SEROLOGICAL DIAGNOSIS OF ENTERIC IN THE INOCULATED

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Introduction

The introduction of preventive inoculation against typhoid and paratyphoid, and in particular its widespread application during the Great War, caused considerable difficulty in the interpretation of the Widal test. It was often impossible to decide whether the agglutinins demonstrated were due to active infection or to previous inoculation.

In the civilian population of this country the problem is not now of any moment, but it is still of importance in inoculated communities, notably in the Navy, Army and Air Force.

Dreyer and his colleagues (1915, 1916, 1917) suggested a means of overcoming the difficulty. They proposed that the test should be repeated during the course of the illness at intervals of about four days. A regular rise of the agglutination titre for one of the typhoid or paratyphoid organisms attaining its maximum between the 16th and 20th day of the disease would indicate active infection with that organism. This procedure has proved of some value, but is often impossible and always laborious.

An attractive and easy solution appeared to be offered by Felix (1923, 1924) with the introduction of his method of "qualitative receptor analysis". Felix's views are too well known to require detailed recapitulation. Briefly, he maintained that inoculation did not produce "O" agglutinins but only "H" agglutinins. If marked "O" agglutination could be demonstrated with serum diluted 1 in 100 or 1 in 200 (the dilutions he recommended), then the patient was suffering from enteric.

In his contentions Felix has been supported by Stuart & Krikorian (1928); but the majority of workers have followed Gardner (1929) in believing that "O" agglutinins are produced in response to inoculation. Even although Felix's fundamental premise can no longer be maintained it does not necessarily follow that the "O" agglutination test is valueless in the diagnosis of enteric in the inoculated.

If it can be shown that the "O" agglutinins produced by inoculation disappear rapidly or are found only in comparatively small quantity, then the test may still be valid. Gardner (1929) believed that this was so, as did Wyllie (1932) and Smith (1932). Giglioli (1933) considered that serological methods of

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diagnosis were useless in persons inoculated less than three months before the test, but that thereafter the "O" agglutination test might be of great value.

Horgan (1932) and Dennis & Berberian (1934) showed that following inoculation "O" agglutinins were produced in quantities comparable to those found in active infection. They concluded that no arbitrary diagnostic titre could be established.

It would obviously be of great value if a limit could be set, particularly so if it enabled the diagnosis to be made on one single test. Opinions being divided on this point, we decided to examine for ourselves the sera of healthy non-inoculated and of inoculated persons. The non-inoculated were students, and the inoculated naval ratings, many of whom had been inoculated on several occasions.

Our main object was to ascertain if it were possible to establish any diagnostic titres for "O" agglutination in the inoculated and non-inoculated. As it is well established that "H" agglutinins active in very high titre may result from inoculation, we had no hope that a diagnostic titre could be set for "H" agglutination in the inoculated. Nevertheless, as a matter of interest, we also examined the sera we obtained for "H" agglutinins.

METHODS

Serum dilutions ranging from 1 in 20 to 1 in 640 were used. Each tube contained 0·2 c.c. diluted serum and 0·2 c.c. antigen. In every case the antigen employed was that supplied by the Oxford Standards Laboratory. The tests were incubated for 6 hours at 37° C. in a dry incubator and thereafter allowed to stand overnight at room temperature. Readings were made next morning. Any agglutination visible to the naked eye was recorded as "positive".

RESULTS

The results are recorded in Tables I–VI.

Table I. *Forty-seven non-inoculated students*

	Number of sera with end-titres of						
Antigen	0	1 in 20	1 in 40	1 in 80	1 in 160	1 in 320	1 in 640
B. typhosus "O"	41	4	2	—	—	—	—
B. paratyphosus B "O"*	32	6	4	5†	—	—	—
B. typhosus "H"	47	—	—	—	—	—	—
B. paratyphosus A "H"	47	—	—	—	—	—	—
B. paratyphosus B "H"	46	—	—	—	—	—	1†

Table II. *One hundred inoculated naval ratings tested more than 1 month after inoculation*

	Number of sera with end-titres of						
Antigen	0	1 in 20	1 in 40	1 in 80	1 in 160	1 in 320	1 in 640
B. typhosus "O"	35	20	34	10	1	—	—
B. paratyphosus B "O"*	25	17	33	21	4	—	—
B. typhosus "H"	3	2	6	19	29	32	9
B. paratyphosus A "H"	4	3	14	28	30	18	3
B. paratyphosus B "H"	2	3	9	14	31	30	11

* The suspension actually used (Tables I–VI) was *B. aertrycke* "O" which is antigenically the same as *B. paratyphosus* B "O".

† One of these had suffered from "food poisoning" four months before the test.

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Table III. *Ten inoculated naval ratings tested within* 1 *month of last inoculation*

Antigen	Number of sera with end-titres of						
	0	1 in 20	1 in 40	1 in 80	1 in 160	1 in 320	1 in 640
B. typhosus "O"	1	—	4	2	1	2	—
B. paratyphosus B "O"*	—	1	2	4	3	—	—
B. typhosus "H"	—	—	—	—	2	2	6
B. paratyphosus A "H"	—	—	—	1	1	4	3
B. paratyphosus B "H"	—	—	—	—	3	1	6

Table IV. *Twenty-nine inoculated naval ratings tested* 7–12 *months after last inoculation*

Antigen	Number of sera with end-titres of						
	0	1 in 20	1 in 40	1 in 80	1 in 160	1 in 320	1 in 640
B. typhosus "O"	12	5	9	2	1	—	—
B. paratyphosus B "O"*	9	6	7	6	1	—	—
B. typhosus "H"	1	—	2	6	11	6	3
B. paratyphosus A "H"	—	1	5	7	12	4	—
B. paratyphosus B "H"	—	1	1	4	6	14	3

Table V. *Forty inoculated naval ratings tested* 1–2 *years after last inoculation*

Antigen	Number of sera with end-titres of						
	0	1 in 20	1 in 40	1 in 80	1 in 160	1 in 320	1 in 640
B. typhosus "O"	11	8	17	4	—	—	—
B. paratyphosus B "O"*	6	7	18	9	—	—	—
B. typhosus "H"	1	1	3	9	9	17	—
B. paratyphosus A "H"	1	1	7	11	10	7	3
B. paratyphosus B "H"	2	2	4	6	15	7	4

Table VI. *Twelve inoculated naval ratings tested over* 2 *years after last inoculation*

Antigen	Number of sera with end-titres of						
	0	1 in 20	1 in 40	1 in 80	1 in 160	1 in 320	1 in 640
B. typhosus "O"	4	3	2	3	—	—	—
B. paratyphosus B "O"*	3	3	2	2	2	—	—
B. typhosus "H"	—	—	—	1	3	5	3
B. paratyphosus A "H"	—	1	—	3	4	4	—
B. paratyphosus B "H"	—	—	1	1	3	4	3

* See footnote to p. 37.

Discussion

The results obtained in the examination for "O" agglutinins of sera from non-inoculated persons correspond to a fair degree with those of Gardner & Stubington (1932) and of Giglioli (1933). These agglutinins were found more frequently than by Horgan (1932) and less frequently than by Alves (1936). Felix (1930) himself stated that normal "O" agglutinins for *B. typhosus* and *B. paratyphosus* B might reach a titre of 1 in 100. He considered however that "agglutination + + +" at a titre of 1 in 100 should be taken as indicating active infection. Our range of dilutions did not include 1 in 100, but from our limited observations we concluded that in normal non-inoculated persons "O"

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agglutinins for *B. typhosus* and *B. paratyphosus* B could not be demonstrated by the technique described in dilutions of 1 in 160 or higher.

The examination for "O" agglutinins of sera from inoculated persons gave results generally comparable to those obtained by Giglioli (1933), but tended to show their persistence for a longer period. Titres as high as those recorded by Horgan (1932) and by Dennis & Berberian (1934) were not obtained. It appeared that within one month after inoculation "O" agglutinins for *B. typhosus* or *B. paratyphosus* B were not likely to be found in serum dilutions of 1 in 640 or higher. At a later period they were not likely to be present in dilutions of 1 in 320 or higher, but might remain active in dilutions of 1 in 160 for two years or more.

Summarizing our results we concluded that the following minimum "O" agglutination titres should be required before a diagnosis of enteric was made on the result of a single test:

(1) In non-inoculated persons 1 in 160 or more.

(2) In persons inoculated more than 1 month before the test 1 in 320 or more.

(3) In persons inoculated less than 1 month before the test 1 in 640 or more.

Criteria such as these would only be of value if it could be shown that the titre of agglutinins in the sera of most enteric patients surpassed them. We therefore proceeded to consider what "O" agglutination titres might be expected in active infection. We ourselves had little opportunity to examine sera from enteric patients. Of the few we did examine some did not possess "O" agglutinins active in a serum dilution of 1 in 30 or more, while in others the agglutination titre did not pass 1 in 60. We have supplemented this information from other sources.

Gardner (1929) obtained the following results:

Thirteen typhoid patients

	Number of sera with end-titres of					
Antigen	0–15	20–50	55–200	250–500	950–3200	3500–10,000
B. typhosus "O"	0	1	2	4	3	3

Whitehead (1930):

Fifteen typhoid patients

	Number of sera with end-titres of						
Antigen	0	1 in 25	1 in 50	1 in 125	1 in 250	1 in 500	1 in 1250
B. typhosus "O"	—	1	—	4	2	5	3

Smith (1932):

	Twenty-eight typhoid patients: Number of sera with end-titres of						
Antigen	0	1 in 20	1 in 40	1 in 80	1 in 160	1 in 320	1 in 640
B. typhosus "O"	7	3	5	3	5	3	2
	Forty-two paratyphoid B patients						
B. paratyphosus B "O"	18	2	5	8	5	3	1

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The time in the course of the illness at which the sera were taken varied from case to case. Some would doubtless have shown a higher titre had the examination been repeated at a later date. The results as they stand are however apposite to our purpose, which is to show what may be expected from a single test.

It will be seen that more than half of Smith's cases do not even reach the minimum titre which appears to be necessary for a certain diagnosis of enteric in non-inoculated persons. Three of Gardner's thirteen cases, five of Whitehead's fifteen, eighteen of Smith's twenty-eight typhoid and thirty-three of his forty-two paratyphoid cases fall below the standard which appears to be required in the inoculated.

It is possible, and theoretically probable, that the development of enteric fever in a previously inoculated person might cause the production of agglutinins in larger amounts than is found in the enteric patient who has not been previously inoculated. Little information is available on this point, but the experience of Stuart & Krikorian (1928), who failed to find "O" agglutinins for *B. typhosus* active in a dilution of over 1 in 200 in three previously inoculated persons suffering from typhoid, is against it.

The examination for "H" agglutinins affords confirmatory evidence that "H" agglutinins for members of the enteric group of organisms are not normally found in healthy non-inoculated persons. The one student whose serum contained "H" agglutinins for *B. paratyphosus* B had an illness with the characteristic symptoms of food poisoning four months before. "O" agglutinins for *B. paratyphosus* B or *B. aertrycke* were present in his serum to a titre of 1 in 80. The results also show that in the inoculated subject "H" agglutinins may remain active in high dilutions over a considerable period.

An analysis of our detailed records, which considerations of space do not justify us in reproducing, shows a wide variation both in the faculty of the inoculated individual to produce agglutinins and in the persistence of these agglutinins. There does not appear to be any correlation between the number of inoculations and the height of the agglutination titre.

The sera of the naval ratings were also examined for agglutinins for *Brucella abortus*. Two showed agglutinins in a titre of 1 in 80, one in a titre of 1 in 40 and one in a titre of 1 in 20. None of these men gave a history of an illness which might have been undulant fever. The previous occupations of three of them are however of interest and significance. One had been a farm servant, another a butcher and the third a carter. In these occupations they had the opportunity of contact with infected animals. No possible specific cause could be found for the presence of agglutinins in the fourth.

Conclusions

We are driven to the conclusion that only rarely will it be possible to make the diagnosis of enteric in a previously inoculated person as the result of a single "O" agglutination test. If serological methods are to be used, there

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appears no other way than to carry out repeated quantitative tests using both "O" and "H" antigens, and, having established an initial level of agglutinins, to look for a rising titre for one of the enteric group. In many cases early blood culture, or culture of the clot from the sample submitted for the Widal reaction, will settle the diagnosis more easily and definitely.

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