EXPERIENCES WITH SMALL-FLAKING OR GRANULAR AGGLUTINATION IN NORMAL, INOCULATED AND ENTERIC FEVER CASES.

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(With 3 Charts.)

CONTENTS.

Introduction.

The recognition of small-flaking, granular or "O" agglutination and the conditions under which it occurs have lately assumed importance in the diagnosis and prognosis of enteric infections.

Felix (1924) has applied the term Qualitative Receptor Analysis to this method in contrast to the method of Quantitative Receptor Analysis in which the serum is titrated in serial dilutions.

The original Widal reaction, when performed with the customary preserved suspensions, is concerned only with large-flaking, floccular or "H" agglutination (Felix and Olitzki, 1928). The test is extensively used as a means of diagnosing enteric fever in suspected individuals, but in inoculated persons at least three agglutination tests are required before a definite diagnosis can be given. The Widal reaction has thus become a complicated serological problem, necessitating a distinction between the inoculation agglutinins arising from preventive inoculation and the infection agglutinins resulting from an attack of the disease.

During the Great War, Dreyer and his colleagues made a great advance in the serial quantitative method by introducing formolised broth standardised suspensions. These suspensions were used to enable agglutinin curves to be drawn in enteric infections in inoculated persons, on the assumption that a differentiation between inoculation and infection agglutinins could be made by repeated quantitative tests.

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Criticism of this method has been made by Felix (1929) who relies on the small-flaking or "O" agglutination method. Working with the sera of enteric fever cases, he described two types of agglutination:

(1) Large-flaking, floccular or "H" agglutination in which large flakes are formed and settle rapidly, producing a loose sediment.

(2) Small-flaking, granular or "O" agglutination which forms and settles slowly in small uniform granules producing a scanty sediment.

Felix (1930) prefers to use living suspensions of special strains of the enterica group of organisms, but he has approved the use of bacterial suspensions preserved with phenol or formalin as a reagent to demonstrate the "H" agglutinin and an alcoholic suspension as a reagent to demonstrate the "O" agglutinin.

It is claimed that the method of Qualitative Receptor Analysis enables a distinction to be made between agglutination due to preventive inoculation and that arising during enterica disease, that it is able to recognise "anamnestic" reactions and to eliminate paradox reactions.

My experience with the test has been confined to sera from normal, inoculated and infected persons.

Technique.

The method followed was recommended by the Standards Laboratory, Oxford, in the use of standardised agglutinable suspensions. Normal sera, *i.e.* sera from cases giving no history of previous enterica infection or of preventive inoculation, were set up in dilutions ranging from 1/25 to 1/500. In sera from inoculated cases the range was 1/25 to 1/1000 and in sera from suspected enterica cases 1/25 to 1/5000. Physiological saline and buffer saline were used alternately, but neither appeared to have any advantage over the other in favouring agglutination. The suspensions used were Typhoid "H," Typhoid "O," Para B "H" specific, Aertrycke "O" and non-specific Salmonella. (Inoculation with T.A.B. vaccine gives rise to non-specific Salmonella group agglutinins.)

For the detection of small-flaking or granular agglutination the tubes were placed in a water bath at 50–55° C. for 4½ hours, and after standing at room temperature for 15 min. the results were read with the aid of a lens of × 10 magnification against a black background. Further incubation in the water bath for 18 hours was found beneficial in bringing down completely the granular agglutination. Examination of the tubes with the lens at frequent intervals during the period of incubation often revealed finely granular flocculi moving in the convection currents—a characteristic feature in my experience of "O" agglutination. This type of agglutination forms and settles slowly in small, uniform granules, producing usually after 24 hours a scanty sediment which is difficult to dislodge.

The results may be expressed in the usual way as the highest dilution of serum in which marked, *i.e.* standard, agglutination occurs or in reduced

titres. With a standard technique, the observed titre of a serum depends on two factors: (*a*) the agglutinating power of the serum, and (*b*) the sensitiveness of the bacterial suspension. The former factor is a fixed quantity, the latter is measured by a standardising process and indicated by a number. The reduced titre (R.T.) is obtained by dividing the observed titre by this number.

In plotting graphs, it is essential to use reduced titres to make accurate comparisons.

SERA FROM NORMAL CASES.

In this series of twenty-eight cases, non-inoculated persons who gave a clear history of never having suffered from enterica were regarded as normal. It is usually considered that the presence of typhoid agglutinins in the sera from presumably normal persons indicates previous exposure to infection or preventive inoculation. The work of Rosher and Fielden (1922) confirms this view. It must be borne in mind that, between the typical case of typhoid fever and the infected subject who remains perfectly healthy, a variety of more or less severe forms of illness occurs. It is well known that in the very young and the very old the disease tends to be atypical. Hence there exists a proportion of cases in which a reaction occurs with little or no obvious disturbance of health, but the blood serum acquires a marked agglutinating action for the typhoid bacillus. Gardner (1929), in forty-seven normal sera, found negative "O" agglutination in approximately 50 per cent. and only one case showed a reduced titre over 2·5.

In the present series only three cases gave a titre for "O" agglutination of 1 : 50, *i.e.* R.T. of 3, while twenty-three cases were negative. While it is recognised that this number is too small on which to base percentages, it is striking that negative "O" agglutination has been found in 78 per cent. The results of the tests are shown in Table I.

SERA FROM INOCULATED CASES.

Felix (1924) asserts that preventive inoculation against typhoid fever produces *exclusively* large-flaking agglutinins and that, if the polyvalent T.A.B. vaccine is used, large-flaking agglutinins against all three species of bacteria are formed. Now Gardner (1930) has pointed out that the present type of inoculation in England normally gives rise to titres of "O" agglutination varying from 1/200 to 1/500 which rapidly fall below 1/100, so that the residual "O" titre seldom interferes with diagnosis in persons whose inoculation is not very recent. Ashby (1931) examined the sera from five cases inoculated with T.A.B. vaccine for the presence of typhoid "O" agglutinins and found four cases positive in a dilution of 1/30 (R.T. 4), two of which had been inoculated 4 years and two 1 month previously; and one case positive in 1/50 (R.T. 6) which had been inoculated 1 month before.

The present series includes two groups of cases: (*a*) those which have received prophylactic inoculation, and (*b*) those which have received thera-

peutic inoculation. Inoculations of T.A.B. vaccine (the vaccine consists of 1000 million *B. typhosus* and 500 million each of *B. Para A* and *B*) issued by the Department of Health of Ontario, were given subcutaneously[1] in Group A and intravenously[2] in Group B in doses sufficient to produce a febrile reaction in different mental states, such as general paralysis, tabes dorsalis, dementia praecox, mania and paranoia.

In thirteen of the twenty-two cases which received a course of prophylactic inoculations between January, 1928 and February, 1931 (Group A), typhoid

Table I. *Normal cases.* (*Unvaccinated and without history of enterica.*)

Patient's initials	Month when blood sample received in 1931	Titre of typhoid "H" agglutination	Titre of typhoid "O" agglutination	Reduced "O" titre
A.C.	Aug.	—	—	—
A.McK.	,,	—	—	—
H.D.	,,	—	1 : 25 (3)	1·5
H.R.	June	—	1 : 50 (2)	3
I.F.M.	Aug.	—	—	—
J.W.	,,	—	—	—
M.F.	,,	—	—	—
M.G.	,,	—	—	—
P.R.	,,	—	—	—
S.C.	,,	—	—	—
M.W.	Sept.	1 : 50 (2)	—	—
B 2	,,	—	—	—
B 3	,,	—	—	—
B 4	,,	—	—	—
B 5	,,	—	—	—
B 6	,,	1 : 100 (2)	1 : 50 (3)	3
B 7	,,	—	—	—
B 8	,,	—	—	—
B 9	,,	—	—	—
B 10	,,	—	—	—
B 11	,,	—	—	—
B 12	,,	—	—	—
B 13	,,	—	—	—
B 14	,,	—	—	—
B 15	,,	—	—	—
B 16	,,	—	—	—
B 17	,,	—	1 : 25 (2)	1·5
B 18	,,	—	1 : 50 (3)	3

Note: (2), (3) indicate strength of reaction as + +, + + +.

"O" agglutinins could not be detected after periods of 6 months or of 3 years and 3 months, whereas in the remaining nine only one serum showed an "O" titre as high as 1/100. In every member of this group typhoid "H" agglutinins were developed and found persisting after periods varying from 6 months to 4 years.

These results show that "O" agglutinins are produced after prophylactic inoculation with the type of vaccine used in Ontario, but that the residual titre falls to limits which probably do not interfere with diagnosis in cases subsequently contracting typhoid fever, as Gardner suggests (See Table II).

[1] Three doses, $\frac{1}{4}$, $\frac{1}{2}$ and 1 c.c. given at weekly intervals.

[2] A series of gradually increasing doses of typhoid vaccine was given over a period of several weeks, the dosage varying according to the reaction produced.

In Group B, however, the matter is different. In Table III the cases inoculated in 1929 show "H" agglutination up to 1/50 and "O" agglutination up to 1/250, while those inoculated in 1930 show "H" agglutination up to 1/500 and "O" agglutination up to 1/200. A titre of 1/100 and over for "O" agglutinins occurred thirteen times in this series, *i.e.* in 54 per cent. of the cases. From this we conclude that the "O" agglutinins when once formed tend to persist longer than the "H" agglutinins and, comparing the "O" agglutinin titres in Groups A and B, that intravenous injection produces higher residual "O" titres than subcutaneous inoculation and in a greater percentage of cases, even after periods varying from 8 to 20 months.

Table II. *Group A.*

Patient's initials	Date of *Prophylactic* inoc. (subcut. inject.)	Doses of vaccine given	Month when blood sample taken in 1931	Titre of typhoid "H" agglutination	Titre of typhoid "O" agglutination
O.W.	Jan. 1927	.	April	1 : 100 (3)	1 : 50 (3)
A.G.B.	Jan. 1928	3 of $\frac{1}{4}$, $\frac{1}{2}$, 1 c.c.	,,	1 : 50 (4)	—
G.N.	,,	3 of $\frac{1}{4}$, $\frac{1}{2}$, 1 c.c.	,,	1 : 50 (3)	1 : 50 (3)
H.S.G.	,,	2 of $\frac{1}{2}$ c.c.	,,	1 : 200 (3)	1 : 100 (2)
P.E.	Jan. 1928	3 of $\frac{1}{4}$, $\frac{1}{2}$, 1 c.c.	,,	1 : 200 (4)	—
	Jan. 1929	1 of 1 c.c.			
R.E.	Jan. 1928	3 of $\frac{1}{4}$, $\frac{1}{2}$, $\frac{1}{2}$ c.c.	,,	1 : 100 (2)	1 : 25 (2)
T.J.O.	,,	1 of $\frac{1}{4}$ c.c.	,,	1 : 25 (4)	1 : 50 (4)
T.S.	,,	1 of 1 c.c.	,,	1 : 200 (3)	—
W.L.B.	,,	1 of 1 c.c.	,,	1 : 100 (3)	—
W.L.C.	,,	1 of 1 c.c.	,,	1 : 50 (3)	—
	Feb. 1931	.	Aug.	1 : 250 (2)	—
	,,	.	,,	1 : 50 (2)	—
	,,	.	,,	1 : 250 (3)	—
	,,	.	,,	1 : 25 (3)	1 : 25 (3)
	,,	.	,,	1 : 200 (2)	—
	,,	.	,,	1 : 100 (3)	1 : 50 (2)
	,,	.	,,	1 : 50 (4)	1 : 25 (2)
	,,	.	,,	1 : 500 (3)	1 : 25 (3)
	,,	.	,,	1 : 250 (2)	—
	,,	.	,,	1 : 100 (3)	—
	,,	.	,,	1 : 50 (2)	—
	,,	.	,,	1 : 100 (3)	1 : 25 (3)
	,,	.	,,	1 : 100 (2)	—

Note: (2), (3), etc., indicate strength of reaction as + +, + + +, etc.

In five of the cases in this group, the R.T. for "O" agglutinins varied between 18 and 22·7 so that even when a course of intravenous injections of typhoid vaccine is administered the R.T. is seldom above 20 if the inoculation is recent. These observations assume importance in considering sera from cases which have received intravenous injections of typhoid vaccine in protein shock therapy, *e.g.* rheumatoid arthritis and various mental states.

SERA FROM UNCOMPLICATED CASES OF ENTERIC FEVER.

The cases examined in Table IV (*i.e.* not previously inoculated) illustrate that (*a*) sera from typhoid patients taken early in the course of the disease usually contain both large-flaking and granular agglutinins ("H" and "O"),

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(*b*) the absence of "H" agglutinins in a few cases (*e.g.* case 4), and (*c*) the possibility of giving a prognosis from consideration of the "O" agglutinin titres.

Table III. *Group B.*

Patient's name or initials	Date of *Therapeutic* inoculation (intrav. inject.)	Month when blood sample taken in 1931	Titre of typhoid "H" agglutination	Titre of typhoid "O" agglutination	Reduced "O" titre
A.D.	Oct.–Nov. 1929	July	1 : 25 (3)	—	—
C.O.	,,	,,	—	1 : 50 (3)	4·5
Durham	,,	,,	1 : 50 (2)	1 : 50 (2)	4·5
Ellis	,,	,,	1 : 25 (2)	1 : 100 (2)	9
Hague	,,	,,	1 : 25 (2)	1 : 100 (2)	9
Vaidnoff	,,	Aug.	1 : 25 (2)	1 : 50 (2)	3
Willis	,,	July	1 : 25 (2)	1 : 100 (3)	9
W.M.	,,	,,	1 : 50 (2)	1 : 250 (2)	22·7
X.	,,	,,	1 : 50 (3)	1 : 250 (3)	22·7
Bullen	Sept.–Oct. 1930	,,	—	1 : 50 (3)	4·5
Caplan	,,	June	1 : 100 (3)	1 : 200 (2)	18
Carson	Oct.–Nov. 1930	July	1 : 100 (2)	1 : 50 (3)	4·5
A.S.	Sept.–Oct. 1930	June	1 : 500 (3)	—	—
F.J.	June-Aug. 1930	,,	1 : 500 (3)	1 : 200 (3)	18
Hope	Oct.–Nov. 1930	July	—	—	—
Legge	Sept.–Oct. 1930	,,	1 : 250 (3)	1 : 100 (2)	9
Parks	,,	June	1 : 250 (2)	1 : 100 (3)	9
Penley	Aug.–Sept. 1930	,,	1 : 250 (2)	1 : 100(2)	9
P.L.	,,	,,	1 : 100 (3)	1 : 200 (2)	18
Spence	Sept.–Oct. 1930	July	—	—	—
S.C.	,,	June	1 : 100 (3)	—	—
T.W.	,,	,,	1 : 50 (3)	1 : 100 (2)	9
Williams	Jan.–Feb. 1930	,,	1 : 250 (3)	1 : 100 (3)	9
W.B.	Sept.–Oct. 1930	,,	1 : 200 (4)	1 : 50 (4)	4·5

Note: (2), (3), etc., indicate strength of reaction as + +, + + +, etc.

Table IV. *Uncomplicated cases of typhoid fever.*

Case No.	Patient's initials	Month when disease contracted in 1931	Date when first blood sample taken in 1931	Titre of typhoid "H" agglutination	Titre of typhoid "O" agglutination	Reduced titre for "O" agglutination
1	Grant	May	May 9	1 : 50 (3)*	1 : 200 (3)*	18·2
2	E.G.	July	July 17	1 : 5000 (4)	1 : 200 (3)	18·2
3	S.C.	Aug.	Aug. 8	1 : 500 (2)	1 : 2000 (2)	182
4	L.C.	,,	Aug. 8	—	1 : 50 (2)	4·5
5	W.C.	,,	Aug. 19	1 : 2000 (3)	1 : 500 (3)	45·5
6	McGrath	,,	Aug. 24	1 : 1000 (3)	1 : 250 (3)	15·5
7	H.L.	,,	Aug. 27	1 : 1000 (3)	1 : 500 (3)	31
8	D.W.	Sept.	Sept. 8	1 : 500 (3)	1 : 1000 (2)	62·5

The blood samples were usually taken towards the end of the first week of illness.

* (2), (3), etc., indicate strength of reaction as + +, + + +, etc.

If the "O" agglutinin titre is high in the early stage of the disease, the prognosis is favourable; if low or absent, the case may be severe or mild; and if entirely absent throughout the course of the disease, the case may end fatally. The mere presence of "O" agglutinins denotes an enteric infection, whereas the "H" agglutination indicates the type of infection.

The following three cases, all members of the same family—Mrs S. C. age 53, Miss L. C. age 26 and Mr W. C. age 18, developed typhoid fever within a few days of each other. They have been observed in detail and present interesting features.

Case I (see Chart I). S. C.'s graphs are interesting because they show a much higher titre for "O" agglutination (1/2000 = R.T. 182) than for "H" agglutination (1/500 = R.T. 62·5) in the first sample of serum taken on admission to hospital on August 8th (the 12th day of illness). A high titre for "O"

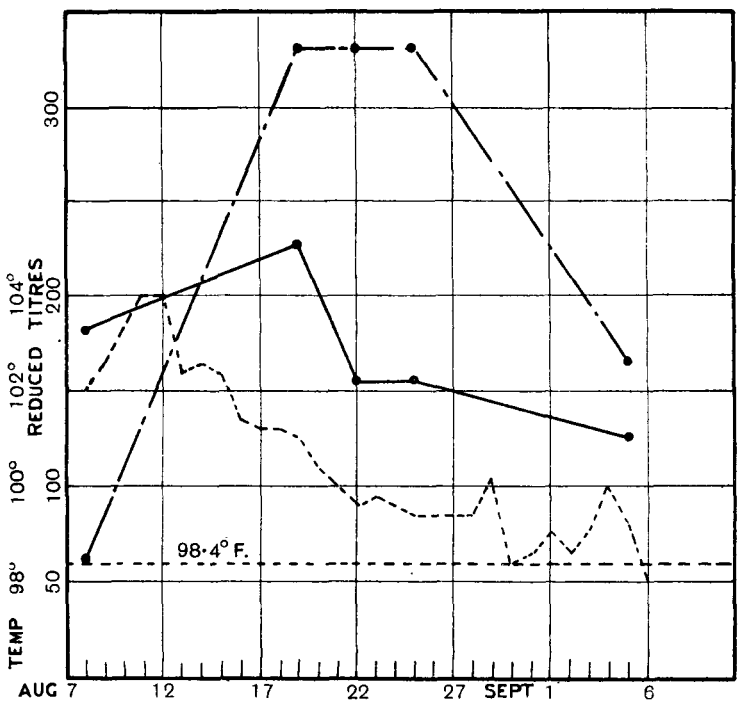

Chart I: S. C. age 53. Typhoid Fever.

— — — "H" agglutinins.
———— "O" agglutinins.
. . . . . highest daily temperature reached.
• blood sample withdrawn and titres determined.

agglutinins in the early stage of typhoid fever indicates a favourable prognosis, and the subsequent history shows that her recovery was uneventful.

Another point is that between August 12th and 17th the temperature varied between 101 and 104° F. and thereafter showed a steady decline by lysis. Reference to the graphs shows that the "O" agglutinins follow the clinical course of the disease more closely than the "H" agglutinins because the "H" agglutinins did not diminish till after August 25th, although the temperature had been closely approaching normal limits for several days.

The clinical condition of the patient was closely observed throughout her

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illness and at no time did she appear gravely ill. She made a good convalescence and was not much emaciated. The "O" agglutinin titre, even in the stage of convalescence, is quite high (1/2000 or R.T. 125).

Case II (Chart II). W. C.'s graph for "O" agglutinins accords with Felix's view that the "O" agglutinin titres are relatively highest in the early and

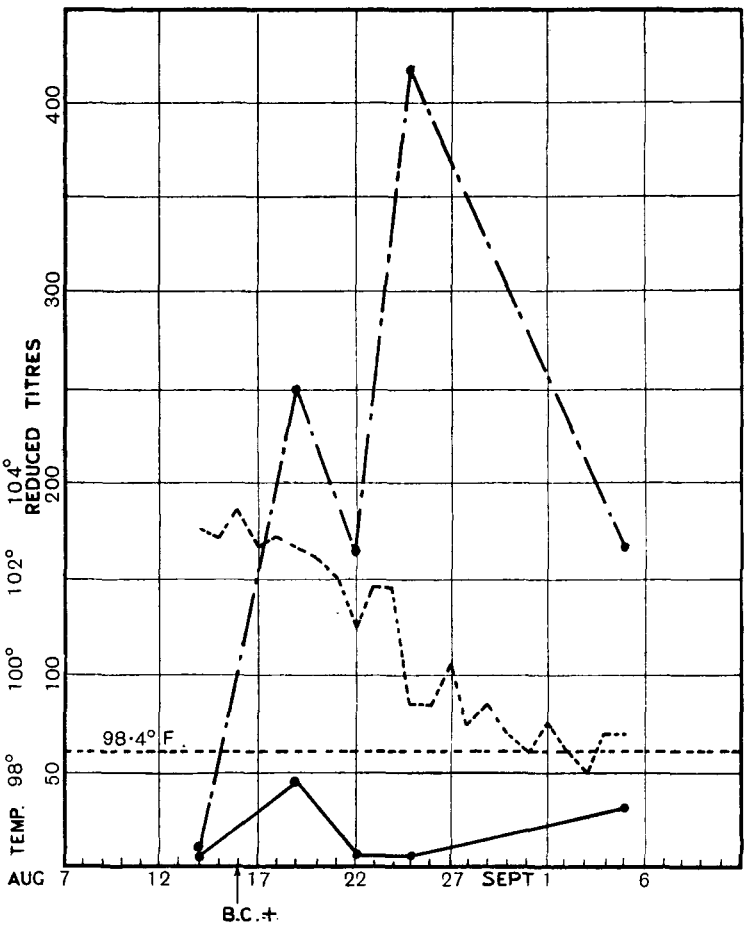

Chart II: W. C. age 18. Typhoid Fever.

—— — —— "H" agglutinins.
———— "O" agglutinins.
- - - - - - - - - - highest daily temperature reached.
• blood sample withdrawn and titres determined.
B.C.+ *B. typhosus* isolated by blood culture.

convalescent stages of typhoid fever. These are 1/500 = R.T. 45·5 on August 19th (10th day of illness) and 1/500 = R.T. 31 on September 7th (27th day of illness).

His graph for "H" agglutinins shows sudden excursions which bear no relation to the clinical course of the disease, for this patient was never at any

stage acutely ill and, although his temperature varied between 102 and 103° F. from August 14th to 22nd, it fell thereafter by lysis to within normal variations.

Case III (Chart III). L.C.'s graphs are particularly interesting. The graph for "H" agglutinins shows a zero limit even during the 3rd week of her illness and after four weeks only reached a titre of 1/25 (R.T. 4).

The titre for "O" agglutinins on the 2nd day of her illness (August 8th) was 1/50 (R.T. 4·5) and this low figure led me to conclude that this patient would either have an extremely mild course or a protracted and severe course. A comparatively low titre of 1/250 (R.T. 15·5) on August 19th, combined with

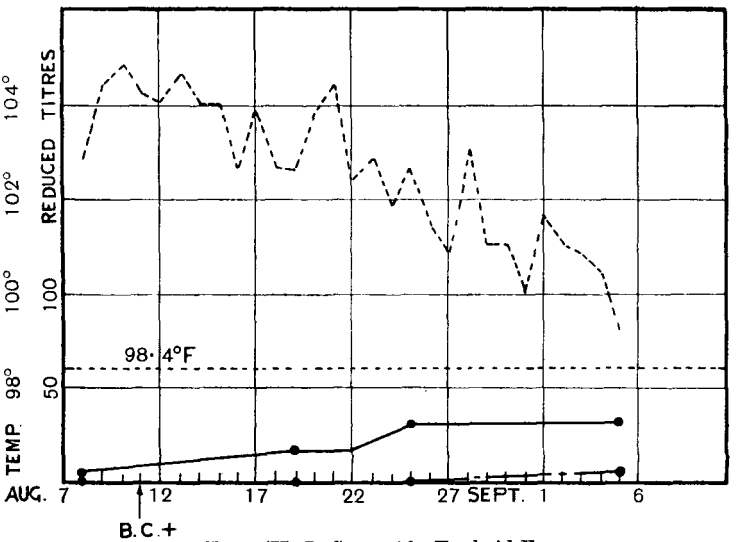

Chart III: L. C. age 26. Typhoid Fever.

—— — —— "H" agglutinins.
———— "O" agglutinins.
- - - - - - - - - - highest daily temperature reached.
• blood sample withdrawn and titres determined.
B.C.+ *B. typhosus* isolated by blood culture.

high variations of temperature of 102·6–104·8° F. since August 8th, inclined to the latter prognosis.

The entire graph of "O" agglutinins shows a comparatively low limit, finally reaching a titre of 1/500 (R.T. 31), and is correlated with a severe clinical course in which the patient became very emaciated. This is an example where positive "H" agglutination occurs late in the disease. The bacillus isolated by blood culture on the 5th day of illness (August 11th) was motile, but Gardner, Hobson and Stenhouse (1930) have recently reported a case with persistent negative "H" and positive "O" agglutination associated with non-motile "O" *B. typhosus* in the blood.

VALUE OF THE "O" AGGLUTINATION TEST.

A consideration of these results indicates the value of the "O" agglutination test. In the laboratory diagnosis of enteric fever we are accustomed

to emphasise (1) that the Widal reaction is not likely to be of diagnostic value prior to the 10th day and (2) that a positive Widal reaction is *not* diagnostic if the patient has ever been previously inoculated with typhoid vaccine. We have learned to depend on (*a*) blood culture during the first 10 days of illness, for during this period the causal organism is nearly always present in the blood stream, and (*b*) isolation of the causal organism from the faeces after the 10th to 12th day of illness and later from the urine also. Practitioners, however, continue to send samples of blood from enterica suspects during the first week of the disease and as the "O" agglutinins are seldom absent in the very early stages of the disease—according to Felix the "O" agglutinins appear before the "H" agglutinins—we have not only an important guide to diagnosis but also to prognosis. It is suggested that laboratory workers could with advantage employ "O" suspensions in addition to the ordinary "H" suspensions in performing the Widal test, so that more data may be accumulated regarding the value of the "O" test in cases showing negative "H" agglutination, in cases where "O" agglutination appears earlier than "H," and in the diagnosis of typhoid fever in inoculated persons.

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