# AGGLUTININS FOUND IN THE SERUM OF SUBJECTS INOCULATED WITH TYPHOID-PARATYPHOID VACCINE.

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In the preceding paper (1933) I have recorded the results of an investigation on typhoid-paratyphoid agglutinins in a random sample of the normal population of British Guiana. It has been shown what a serious cause of error these "natural" agglutinins may represent in the carrying out of routine serological work in certain countries.

Typhoid-paratyphoid vaccination also gives rise to the production of agglutinins, rendering the serological diagnosis of enteric particularly difficult in the case of vaccinated patients.

On these agglutinins of vaccinated subjects much work was carried out during the war; but our knowledge of the antigenic structure of bacteria has since advanced so considerably as to render a revision of much of this research necessary. This problem, in fact, remains one of much practical importance in countries where conscription exists and compulsory typhoid-paratyphoid inoculation is practised on recruits, and in the tropics where vaccination is frequent among Europeans and in large labour forces.

The exact nature of the agglutinin reaction which follows inoculation is still a matter of controversy: Felix and Olitsky (1928) deny the existence of somatic agglutinins in the blood of inoculated subjects. These authors regard the "O" antibodies as the only important immunological substance for the production of immunity; they therefore conclude that anti-typhoid vaccination does not produce immunity and is useless as a preventive. Stewart and Krikorian (1928) have come to similar conclusions. All these observers, it should be noted, have accepted, as positive, only results obtained at a titre of 1 : 100 or over.

Gardner (1929), with an "O" suspension of *Bact. typhosum* as antigen, found in a series of 47 normal subjects, as an average, 0·65 agglutination units per serum examined, with a range from 0 to 3 units. In 17 recently vaccinated cases he found an average of 4·3 units per serum, with a range from 0 to 18 units. In 16 subjects vaccinated in the past he found 1·8 units per serum. These findings have been recently confirmed by Horgan (1932) in a series of 70 normal and 20 vaccinated subjects, and by Wyllie (1932) on 46 inoculated persons.

The evidence, so far, appears to tilt the balance in favour of Gardner's views, *i.e.* that prophylactic inoculation for enteric should give rise to "O" agglutinin

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production. But it is certain that the material on which this important discussion has been based, up to the present, is excessively small. There also appears to be some discrepancy between the low dilution positive results recorded by Gardner and the definitely high titres obtained by Horgan and by Wyllie in some of their cases. Further studies on more ample material are required.

During 1930–2, in British Guiana, I carried out a series of serological examinations on vaccinated subjects; the technique employed was the same as has been described in my first paper.

## Material.

The sera of 137 persons vaccinated by hypodermic injection, and of 10 vaccinated *per os* have been studied; these were in the majority males of the Negro race, between the ages of 18 and 40 years. Cases were in no way selected, but were taken at random amongst vaccinated subjects coming up for treatment as in- or out-patients at Mackenzie Hospital.

## Vaccine.

As regards the composition of the hypodermic vaccine employed, this series of cases is not uniform. In 1928 I made use of a tetravalent (T.A.B.C.) vaccine of the following composition:

| | | |
|---|---|---|
| *Bact. typhosum* ... | ... | 500 millions per c.c. |
| *Bact. paratyphosum* C | ... | 500 ,, |
| *Bact. paratyphosum* A | ... | 250 ,, |
| *Bact. paratyphosum* B | ... | 250 ,, |

The standard vaccination consisted of three injections of 0·25, 1 and 1 c.c. respectively at one week's interval.

No cases of paratyphoid A or B having been recorded during 4 years, in 1930 I modified the composition of the vaccine so as to reduce the number of injections. This new vaccine (T.C.) contained 1000 millions of both *Bact. typhosum* and *Bact. paratyphosum* C per c.c.; the standard vaccination consisted of two injections of 0·5 and 1 c.c. respectively at 7 days' interval.

Many cases which had been inoculated in 1928 with T.A.B.C. were re-vaccinated in 1930 with a single 1 c.c. dose of T.C. vaccine. For the preparation of both these vaccines five local strains of each species were employed; saline suspensions were made from 24-hour agar cultures and killed by heat (56° C. for 2 hours); for preservation 0·5 per cent. of carbolic acid was added.

In conclusion our series of vaccinated subjects can be classified as follows:

| | |
|---|---|
| Vaccinated with T.A.B.C. in 1928 ... | 17 cases |
| Vaccinated with T.C. in 1930 ... ... | 79 ,, |
| Vaccinated with T.A.B.C. in 1928 and re-vaccinated with T.C. in 1930 ... ... | 41 ,, |
| Vaccinated *per os* ... ... ... ... | 10 ,, |

In the present investigation, therefore, we are essentially interested in agglutinins for *Bact. typhosum* and *Bact. paratyphosum* C.

Table I summarises the findings obtained in the whole series of 137 examinations; it supplies an index as to the average agglutinin conditions to be found in a random sample of a vaccinated population during the first 3 years following inoculation.

In Tables II–VII cases have been classified into groups according to the period, in respect to the date of vaccination, at which they were examined.

In order to render possible a comparison of the findings obtained in each group, the percentage of sera giving positive agglutination at a *significant titre* has been calculated and is given at the end of each table. The choice of a significant titre is evidently arbitrary: a titre 1 : 40 has been adopted; agglutination at such a low titre, in fact, may not rarely be found in the earlier stages of actual typhoid infection; the choice of a higher titre (1 : 100 for instance, as practised by Felix and Olitsky) would easily lead one to overlook the slight, but definite, somatic agglutinin reaction which occurs in many vaccinated cases.

Table VIII summarises these findings; the incidence of agglutinins in a random sample of the normal, non-inoculated population is also given for the purpose of comparison. This table gives a clear view of the chronological variations, qualitative and quantitative, of the various agglutinins in the sera of inoculated subjects.

In all our cases inoculation was carried out with equal proportions of *Bact. typhosum* and *Bact. paratyphosum* C; it is interesting therefore to note how differently individuals react to these two species; as a rule agglutinins for *Bact. typhosum* are more frequent, more abundant, and tend to be much more persistent than those for *Bact. paratyphosum* C. In individual cases one may note a very marked agglutinin reaction to one species and none, or next to none, to the other.

The reaction to the various antigens of the same species is also very variable, even in subjects inoculated from the same supply of vaccine. This is particularly evident with the "H" antigens of *Bact. paratyphosum* C; the serum of one subject may present abundance of both type and group agglutinins; in another only group or only type agglutinins may be found. Group agglutinins tend to be considerably more frequent than type agglutinins; this same fact we have already observed in the study of normal sera. It does not appear necessary therefore to invoke the influence of other Salmonella infections to explain this preponderance of group agglutinins as was suggested in my first paper.

Our findings tend to confirm the known fact that "H" agglutinins are abundantly produced in the sera of vaccinated subjects, and that very frequently they may still be present in the blood, two and three years after inoculation, in titres which could easily be found in actual cases of enteric infection. It is evident that it would scarcely be practical to try to fix a "limit titre" to "H" agglutinins caused by inoculation, as has been suggested by some, as a criterion for the serological diagnosis of enteric in vaccinated subjects.

Table I. "H" *and* "O" *agglutinins in sera from a random sample of a vaccinated population. Collected during the first three years following inoculation.*

| Vaccine | No. of sera |
|---|---|
| T.A.B.C. 1928 | 17 |
| T.C. 1930 | 81 |
| T.A.B.C. 1928 + T.C. 1930 | 39 |
| Total | 137 |

| | Agglutination titre* | | | | | | | | | | | Percentage of sera examined agglutinating at a titre of |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| "H" antigens | Negative | 1:10 | 1:20 | 1:40 | 1:80 | 1:160 | 1:320 | 1:640 | 1:1280 | 1:2560 | 1:5120 | 1:10240 | 1:40 or + |
| *Bact. typhosum* | 8 | 4 | 13 | 10 | 12 | 24 | 23 | 12 | 15 | 12 | 2 | 2 | 81·7 |
| *Bact. paratyphosum* A | 101 | 11 | 9 | 7 | 3 | 3 | 3 | . | . | . | . | . | 11·7 |
| *Bact. paratyphosum* B type | 116 | 6 | 7 | 5 | 2 | 1 | . | . | . | . | . | . | 5·8 |
| *Bact. paratyphosum* C type | 59 | 13 | 9 | 16 | 10 | 8 | 6 | 4 | 1 | 9 | . | 2 | 40·8 |
| Group phase antigen | 42 | 6 | 14 | 19 | 17 | 15 | 14 | 3 | 1 | 4 | 1 | 1 | 54·7 |
| "O" antigens | | | | | | | | | | | | | |
| *Bact. typhosum* | 59 | 23 | 28 | 17 | 7 | 2 | 1 | . | . | . | . | . | 19·7 |
| *Bact. paratyphosum* B | 87 | 20 | 23 | 6 | 1 | . | . | . | . | . | . | . | 5·1 |
| *Bact. paratyphosum* C | 70 | 16 | 19 | 18 | 12 | 2 | . | . | . | . | . | . | 23·3 |

Table II. "H" *and* "O" *agglutinins in the sera of* 17 *vaccinated subjects examined within the first month following inoculation.*

| Vaccine | No. of sera |
|---|---|
| T.C. 1930 | 17 |

| | Agglutination titre* | | | | | | | | | | | Percentage of sera examined agglutinating at a titre of |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| "H" antigens | Negative | 1:10 | 1:20 | 1:40 | 1:80 | 1:160 | 1:320 | 1:640 | 1:1280 | 1:2560 | 1:5120 | 1:10240 | 1:40 or + |
| *Bact. typhosum* | . | . | . | 1 | 3 | 1 | 1 | 1 | 2 | 6 | . | 2 | 100·0 |
| *Bact. paratyphosum* A | 14 | 1 | . | . | . | . | 2† | . | . | . | . | . | 5·8 |
| *Bact. paratyphosum* B type | 15 | 1 | 1 | . | . | . | . | . | . | . | . | . | 0·0 |
| *Bact. paratyphosum* C type | 5 | . | . | 2 | . | 2 | 1 | . | 1 | 4 | . | 2 | 40·5 |
| Group phase antigen | 1 | 2 | 3 | 1 | 3 | 2 | 1 | . | . | 2 | 1 | 1 | 64·7 |
| "O" antigens | | | | | | | | | | | | | |
| *Bact. typhosum* | 4 | 3 | 3 | 5 | 1 | . | 1 | . | . | . | . | . | 41·0 |
| *Bact. paratyphosum* B | 8 | 4 | 4 | 1 | . | . | . | . | . | . | . | . | 5·8 |
| *Bact. paratyphosum* C | 7 | . | 3 | 2 | 5 | . | . | . | . | . | . | . | 41·0 |

* The figures given under each titre are the number of sera reacting at that dilution, but not above.

† In both cases these agglutinins were present also before vaccination.

Table III. "H" *and* "O" *agglutinins in the sera of* 21 *vaccinated subjects examined during the second and third month after inoculation.*

| Vaccine | No. of sera |
|---|---|
| T.C. 1930 | 14 |
| T.A.B.C. 1928 + T.C. 1930 | 7 |
| Total | 21 |

| | Agglutination titre* | | | | | | | | | | | Percentage of sera examined agglutinating at a titre of |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| "H" antigens | Negative | 1:10 | 1:20 | 1:40 | 1:80 | 1:160 | 1:320 | 1:640 | 1:1280 | 1:2560 | 1:5120 | 1:10240 | 1:40 or + |
| *Bact. typhosum* | 2 | . | . | . | 1 | 3 | 5 | 7 | 3 | . | . | . | 90·4 |
| *Bact. paratyphosum* A | 19 | 1 | . | . | 1 | . | . | . | . | . | . | . | 4·7 |
| *Bact. paratyphosum* B type | 17 | 1 | 1 | 1 | . | 1 | . | . | . | . | . | . | 9·0 |
| *Bact. paratyphosum* C type | 7 | 2 | 4 | 3 | . | 1 | 1 | . | . | 3 | . | . | 38·1 |
| Group phase antigen | 8 | 1 | 1 | 4 | 1 | 2 | 2 | . | . | 2 | . | . | 52·3 |
| "O" antigens | | | | | | | | | | | | | |
| *Bact. typhosum* | 7 | 3 | 4 | 4 | 2 | 1 | . | . | . | . | . | . | 33·3 |
| *Bact. paratyphosum* B | 18 | 2 | 1 | . | . | . | . | . | . | . | . | . | 0·0 |
| *Bact. paratyphosum* C | 9 | 4 | 5 | 2 | 1 | . | . | . | . | . | . | . | 14·2 |

Table IV. "H" *and* "O" *agglutinins in the sera of* 29 *vaccinated subjects examined during fourth, fifth and sixth month following inoculation.*

| Vaccine | No. of sera |
|---|---|
| T.C. 1930 | 16 |
| T.A.B.C. 1928 + T.C. 1930 | 13 |
| Total | 29 |

| | Agglutination titre* | | | | | | | | | | Percentage of sera examined agglutinating at a titre of |
|---|---|---|---|---|---|---|---|---|---|---|---|
| "H" antigens | Negative | 1:10 | 1:20 | 1:40 | 1:80 | 1:160 | 1:320 | 1:640 | 1:1280 | 1:2560 | 1:5120 | 1:40 or + |
| *Bact. typhosum* | 1 | 1 | 3 | 2 | 3 | 4 | 3 | 3 | 3 | 4 | 2 | 82·7 |
| *Bact. paratyphosum* A | 20 | 2 | 5 | 2 | . | . | . | . | . | . | . | 6·9 |
| *Bact. paratyphosum* B type | 26 | 1 | 1 | 1 | . | . | . | . | . | . | . | 3·4 |
| *Bact. paratyphosum* C type | 13 | 4 | 2 | 4 | 2 | 1 | 3 | . | . | . | . | 34·4 |
| Group phase antigen | 11 | . | 2 | 6 | 3 | 3 | 4 | . | . | . | . | 55·1 |
| "O" antigens | | | | | | | | | | | | |
| *Bact. typhosum* | 12 | 6 | 7 | 3 | . | 1 | . | . | . | . | . | 13·7 |
| *Bact. paratyphosum* B | 20 | 8 | 1 | . | . | . | . | . | . | . | . | 0·0 |
| *Bact. paratyphosum* C | 14 | 3 | 6 | 6 | . | . | . | . | . | . | . | 20·6 |

* The figures under each titre are the number of cases reacting at that dilution, but not above.

Table V. "H" *and* "O" *agglutinins in the sera of* 34 *vaccinated subjects examined from the seventh to the twelfth month following inoculation.*

| Vaccine | No. of sera |
|---|---|
| T.C. 1930 | 22 |
| T.A.B.C. 1928 + T.C. 1930 | 12 |
| Total | 34 |

| | Agglutination titre* | | | | | | | | | | Percentage of sera examined agglutinating at a titre of |
|---|---|---|---|---|---|---|---|---|---|---|---|
| "H" antigens | Negative | 1:10 | 1:20 | 1:40 | 1:80 | 1:160 | 1:320 | 1:640 | 1:1280 | 1:2560 | 1:5120 | 1:40 or + |
| *Bact. typhosum* | 1 | 3 | 4 | . | 1 | 8 | 8 | 1 | 6 | 2 | . | 76·4 |
| *Bact. paratyphosum* A | 27 | 3 | 1 | . | 1 | 2 | . | . | . | . | . | 8·8 |
| *Bact. paratyphosum* B type | 28 | 2 | 3 | 1 | . | . | . | . | . | . | . | 2·9 |
| *Bact. paratyphosum* C type | 14 | 5 | . | 1 | 6 | 2 | 1 | 3 | . | 2 | . | 44·1 |
| Group phase antigen | 10 | 1 | 2 | 2 | 7 | 6 | 4 | 2 | . | . | . | 61·7 |
| "O" antigens | | | | | | | | | | | | |
| *Bact. typhosum* | 18 | 8 | 4 | 2 | 2 | . | . | . | . | . | . | 11·7 |
| *Bact. paratyphosum* B | 22 | 3 | 6 | 3 | . | . | . | . | . | . | . | 8·8 |
| *Bact. paratyphosum* C | 24 | 2 | 1 | 3 | 3 | 1 | . | . | . | . | . | 20·5 |

Table VI. "H" *and* "O" *agglutinins in the sera of* 19 *vaccinated subjects examined in the second year after inoculation.*

| Vaccine | No. of sera |
|---|---|
| T.C. 1930 | 10 |
| T.A.B.C. 1928 + T.C. 1930 | 9 |
| Total | 19 |

| | Agglutination titre* | | | | | | | | | | Percentage of sera examined agglutinating at a titre of |
|---|---|---|---|---|---|---|---|---|---|---|---|
| "H" antigens | Negative | 1:10 | 1:20 | 1:40 | 1:80 | 1:160 | 1:320 | 1:640 | 1:1280 | 1:2560 | 1:5120 | 1:40 or + |
| *Bact. typhosum* | 2 | . | 4 | 2 | 3 | 2 | 5 | . | 1 | . | . | 68·4 |
| *Bact. paratyphosum* A | 16 | 2 | 1 | . | . | . | . | . | . | . | . | 0·0 |
| *Bact. paratyphosum* B type | 18 | . | 1 | . | . | . | . | . | . | . | . | 0·0 |
| *Bact. paratyphosum* C type | 8 | 1 | 2 | 5 | 1 | 2 | . | . | . | . | . | 42·1 |
| Group phase antigen | 8 | 1 | 4 | 2 | 1 | 1 | 1 | 1 | . | . | . | 31·5 |
| "O" antigens | | | | | | | | | | | | |
| *Bact. typhosum* | 7 | 2 | 8 | 1 | 1 | . | . | . | . | . | . | 10·5 |
| *Bact. paratyphosum* B | 8 | 2 | 7 | 2 | . | . | . | . | . | . | . | 10·5 |
| *Bact. paratyphosum* C | 6 | 5 | 3 | 3 | 2 | . | . | . | . | . | . | 26·3 |

* The figures under each titre are the number of cases reacting at that dilution, but not above.

Table VII. *"H" and "O" agglutinins in the sera of 17 vaccinated subjects examined in the third year following vaccination.*

Vaccine T.A.B.C. 1928 — No. of sera 17

| "H" antigens | Agglutination titres* Negative | 1:10 | 1:20 | 1:40 | 1:80 | 1:160 | 1:320 | 1:640 | 1:1280 | 1:2560 | 1:5120 | Percentage of sera examined agglutinating at a titre of 1:40 or + |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Bact. typhosum* | 2 | . | 2 | 5 | 1 | 6 | 1 | . | . | . | . | 76·4 |
| *Bact. paratyphosum* A | 5 | 2 | 2 | 5 | 1 | 1 | 1 | . | . | . | . | 47·0 |
| *Bact. paratyphosum* B type | 12 | 1 | . | 2 | 2 | . | . | . | . | . | . | 23·5 |
| *Bact. paratyphosum* C type | 12 | 1 | 1 | 1 | 1 | . | . | 1 | . | . | . | 17·6 |
| Group phase Antigen | 4 | 1 | 2 | 4 | 2 | 1 | 2 | . | 1 | . | . | 58·8 |
| "O" antigens | | | | | | | | | | | | |
| *Bact. typhosum* | 11 | 1 | 2 | 2 | 1 | . | . | . | . | . | . | 17·6 |
| *Bact. paratyphosum* B | 11 | 1 | 4 | . | 1 | . | . | . | . | . | . | 5·8 |
| *Bact. paratyphosum* C | 10 | 2 | 1 | 2 | 1 | 1 | . | . | . | . | . | 23·5 |

* The figures under each titre are the number of cases reacting at that dilution, but not above.

Table VIII. *Percentage of sera containing "O" and "H" agglutinins at the titre of 1 : 40 or over at different periods after inoculation.*

| Vaccine | No. of sera |
|---|---|
| T.A.B.C. 1928 | 17 |
| T.C. 1930 | 81 |
| T.A.B.C. 1928 + T.C. 1930 | 39 |
| Total | 137 |

| "H" antigens | Percentage of sera of vaccinated subjects examined agglutinating at a titre of 1:40 or + 1st month No. of sera 17 | 2nd and 3rd month No. of sera 21 | 4th to 6th month No. of sera 29 | 7th to 12th month No. of sera 34 | 2nd year No. of sera 19 | 3rd year No. of sera 17* | Percentage of normal sera showing agglutinins at a titre of 1:40 or over. No. of sera 350 |
|---|---|---|---|---|---|---|---|
| *Bact. typhosum* | 100.0 | 94·4 | 82·7 | 76·4 | 68·4 | 76·4 | 16·0 |
| *Bact. paratyphosum* A | 5·8 | 4·7 | 6·9 | 8·8 | 0·0 | 47·0 | 6·8 |
| *Bact. paratyphosum* B type | 0·0 | 9·0 | 3·4 | 2·9 | 0·0 | 23·5 | 3·1 |
| *Bact. paratyphosum* C type | 40·5 | 38·1 | 34·4 | 44·1 | 42·1 | 17·6 | 11·7 |
| Group phase antigen | 64·7 | 52·3 | 55·1 | 61·7 | 31·5 | 58·8 | 20·0 |
| "O" antigens | | | | | | | |
| *Bact. typhosum* | 41·0 | 33·3 | 13·7 | 11·7 | 10·5 | 17·6 | 3·7 |
| *Bact. paratyphosum* B | 5·8 | 0·0 | 0·0 | 8·8 | 10·5 | 5·8 | 5·7 |
| *Bact. paratyphosum* C | 41·0 | 14·2 | 20·6 | 20·5 | 26·3 | 23·5 | 9·4 |

* All these cases were vaccinated for *B. paratyphosum* A and B.

All our tables demonstrate the existence of definite somatic agglutinin response both for *Bact. typhosum* and *Bact. paratyphosum* C. This reaction is very distinct in the first month after inoculation, but tends rapidly to become less evident. After the third month agglutination of the "O" antigen of *Bact. typhosum* in significant titre (1 : 40 to 1 : 160) occurs in approximately 1/7 of cases examined; for *Bact. paratyphosum* C in something more than 1/5 of cases examined. Such proportions are maintained, more or less uniformly, up to the third year. In the normal, non-inoculated population "O" agglutinins for *Bact. typhosum*, in titres of 1 : 40 or over, are found in 1/25 and for *Bact. paratyphosum* C in 1/10 of cases examined (350).

In the whole series of 137 cases, "O" agglutinins for either species were never observed at a titre above 1 : 320; this same titre was recorded in a single instance, and that for *Bact. typhosum* in a serum collected 7 days after the last injection of vaccine.

"O" agglutinins for *Bact. paratyphosum* B were very frequent in low dilution (1 : 10, 1 : 20) as has been shown to occur in normal sera; but in titres of 1 : 40 or over somatic agglutinins were, in most groups, no more frequent in inoculated than in non-inoculated subjects; it should be noted, however, that only 58 of our cases had been vaccinated with this species in 1928, *i.e.* two years before the present investigation was started, so that the figures for these particular agglutinins have little significance from our present point of view.

No important difference has been noted in the incidence of "H" and "O" agglutinins in re-vaccinated subjects; possibly "H" agglutinins for *Bact. typhosum* tend to occur in higher dilutions and to be more persistent in such cases.

Malaria does not appear to have any importance as an exciting factor of "anamnestic agglutinin reaction": no substantial difference has been noted in the average findings in subjects suffering from this disease and others admitted to hospital for surgical and traumatic conditions. In three cases which were undergoing a course of shock treatment (intravenous Dmelcos vaccine for venereal ulcers), no marked agglutinin changes were noted by repeated examination carried out before, during and after treatment.

### *Agglutinins in the sera of subjects vaccinated* per os.

Only a small series of ten cases has been studied; subjects who showed no "natural" agglutinins in their blood were selected.

The vaccine employed contained 25,000 millions of both *Bact. typhosum* and *Bact. paratyphosum* C per dose of 4 c.c. (saline suspension of 24 hours' agar cultures killed by heating at 56° C. in the water bath for 2 hours); it was given on an empty stomach ten minutes after a preliminary dose of 4 c.c. of ox bile; this treatment was repeated for five consecutive days. Sera were collected from the 5th to the 15th day following the last dose of vaccine.

In none of these cases did examination reveal "H" or "O" agglutinins for either of the organisms contained in the vaccine.

## DISCUSSION AND CONCLUSION.

Our results confirm Gardner's views: typhoid-paratyphoid inoculation gives rise to production of somatic agglutinins. This reaction is marked in the first few weeks following vaccination, but it is always limited to low and medium dilutions; the highest titre at which a positive result was recorded, in the present series of cases, with an "O" antigen, was 1 : 320.

In actual enteric infection "H" agglutinins usually occur in much higher titres than the corresponding "O" agglutinins; but even so the "O" titres found in enteric patients are generally much higher than the "O" titres found in vaccinated subjects, especially after more than 2 months have elapsed from the date of vaccination.

Smith (1932), in his recent work on cases of typhoid and paratyphoid B, has shown that a single test may not be sufficient for the demonstration of somatic agglutinins. Repeated tests invariably revealed their existence, usually in considerable titres (1 : 100 to 1 : 400).

In my own experience, in typhoid (4 cases) and paratyphoid C (12 cases), "O" agglutinins have been found constantly after the 1st week of the disease, in dilutions from 1 : 80 to 1 : 2560, usually appearing at a somewhat earlier date in the course of the disease than the corresponding "H" agglutinins.

The presence of "O" agglutinins in the sera of vaccinated subjects does not destroy the utility of qualitative receptor analysis as a means for the serological diagnosis of enteric in vaccinated subjects; but it is necessary to make this a quantitative as well as a qualitative test, by establishing the end titre of agglutination for each antigen. The test moreover should be repeated after an interval of a week or more in negative or doubtful cases.

In conclusion, for the diagnosis of enteric in vaccinated subjects, one should not expect much from serological methods during the first 3 months following inoculation; but after this period has elapsed the finding of "O" agglutinins in a high dilution constitutes a datum which is very strongly suggestive of active infection; negative findings, on the other hand, have little value unless confirmed by repeated tests, carried out at intervals of 5 or 6 days.

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