QUALITATIVE OR QUANTITATIVE METHODS IN THE SEROLOGICAL DIAGNOSIS OF ENTERIC INFECTIONS?

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Since the pioneer studies of Weil and Felix (1920) and the further studies of Felix (1924–9) on the principles of qualitative receptor analysis, a considerable amount of attention has been devoted to this subject. Recent workers, especially Stuart and Krikorian (1928), Gardner (1929), Pijper (1930), Whitehead (1930) and Smith (1932), have made clinical studies of the qualitative method and in the main have confirmed the contentions of Felix, but with the exception of Gardner's valuable paper (1929) little critical study appears to have been made of certain fundamental assumptions underlying this method.

The general principles of qualitative receptor analysis are too well known to most recent workers to need repetition in this present paper, which in no way pretends to be a comprehensive study of the question. An endeavour is, however, made to examine certain unproved assumptions of the method and to point out some of its possible fallacies when used for clinical diagnosis. The basis of the method may be reduced to two fundamental principles:

(1) O agglutinins may be present in low titres in normal sera but are not produced by inoculation of T.A.B. vaccine, save in negligible amounts.

(2) O agglutinins are almost always present to significant titres in enteric infections and are more closely associated with the infection than the H agglutinins. Hence if the limit of normal O agglutinins be established, a positive diagnosis of an enteric infection can be readily made in any case inoculated or not which shows O agglutination above this titre.

Methods. The macroscopic method was used, the dilutions of serum ranging from 1 in 25 to 1 in 5000. Incubation was in a water bath at 52° C. for 8 hours; the racks were then removed, allowed to stand overnight at bench temperature (approximately 32° C. in Khartoum) and read with a lens after 24 hours. A trace of agglutination was taken as the end-titre.

Strains. The following strains were used:

B. typhosus H and O Felix 901, *B. paratyphosus* A (Schottmüller), *B. paratyphosus* B (Rowlands), all of which were obtained from the National Collection of Type Cultures.

Emulsions. For H agglutination the organisms were grown on ordinary agar for 24 hours, washed off, and diluted with 0·5 per cent. carbol saline. For O agglutination alcoholic suspensions were employed according to Gardner's method (1929).

Sufficient emulsions of both types were made to last the whole series and stored in an ice box; all remained perfectly stable.

Sera. In view of Felix's observations on the weakening by heat of O agglutinins, all sera were used unheated after standing for 24 hours.

TYPES OF CASE.

Investigations were made of the following types of case:

(1) O agglutinins in normal sera, *i.e.* in non-inoculated persons with no previous history of enteric infection.

(2) O titres after inoculation by T.A.B. vaccine.

(3) O titres in enteric fevers principally typhoid.

(1) *Agglutinins in normal human sera (non-inoculated).*

In all, 70 sera were selected at random from natives of the Sudan in whom there was no history of either inoculation or of previous enteric infections (typhoid or paratyphoid fevers).

Table I.

Titres of O agglutinins	T	A	B
0–25 (<25)	65	70	67
25–50	5	0	3

It may be remarked that in all these sera H agglutination was negative.

Few figures of the absolute magnitude of O titres in normal persons appear to be available. Gardner (1929) found that out of 47 cases, 24 were negative, 22 lay between titres of 20 and 50 (0·5–2 units), and one only gave more than 8 standard units (trace 1 in 200). These figures refer to *B. typhosus* only. Gibson (1930) in his studies on normal agglutinins has found in human sera a range of titres from 0 to 1 in 32 for *B. typhosus*, from 0 to 1 in 16 for *B. paratyphosus* B and no agglutination against *B. paratyphosus* A, but his samples are very few. Pijper (1930) does not quote figures but remarks that his "experience with normal agglutinins so far has been that they very rarely occur in such quantity as to give even just visible agglutination in a dilution of 1 in 100 with live suspensions of *B. typhosus* 901."

Taking all the above results into account, it would seem reasonable to accept 1 in 50 as the limit of normal agglutination in the great majority of cases for *B. typhosus* and *B. paratyphosus* B. In the author's opinion Felix's figure of 1 in 100 for *B. typhosus* is rather high.

Normal agglutinins for *B. paratyphosus* A appear to be uniformly absent at a titre of 1 in 25.

(2) *Titres of O agglutinins in persons who have been inoculated with T.A.B. vaccine.*

Twenty boys aged 14–16, in whom there was no history of enteric infection, were selected at random from the entrants to a large secondary school in Khartoum, and each received the two standard doses of T.A.B. vaccine at 8 days' interval. The vaccine was the usual stock vaccine prepared from the following strains: *B. typhosus* "Rawlings," *B. paratyphosus* A "Schottmüller," and *B. paratyphosus* B "Rowland." Before inoculation, the sera were examined and all were negative (<1 in 25) for T.A.B.—O agglutination. The sera were again examined 8 days and 6 weeks after the second dose.

As the results are rather unexpected, the details are given in Table II.

Table II. *O agglutination titres in inoculated individuals.*

Number of boy	T			A			B		
	8 days	6 weeks	1 year	8 days	6 weeks	1 year	8 days	6 weeks	1 year
1	125	50	25	0	0	0	125	125	0
2	125	0	0	0	0	0	250	250	125
3	250	125	25	0	0	0	250	500	125
4	125	125	25	0	0	0	250	25	0
5	125	125	0	0	0	0	250	250	50
6	125	50	25	0	0	0	125	125	0
7	125	50	0	0	0	0	50	25	0
8	500	–	25	0	–	–	250	–	25
9	250	125	50	250	0	0	500	500	125
10	500	–	–	250	–	25	500	–	–
11	50	0	0	0	50	0	0	250	0
12	125	50	50	125	250	0	50	500	25
13	500	250	50	50	250	0	125	500	25
14	500	250	50	125	500	50	125	250	50
15	250	250	125	25	50	0	50	500	125
16	125	125	0	50	250	25	125	500	50
17	500	250	0	50	125	0	125	125	0
18	1250	500	125	50	0	0	125	125	0
19	1250	–	125	0	0	0	125	50	0
20	250	–	–	25	0	0	50	125	25

– =not examined. 0 =less than 1 in 25 titre.

Discussion of results. The following conclusions may be drawn from Table II.

(1) Anti-enteric inoculation stimulates a production of O agglutinins in man as in other animals. This response may be of comparatively high titre and remain constant for some weeks.

(2) The irregular and rather poor response to *B. paratyphosus* A. This is of some interest in view of the fact that O agglutinins for this organism never appear to be present in normal human sera.

(3) After a period of a year the O agglutinins have sunk to a very low titre, but one which on the average is higher than the titre of normal sera.

It is interesting to compare these figures with those of Whitehead and Gardner.

Whitehead (1930) reports 54 cases ranging from 1 to 15 months after inoculation; his titres range from 1 in 25 to 1 in 250, but there appears to be no correlation between the higher titres and the more recent inoculations. His figures after any one period are, however, very small.

Gardner's (1929) recently inoculated (4 weeks) group of 11 cases showed titres from 20 to 800 averaging 5·0 standard units. His group of 16 cases headed "Inoculation" (probable or certain) in the past averages 1·8 units per case as against a normal average of 0·5 unit.

Summarising the above it seems fair to state:

(1) Inoculation of T.A.B. vaccine produces very considerable quantities of O agglutinins.

(2) Such titres persist practically unchanged for at least 2 months.

(3) At a year or more after inoculation O titres are low and irregular, but on the average are higher than those of normal sera.

(4) It is impossible to fix any definite titre as a limit for recently inoculated persons.

(3) *Titres of O agglutinins in enteric fevers.*

Table III. *Proved cases (by culture) of typhoid fever.*

		B. typhosus		*B. paratyphosus* B	
Case	Day	H	O	H	O
1	20	2500	500	125	125
2*	21	0	125	0	50
3	15	1250	250	0	0
4	15	250	500	0	0
5	8	0	50	0	0
6	14	1250	500	0	0
	20	500	500	0	0
	28	500	250	0	0
7	11	0	50	0	0
	28	0	50	0	0
8	20	50	250	0	0
9	10	125	125	0	25
	17	125	125	0	25
	31	50	50	0	0
10	10	0	0	0	25
(very ill-marked rash)	17	0	0	0	25
11	21	0	250	0	0
12	14	0	500	0	0
13	21	500	125	0	0
14*	12	1250	2500	0	50

* = previous inoculation of T.A.B.

Table IV. *Proved cases of paratyphoid B fever.*

		B. paratyphosus B		*B. typhosus*	
Case	Day	H	O	H	O
1	12	5000	500	0	0
2*	11	1250	500	50	0
3*	10	1250	50	125	50

* = previous inoculation of T.A.B.

	Inoculated (T.A.B.)
Table III. Case 2	1927
,, ,, 14	1928
Table IV. Case 2	1928
,, ,, 3	1928

Reference to the above tables shows:

(1) The well-marked production of homologous O agglutinins in most of the cases.

(2) The almost negligible production of heterologous agglutinins either H or O in the majority of cases.

(3) The remarkable number of sera in which H agglutinins were absent, 6 out of 17. It is of course possible that blood taken at different stages would have shown in some samples the presence of H agglutinins, but in the above cases the blood was taken on the second or third week when one might reasonably expect the presence of agglutinins. It will also be noticed that in

these cases the titre of O agglutination is so low as to be of doubtful diagnostic significance, if 1 in 100 is taken as the normal threshold.

Cases showing only O agglutination are of considerable interest and have been reported from Palestine (Felix, 1924), England (Gardner, 1930), South Africa (Pijper, 1930), Federated Malay States (Martin, 1930). One serum (10) in the above series, although from a typical clinical case of typhoid in which the blood culture was positive, was negative on two occasions for both types of agglutinins.

It may be mentioned that the above sera with low or negative O titres were retested against a live emulsion of O 901; the titres in all cases were the same as given by the alcoholic emulsions.

DISCUSSION.

It is clear from Table II the residual O agglutinins after inoculation show very variable titres, and hence it is most important that quantitative estimation of these titres be made as early as possible in the course of an infection "to establish a point on the early part of the curve, from which the extent of any subsequent rise can be measured" (Topley and Wilson, 1929).

The fallacies of a purely qualitative method may be stated as follows:

(1) There is no *a priori* justification for fixing an arbitrary titre as a limit for O agglutinins either in normal or inoculated subjects. The acceptance of such a limit was logical only so long as one denied the production of O agglutinins after T.A.B. inoculation. In view of the above figures and also of Gardner's observations (1929) such a contention is no longer valid. In fact it is probable that there is no quantitative or qualitative difference between the response of O agglutinins to a vaccine and the response to an enteric infection.

(2) Considerable stress has been laid on the anamnestic reaction, *i.e.* the non-specific stimulation of agglutinins in an inoculated person by some non-enteric infection. A discussion of this reaction is outside the scope of the present paper, but there appears to be little doubt that the reaction is concerned essentially with H agglutination, the effect on O titres being negligible.

This fact may be of considerable theoretical interest, but it cannot be adduced as support for a qualitative Widal reaction. The criticism previously mentioned also applies here, viz. the impossibility of fixing any definite titre as a limit of O agglutination in inoculated individuals.

(3) Cases are frequently encountered, as above or as reported by Whitehead (1930), in which O titres remain consistently at a low level, 1 in 50 to 1 in 125, and there is a considerable risk of missing such cases if an arbitrary normal limit of 1 in 100 or 1 in 200 is accepted as a base line. In such cases the value of a negative Widal early in the disease is very considerable in arriving at a serological diagnosis.

In conclusion one must emphasise the especial importance of accurate

quantitative Widals in the diagnosis of fevers in the tropics for the following reasons:

(1) The common practice of mass T.A.B. inoculation, *e.g.* government officials, pilgrims in quarantine, etc.

(2) The difficulty of obtaining any reliable history from natives as to whether they have been inoculated, and if so, the time that has elapsed since inoculation.

(3) The common occurrence of various obscure types of pyrexia simulating enteric infections in both normal and inoculated persons.

As such cases are frequently outside the range of full laboratory facilities, the Widal reaction assumes proportionately greater importance in arriving at a diagnosis.

A quantitative technique using both types of antigens (H and O) for each organism and carrying out agglutination to end-titre is alone capable of giving such assistance.

Summary.

1. "Normal" agglutinins for *B. typhosus* and *B. paratyphosus* B appear to be conclusively of the O type.

2. Such agglutinins appear to be of consistently low titre and are absent in many sera.

3. Normal agglutinins for *B. paratyphosus* A have not been encountered.

4. O agglutinins are readily produced by inoculation of a T.A.B. vaccine, and may rise to as high titres as those encountered during an enteric infection.

5. It is impossible to fix any limit for residual O agglutinins in inoculated persons.

6. The value of examining for O agglutinins in enteric cases is confirmed.

7. Some fallacies of a purely qualitative method are pointed out, and a plea is made for the inclusion of the principles of qualitative receptor analysis in a quantitative Widal.

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