

THE DIAGNOSTIC VALUE OF THE WIDAL TEST IN THE INOCULATED

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(With 1 Figure in the Text)

The first widespread application of the quantitative (Dreyer) Widal test during the 1914-18 war coincided with a thorough inoculation campaign against the enteric fevers. In an inoculated patient, the similar actions of antibodies produced by inoculation and by natural infection have often prevented a clear-cut interpretation of the results of the test, and claims for the test have had to be revised repeatedly in the light of further experience. For example Walker (1917) stated 'In every case proved bacteriologically...the diagnosis independently arrived at by means of agglutination tests... was proved to be correct'. Later it was found that the test material used in the 1914-18 war gave results dependent almost entirely on the 'H' agglutinin, and it is now accepted that, in inoculated persons, 'H'-type agglutinins are quite unreliable as a diagnostic test (Topley & Wilson, 1946). It was originally considered that 'a regular rise and subsequent fall of only 100 or 200%' in titre at the appropriate period made it likely that the case was one of typhoid or paratyphoid infection (Dreyer, 1916). Later, 'a change in titre for one organism of not less than 200%' was usually accepted (Perry & Bensted, 1929), but now 'relatively large fluctuations in the titre of "O" agglutinins' seem to be considered necessary (Topley & Wilson, 1946).

The larger the rise in titre which is necessary for a 'positive' diagnosis, the smaller will be the percentage of diagnoses and the less the clinical value of the Widal as a diagnostic test. There are few published reports giving the actual titres of serial Widal tests in cases of enteric fever from which the frequency of positive tests can be discovered, even in the uninoculated. No such report has been found for tests in the inoculated so that the results on sixteen inoculated individuals are presented here. All were British Service personnel and had been inoculated during the 9 months preceding the onset of enteric fever, so that, as a group, they should have provided the least favourable material for the evaluation of the Widal test. The majority had received the alcohol-killed vaccine but many of these had also received the standard vaccine at one time or another. No special selection was made of the cases in which serial tests were done except

for the inevitable exclusion of patients dying early in the course of the disease before the later tests could be carried out, and of others from whom no early blood sample was obtained.

METHOD

The technique described by Wilson (1945) was employed except that the serum dilutions were prepared with graduated pipettes, not by the method of doubling dilutions. Standard agglutinable cultures were provided by the Central Military Pathological Laboratory, Poona.

RESULTS

In Fig. 1 are given the O-agglutination titres against *Bact. typhosum* and *Bact. paratyphosum* A in eight cases of typhoid fever and eight cases of fever due to infection by *Bact. paratyphosum* A. The cases were all proved by blood culture and ran a typical course, the duration of the initial fever averaging 23 days in the typhoid and 19 days in the paratyphoid A infections. There did not seem to be any significant differences attributable to the different vaccines with which the patients had been inoculated.

The initial titres in some cases were perhaps surprisingly high, but the whole service population was inoculated 'up to date'. In 35 control sera, 12 normal (W.R.) specimens and 23 from cases of definitely diagnosed non-enteric febrile illnesses, T 'O' titres of 1:400 were found four times and of 1:200 another six times. Wilson (1945), also working in India and using a similar technique, obtained higher figures for healthy inoculated men than most other workers. The recurrent annual inoculation of the subjects examined by Wilson and by the author may be a partial explanation of their findings.

The alterations in titre during the course of the illness were usually small, and a rise of over 200% was seen in only one case of typhoid out of eight, and in only three cases of paratyphoid A out of eight. This failure to find a consistent rise in titre could conceivably have been due to a serious under-estimation of the duration of illness at the time the first blood specimen was obtained. Great care was

taken in establishing the day of onset, however, as the incubation period of enteric fever was also the subject of inquiry. This makes all the more noteworthy the tendency to a falling titre in the homologous reaction, T 'O' agglutination in typhoid fever and A 'O' agglutination in paratyphoid A infection.

being diagnostic would depend on the ratio of the numbers of patients with enteric infection to those with some other febrile illness, and on the proportion of the latter in which Widal tests were performed, i.e. on the fashionableness of the Widal test. For example, if there were 500 febrile patients without enteric to every patient with enteric, there

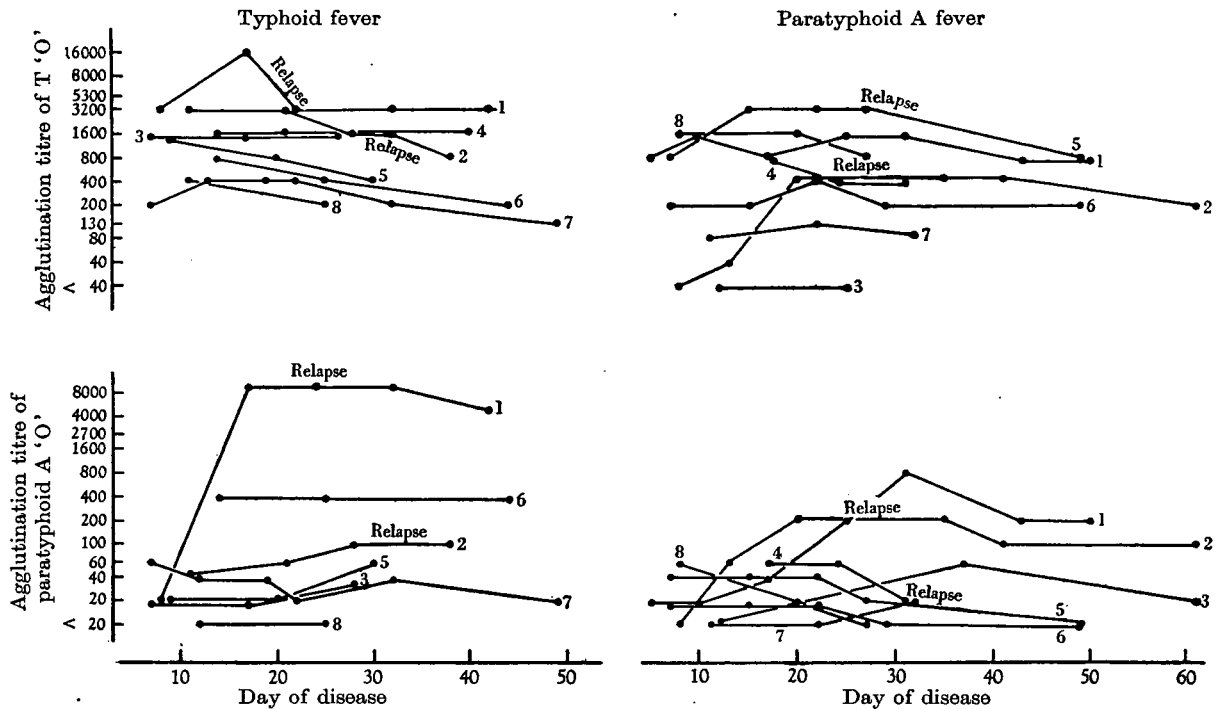


Fig. 1. Serial Widal tests on cases of enteric fever in which blood culture was positive. The cases are distinguished by serial numbers. The titres are shown on a logarithmic scale.

DISCUSSION

Diagnostic value of a single estimation of 'O' titre in inoculated persons suffering from possible enteric fever

The diagnostic value of a single titration cannot be directly assessed by comparing the titre in a suspect individual with the titres in healthy individuals unless it is certain that illness other than enteric fever does not affect the 'O' titre. On this there is very little published evidence, and the data mentioned above are too meagre for consideration. Wilson (1945) found a T 'O' titre of 1:320 or over in 1.6% of patients with diseases other than enteric as compared with 0.5% of healthy inoculated individuals.

However, even if it were established that a titre of 1:320 or over was given by, say, 32% of patients with proved enteric fever, such a titre in a febrile individual would not necessarily mean that there was a 32 to 1.6, i.e. 20 to 1 chance of his having enteric fever. The actual probability of such a titre

would be 25 individuals with titres of 1:320 and over who did not have enteric fever to every one who had. While if the Widal test happened to be carried out on only 4 patients in each 100 with febrile illnesses, there would be an even chance that an individual with such a titre had enteric fever. The paradox is reached that the greater the reliance upon the diagnostic value of the Widal test the less valuable become the results.

The higher the level of the titre chosen for the diagnostic dividing line, the more likely is such a titre to indicate infection, but at the same time the smaller will be the proportion of cases of enteric fever thus diagnosed. A diagnostic criterion to which the answer is correct nine times out of ten, is not likely to be of much help if it occurs in only one case out of ten. It is, therefore, correct to conclude that a single estimation of the 'O' titre in inoculated persons can never be of any real help in the diagnosis of enteric fever, at least if inoculation has been performed within the past year as was true of Wilson's material and that analysed here.

Diagnostic value of serial estimations of 'O' titre in inoculated persons suffering from possible enteric fever

The diagnostic failure of the serial Widal test in the inoculated seems to be the consequence of the high titres produced by inoculation. Table 1 gives the alteration in titre during the first 3 weeks of illness in all the bacteriologically proved cases of typhoid fever in which serial Widal tests were performed during this period of the disease in the years 1942-5 at the District Laboratory, Karachi. These tests were made by various workers and the techniques used are not in all cases known. The lower the initial titre, the higher was the rise in titre during the first 3 weeks of the disease, and vice versa. It appears, therefore, that it is a high initial titre, not just the fact of being inoculated, that prevents a rise in titre during enteric fever. This may in fact be the reason why a significant rise in titre occurred more commonly in paratyphoid A than in typhoid fever (Fig. 1).

Table 1

Comparison of the lowest titre for agglutination of T 'O' in first 11 days with the highest titre found between the 12th and 24th days in 44 cases of proved typhoid fever. At least 36 cases are known to have been inoculated: few, if any, are likely to have had the alcohol-killed vaccine. Thirty patients were of British stock, 14 Indian or Anglo-Indian.

Alteration in titre as n where $\frac{\text{highest titre}}{\text{lowest titre}} = 2^n$,
i.e. using doubling dilutions n = the no. of tubes the end-point shifted

Initial titre	-1	0	+1	+2	+3	+4	+5	+6
0	.	4	2	4	1	8	2	2
20	1	1	5	3	1	.	.	.
40	.	.	1	1
80	.	1	.	.	2	.	.	.
160	.	.	.	2
320	.	1
640	.	1
1280	1

Orthodox teaching is that, even in the inoculated, the 'O' titre rises during the first 3 weeks of enteric fever. The data of Fig. 1 suggest that a significant rise occurs only in about a quarter of the cases, less often than a fall. Wilson (1945) also found that 'no variation in titre during the disease can be taken as diagnostic for enteric fever', although 'rising or fluctuating titres appear more frequently in enteric fever than in other diseases'. The lack of value of the Widal test in the diagnosis of enteric fever in Service personnel, i.e. in the inoculated, was widely

appreciated over many areas of the Middle East and India during the last war, and in some Commands its use was prohibited for this reason.

All the evidence showing the diagnostic failure of the Widal test was obtained from 'recently' inoculated individuals. How long this effect of inoculation may last is unknown but its persistence may become a diagnostically important matter now that conscription ensures the inoculation in their youth of nearly all the adult males in the country.

Blood culture

The usual emphasis, in clinical teaching, on serial agglutination tests as an aid to diagnosis in cases of continued fever is therefore misplaced, at least in so far as recently inoculated men are concerned. A consequence of the usual teaching, more important than the waste of effort and time in doing the Widal test, is the neglect of blood culture which seems to follow reliance on serial Widal tests. The author was guilty of this neglect as a resident when the theory of the serial Widal seemed so convincing that it was always the test of choice, and subsequent experience has shown that newly qualified residents and service medical officers have usually shared the same belief. Blood culture provides a more exact diagnosis than does the serial Widal test, and has the additional advantage of providing the diagnosis more quickly. In 16 out of 19 cases, the first blood culture provided a correct diagnosis in 2 to 4 days, and in the remaining three cases and in others in which the first blood culture appeared sterile after 48 hr., the second blood culture was successful.

SUMMARY

1. Evidence is given that in recently inoculated persons the Widal reaction, even when serial quantitative tests are done, has no practical value in the diagnosis of enteric fever. Conscription enlarges permanently the inoculated proportion of the whole population.

2. The diagnostic value of any particular titre depends not only on the relative frequencies with which this titre occurs in normal and infected individuals but also on the incidence of enteric fever in the population and on the fashionableness of the Widal test as a diagnostic aid.

3. It is emphasized that blood culture is a more reliable aid to diagnosis than the Widal test.

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