosus* (O.901) kindly given me by Dr. Felix, from whom I have received every kind of assistance.

Gardner's method is the one employed in making up the alcoholized emulsion, and a sufficient quantity was made to last the whole period of the investigation.

TABLE I.—SHOWING THE FLOCCULAR ("H") AND GRANULAR ("O") AGGLUTININS IN THE BLOOD OF PERSONS DURING AND AFTER AN ATTACK OF TYPHOID FEVER.

Number	Day of disease	"H" agglutination	"O" agglutination	Particulars
1	14th After 6 months	1/1,250 1/50	1/500 Nil	<i>B. typhosus</i> in stool. Moderately severe.
2	11th After 6 months	1/65 Nil	1/125 1/50	<i>B. typhosus</i> in blood and stool. Severe, relapse and hæmorrhage.
3	16th After 6 months	1/1,250 1/32	1/500 1/50	<i>B. typhosus</i> in stool. Moderately severe.
4	25th After 6 months	Nil 1/500	1/1,250 1/250	<i>B. typhosus</i> in stool. Severe, delirium, high fever.
5	23rd After 6 months	1/125 Nil	1/25 1/50	<i>B. typhosus</i> in stool. Severe, two relapses.
6	18th After 6 months	1/365 Nil	1/2,500 1/50	<i>B. typhosus</i> in stool. Severe, delirium, high fever.
7	50th After 6 months	1/645 1/73	1/500 1/125	<i>B. typhosus</i> in stool. Moderately severe.
8	41st After 6 months	1/5,000 1/25	1/500 Nil	<i>B. typhosus</i> in urine. Moderately severe.
9	16th After 6 months	1/730 1/73	1/250 1/50	Agglutinated <i>paratyphosus</i> A, 1/25. No agglutination with <i>paratyphosus</i> B or <i>melitensis</i> . Mild attack.
10	18th After 6 months	1/250 1/25	1/125 Nil	No agglutination with <i>paratyphosus</i> A, B, or <i>melitensis</i> . Moderately severe.
11	10th	1/250	1/500	<i>B. typhosus</i> in blood. Moderately severe.
12	10th	1/50	1/250	<i>B. typhosus</i> in stool. Moderately severe.
13	21st	1/500	1/2,500	<i>B. typhosus</i> in stool. Moderately severe.
14	19th	1/125	1/125	No agglutination with <i>paratyphosus</i> A, B, or <i>melitensis</i> . Moderately severe.
15	16th	1/50	1/125	No agglutination with <i>paratyphosus</i> A, B, or <i>melitensis</i> . Very severe—died from perforation.

TABLE II.—SHOWING FLOCCULAR (“H”) AND GRANULAR (“O”) AGGLUTININS IN THE BLOOD OF INOCULATED SOLDIERS.

Number	When inoculated	“H” agglutination	“O” agglutination	Remark
18	15 months previously	1/1,500	—	*
19	14	1/250	1/125	P.
20	12	1/125	1/25	P.
21	12	1/250	1/25	—
22	9	1/125	—	—
23	9	1/280	1/50	—
24	8	1/28	—	*
25	8	1/280	1/50	*
26	8	1/250	1/25	*
27	7	1/325	1/50	*
28	7	1/56	1/25	*
29	6	1/2,500	—	—
30	6	1/50	1/125	P.
31	6	1/325	1/250	—
32	6	1/50	1/50	—
33	6	1/300	—	—
34	6	1/280	1/25	*
35	5	1/500	1/25	—
36	5	1/500	—	*
37	4	1/125	1/50	—
38	4	1/250	1/250	—
39	4	1/125	1/125	—
40	4	1/500	—	*
41	3	1/125	1/25	—
42	3	1/250	1/125	—
43	3	1/125	1/50	—
44	2	1/125	1/25	P.
45	2	1/125	1/25	—
46	2	1/500	1/50	—
47	2	1/125	—	P.
48	2	1/322	1/50	—
49	1	1/500	1/25	—
50	1	1/50	1/50	—
51	1	1/250	—	P.
52	1	1/1,250	1/125	—
53	1	1/50	1/25	—
54	1	1/2,500	—	—
55	1	1/2,500	1/50	—
56	1	1/250	—	—
57	1	1/500	1/50	—
58	1	1/250	1/125	—
59	1	1/125	1/25	—
60	1	1/1,250	1/50	—
61	1	1/1,250	1/125	*
62	1	1/500	1/250	—
63	1	1/125	1/50	—
64	1	1/250	—	—
65	1	1/500	1/25	—
66	1	1/125	—	—
67	1	1/1,250	1/25	—
68	?	1/125	—	—
69	?	1/645	—	—
70	?	1/300	—	—
71	?	1/1,612	1/50	—

Note 1.—All the soldiers have been inoculated more than once except those marked “P,” who have only received their first full inoculation, that is to say, two injections of $\frac{1}{2}$ and 1 c.c. at ten days' interval.

Note 2.—Those marked with an * gave no history of having ever suffered from enteric fever, and it must be noted that two out of the eleven of these men show a significant amount of “O” agglutinins, but as regards the remainder of the men they were not questioned on this point.

It is conceivable that a person with a previous history of enteric fever would be more likely to produce “O” agglutinins after inoculation than a person with no such history, but in view of the fact that these men were quite young soldiers, it is permissible to regard the incidence among them of a past history of enteric as being the same as it would be among a civilian population in the British Isles. This incidence would, I submit, make no real difference to the conclusions that have been drawn in this article.

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The "O" agglutinin content of a serum was indicated by the highest dilution in which clear-cut granulation could be seen in the supernatant fluid with a watchmaker's glass (by $3\frac{1}{2}$) after the tubes had been eighteen hours in a water bath at 56° C.

Besides the usual saline control tube with each serum tested, the alcoholized suspension was put up against a range of saline concentrations both at the beginning and the end of the investigation, and there was no sign of any salt agglutination (Arkwright). The strengths of saline used in these tests were as follows: 0.85 per cent, 1.7 per cent, 3.4 per cent, 6.8 per cent.

TABLE III.—GIVING A SUMMARY OF THE FLOCCULAR ("H") AND GRANULAR ("O") AGGLUTININS IN THE BLOOD OF SIXTY-SEVEN UNINOCULATED NORMAL PERSONS TAKEN AT RANDOM.

Number of cases				Dilution in which the standard "O" agglutination occurred
12	1/25
2	1/50
53	No "O" agglutination
—				
67				

None of the 67 persons gave any "H" agglutination at all.

The alcoholized suspension was also tested against a pure typhoid "O" serum and it granulated up to 1/5,000, which was the titre of the serum. It was also tested against a typhoid serum in which the "H" antibody was greatly in excess, the flocculation titre being 1/10,000. In this case the suspension gave a granulation up to 1/1,000, but there was no sign of any flocculation.

These control tests give ample proof that the alcoholized suspension used during this investigation was a stable suspension, which was unaffected by floccular agglutinins but reacted well with granular agglutinins.

SUMMARY AND CONCLUSIONS.

(1) That "O" agglutinins are more closely identified with the actual disease of typhoid fever than are "H" agglutinins.

(2) That in a large proportion of cases the estimation of the "O" agglutinins provides a method of differentiating a typhoid case from an inoculated person uninfected with the disease.

(3) That this method has definite limitations.

(4) That the presence of "O" agglutinins in the blood is some indication of immunity.

(5) That the absence of "O" agglutinins in the blood is no proof that there is no immunity.

(6) That the significance of immunity may lie in the ability to produce a large amount of antibody rapidly and in response to a minimal stimulus.

(7) That the rate of antibody response to a minimal stimulus might supply the much-needed test for immunity.

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ADDENDUM.

Since the above was written I have examined an additional 30 sera from inoculated soldiers, none of whom gives any past history of enteric fever. Amongst these men there were four who showed a significant amount of "O" agglutinin.

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