THE DISTRIBUTION AND SIGNIFICANCE OF TYPHOID Vi AGGLUTININS IN NORMAL SERA OF AFRICAN NATIVES

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Introduction

This communication records the results of testing random samples of sera from Africans in Southern Rhodesia for the presence of agglutinins against the Vi antigen of *Bact. typhosum*, together with the results of cultural examinations for *Bact. typhosum* of the excreta from some of the positive reactors.

The objects of the investigation were twofold. First, in view of the paucity of published reports on the subject it was considered desirable to secure further information concerning the distribution of Vi agglutinins in normal sera. In the literature available, the only references are the following. Horgan (1936), who examined 100 normal sera (presumably from Sudanese), found that four yielded traces of Vi agglutination in a titre of 1 in 5, and six in a titre of 1 in 10, the remaining ninety showed no agglutination at 1 in 5; the technique employed is not stated. Pijper & Crocker (1937) tested seventy normal sera in South Africa by the absorption method over a range of dilutions from 1 in 20 to 1 in 160 and found all sera to be negative. Bhatnagar, Freeman & Dhilon (1937) detected Vi antibody in a titre of 1 in 50 in the sera of two of fifty uninoculated individuals examined in India. Both of these individuals gave a history suggestive of typhoid fever within the previous year, but examinations of their excreta for typhoid bacilli were negative. These authors employed the absorption method and did not test below a dilution of 1 in 20. Lewin (1938) reports that of sixty non-European South African sera examined for Vi agglutinations by the absorption method over a range of dilutions not lower than 1 in 40, one serum agglutinated in a titre of 1 in 40, and one in 1 in 160.

In the second place this inquiry had for its object the acquisition of data that might help to determine the value of the Vi agglutination test as an aid to the detection of chronic typhoid carriers. Felix (1938*a*) recommended the test for this purpose. He considered that definite agglutination of the "Watson" strain of *Bact. typhosum* in a serum dilution of 1 in 5, when established under standard conditions, should be regarded as significant. He also stated, however, that "It must be remembered that sera from persons who cannot be shown to be carriers occasionally also give positive reaction in 1 in 5 or even in higher dilutions". It was hoped, then, that cultural examinations of the excreta of persons whose sera had been shown to contain Vi agglutinins might

provide an indication of the frequency with which such antibodies occur in the absence of evidence of the carrier state.

METHODS

Most of the sera examined were selected at random from those submitted for Wassermann reaction, but the series included eighty-six sera from native servants who were examined in a routine search for carriers in connexion with a small outbreak of typhoid fever. In selecting them only clean and clear specimens were chosen, and only those from natives of Africa. No record of sexes was kept, but the majority of the sera were from adult males. Although practically all the samples were from natives in Bulawayo it is not justifiable to assume that these were necessarily indigenous natives of Southern Rhodesia, for owing to the migratory tendency of the people it is probable that a considerable proportion of them were from Northern Rhodesia, Nyasaland or other neighbouring territories. It may safely be assumed that practically all the individuals in question had not previously been inoculated with T.A.B.

The antigen used was a formolized suspension of Bhatnagar's "Vi 1" variant of *Bact. typhosum* (Bhatnagar, Speechly & Singh, 1938). In addition to this a formolized suspension of the "Watson" strain of *Bact. typhosum* was also used in the earlier part of the investigation, but as this antigen behaved very similarly to the "Vi 1" suspension its use as a routine was discontinued latterly.

Fresh suspensions of antigen containing approximately 600 million organisms per c.c. were prepared every 6 weeks or so during the course of the investigation, which lasted 5 months. The sera were examined in batches of thirty to forty at weekly intervals, and every batch of antigen was tested against "Provisional Standard serum for Vi agglutination" (Felix, 1938*b*) and pure Vi serum kindly supplied by Dr A. Felix, and also against H and O sera. The suspensions were required to satisfy the criteria of sensitivity to the Vi sera and non-agglutinability in H and O (Oxford) sera, even in dilutions of 1 in 5.

The agglutination tests were put up in Kahn test-tubes which were afterwards placed in the water-bath at 37° C. for 2 hr. and then left at room temperature overnight before the readings were made. In making the dilutions Dreyer's drop technique was employed, and in the first place each serum was put up in a final dilution of 1 in 5. Sera giving any sort of positive reaction were subsequently retested over a range of dilutions of 1 in 5 to 1 in 25 and also put up against H and O suspensions. The positive agglutinations were read as a "trace" (±), "weak positive" (+), and "positive" (++ or +++). Traces of agglutination were ignored. "Weak positive" was used to designate whole or partial agglutination of the bacteria to the bottom of the tube but of such a nature that on shaking the tube the flocculation, being in a fine state and barely discernible to the naked eye, required a magnifying glass for its clear recognition. "Positive" was the term used for agglutination reactions

at least as well marked as those produced by the "Provisional Standard Vi Serum" in a titre of 1 in 600 against the particular antigen suspension in use, as recommended by Felix (1939). In this type of reaction the bacteria tend to be completely deposited around the bottom of the tube, and on shaking the clumps are readily visible to the naked eye.

In all cases of sera yielding "positive" agglutination reactions in titres of 1 in 5 or higher, arrangements were made, whenever practicable, for the submission from the individual concerned of at least one sample each of freshly voided faeces and urine for bacteriological examination. The faeces were usually received in a fluid form as the result of a saline purge. Undoubtedly it would have been desirable to have investigated the medical histories of these individuals for evidence of previous illnesses suggestive of typhoid fever, but owing to the impracticability of securing reliable statements from these people it was considered that such data as might be obtained would not be worth recording.

For the culture of the faeces and urines several methods were at first employed in parallel. These were: direct plating on to MacConkey's medium, and on to Wilson and Blair's iron bismuth medium; preliminary enrichment in brilliant green broth and in tetrathionate broth (Jones, 1936) with subsequent plating on to both of the media already mentioned. Latterly the use of Wilson and Blair's medium and of brilliant green enrichment broth was discontinued and the standard procedure adopted was direct plating on the MacConkey's medium, and plating on to this medium subsequent to enrichment in tetrathionate broth over periods of approximately 5 and 20 hr. From time to time the reliability of the technique was controlled by recovering typhoid bacilli from faeces that had been deliberately contaminated with small volumes of diluted cultures of this organism.

Findings

A. *Serological*

656 samples of normal sera were examined for the presence of typhoid Vi agglutinins. The results are set forth in Table 1.

Table 1. *Vi agglutinins in* 656 *normal sera*

Agglutination titres against strain "Vi 1"		Number of sera	Percentage showing agglutination
Positive (+ + or + + +)	1 in 5	49	7·47
Positive (+ + or + + +)	1 in 12·5	13	1·98
Weak positive (+)	1 in 5 to 1 in 25	37	5·64

None of the sera showed full positive agglutination in titres higher than 1 in 12·5.

The results of the examination of Vi-positive sera for H and O agglutinins are shown in Table 2.

Table 2. *H and O agglutination titres of forty-nine sera containing Vi agglutinins*

Titre	Number of sera reacting with "H 901"	"O 901"
1 in 5	6	14
1 in 12·5	10	19
1 in 25	6	9
1 in 50	7	3
1 in 125	1	—
1 in 250	1	—
Negative	18	4

Five of the Vi-agglutinating sera that also showed H and O agglutinins were further tested after absorption with the "H 901" strain of *Bact. typhosum*. The undiluted sera were absorbed with very heavy doses of living emulsion suspended in volumes of saline equal to those of the sera to be absorbed. Subsequently the sera were found to have lost their H and O agglutinins but still agglutinated the "Vi 1" antigen in the original titres.

The first 173 sera tested were also put up against the Vi containing "Watson" strains side by side with the "Vi 1" antigen. The two suspensions behaved very similarly, in no instance resulting in a definite positive reading in the one tube and not in the other. As previously mentioned the "Vi 1" suspension alone was used in the later routine tests, but all sera showing agglutination were afterwards retested with both suspensions. Here again the results were very similar, and while in some sera the agglutination would be somewhat stronger with one suspension, in others the position would be reversed. In no instance was a definite discrepancy encountered.

It is of interest to note that during the course of the investigation of batches at weekly intervals the incidence of Vi-positive sera was distributed with considerable regularity, two to four positives being found in almost every batch.

B. *Cultural*

Faeces and urines from twenty-six of the individuals whose sera had been shown to produce full positive Vi agglutination in titres of 1 in 5 or 1 in 12·5 were examined for typhoid bacilli. In fourteen cases only one examination was practicable, in six the excreta were examined twice at intervals of 2 or 3 weeks, in five cases three examinations were made and in one case the excreta were examined on six occasions. Altogether forty-seven examinations of faeces and urine were made.

Only one of the individuals examined was found to be excreting typhoid bacilli. In this case organisms were readily demonstrated in cultures from the urine at the first attempt, as well as on each of several subsequent examinations during the ensuing weeks. Typhoid bacilli were never found in his faeces. This native (Kachamba) was one of a group of camp servants who were being

investigated for evidence of the carrier state on account of two cases of typhoid fever occurring among Europeans living in the camp. This group consisting of eighty-six natives was first examined for typhoid Vi, H and O agglutinins and the excreta of all those yielding significant titres were subsequently cultured. Seven of the sera showed Vi agglutination in titres of 1 in 5.

It is of interest to note Kachamba's agglutinin titres which were: Ty "Vi 1", 1 in 5; Ty "O 901", 1 in 12·5, and Ty "H 901", nil in 1 in 5. Had it not been for the presence of the Vi agglutinins he would almost certainly have escaped detection as a carrier, for the low titre of his O agglutinin would not have been considered significant. His serum was retested on several occasions with materially the same results; the Vi titre remained at 1 in 5, on one occasion a trace of O agglutination was observed in 1 in 25. H agglutinins were never revealed. Against his own culture his serum caused granular agglutination in 1 in 25.

The strain of *Bact. typhosum* isolated from Kachamba had the following characters: feebly motile, agglutinated in "Oxford" H serum at 1 in 125; in pure O serum (titre 1 in 50,000) it agglutinated in 1 in 12,500, in pure Vi serum agglutination occurred in 1 in 250. It is therefore assumed that the organism belongs to the VW type in that it possesses Vi antigen but is also O agglutinable. No facilities existed for virulence tests.

Discussion

The presence of low-titre Vi agglutinins in a moderately high percentage of sera from apparently healthy uninoculated African natives seems capable of three explanations:

(1) The agglutinins may be non-specific in origin; in this respect resembling the low-titre typhoid O agglutinins in normal sera. For the ubiquitous geographical distribution of the latter antibodies, irrespective of the prevalence of typhoid infection in the populations concerned, supports the belief that they commonly arise apart from any response to infection. This question is adequately discussed by Topley & Wilson (1936).

More light will doubtless be thrown upon the likelihood of this being an explanation of the presence of low-titre Vi agglutinins in normal sera by future studies on their frequency in uninoculated samples of population in countries where typhoid infection is rare.

(2) The occurrence of the Vi agglutinins may be a sequel to widespread experience on the part of the population concerned with the specific antigen in the course of previous clinical or "subclinical" infections.

It is probable that the presence of H agglutinins in normal sera is to be explained in this way, for reports of investigators in different countries indicate that typhoid H agglutinins in the sera of normal uninoculated samples of population tend to be generally higher both in frequency and in titre in countries where typhoid fever is common than where it is rare. The findings of Lewin (1934) in South Africa, of Alves (1936) in Southern Rhodesia and of Dowdeswell

(1937) in Kenya are probably representative of Africa; a more extensive bibliography is given by Topley & Wilson (1936). Admittedly, lack of standardized technique renders strict comparison between the findings of different investigators difficult, but the findings already published seem to justify the conclusion that a correlation exists between the frequency in a population of typhoid H agglutinins and the prevalence within it of typhoid infection. It is perhaps of interest in this connexion to note that Cluver (1936) estimated that half the native population of South Africa at one time or another actually becomes infected with typhoid fever.

(3) The presence of low-titre Vi agglutinins in the sera of apparently normal individuals is usually a consequence of the chronic carrier state. This appears to be the contention of Felix (1938*a*), with the added proviso that the term "carrier" be used to embrace not only excretors but those harbouring while not excreting typhoid bacilli.

The data published by Felix (1938*a*) support this claim in that the great majority of individuals proved to be carriers showed Vi agglutinins in their sera in titres of 1 in 5 or higher. He does not, however, record the frequency of these antibodies in any large group of non-carriers. Bhatnagar *et al.* (1938) and Bhatnagar (1938), advocating the use of the Vi agglutination test for the detection of carriers and employing suspensions of Bhatnagar's "Vi 1" strain as the testing antigen, state that no proved carrier had been found who did not produce evidence for the presence of the Vi antibody in his serum. The titres are not given except those of four carriers in the last paper, and these are 1 in 50 in three of the cases and in the fourth 1 in 50 falling to 1 in 10 coincident with the termination of the carrier-state. Pijper & Crocker (1937) using the absorption method examined the sera of four chronic typhoid carriers and demonstrated Vi agglutinins in all of them, the titres ranging from 1 in 80 to 1 in 200. These authors, however, also found Vi agglutinins in titres of 1 in 20 to 1 in 80 in the sera of three persons formerly proved to be carriers, but whose excreta on repeated cultural examination during recent years had yielded negative results. In seven other former carriers no Vi agglutinins were found at 1 in 20.

On the other hand, Lewin (1938) examined the serum of a proved chronic carrier for Vi agglutinins with negative results in a titre of 1 in 25. Furthermore, Boyd (1939) states that in temporary and convalescent typhoid carriers examined in India approximately half failed to show Vi agglutinins despite the fact that the organisms were readily recovered from the stools.

It will be seen then that though there is ample evidence that Vi agglutinins, for the most part in moderate titres, are commonly present in the sera of proved carriers, there are but scanty data for the contention that low-titre Vi agglutinins of the order of 1 in 5 or 1 in 10 are necessarily indicative of the carrier state or that they are rare in normal sera.

The cultural findings of the present investigation provide little support for the view that low-titre Vi agglutinins are necessarily of diagnostic signi-

ficance in the carrier state, at least as far as the native population of this country is concerned. Admittedly the number of specimens cultured was smaller than I would have wished, for the intermittency of bacillary excretion on the part of typhoid carriers is well known. Nevertheless it is probable that had the majority of the twenty-six Vi reactors whose excreta were examined been intermittent carriers more than one would have been detected. Moreover it seems improbable that failure to isolate the organisms is to be explained on the grounds that many of the reactors were harbourers but not excretors of the bacilli.

Cluver (1936) has computed on theoretical grounds that the proportion of South African natives who are typhoid carriers may be as high as 2%. This figure is, however, considerably lower than the 7·47% of the present series of individuals showing low-titre Vi agglutinins in their sera.

It is suggested that the most plausible conclusion to draw from the findings recorded in this paper is that, while in some instances the presence of low-titre Vi agglutinins of the order of 1 in 5 may be a consequence of the carrier state, in the sera of natives of Southern Rhodesia it frequently has no such significance.

However, in view of the relatively infrequent distribution of low-titre Vi agglutinins in normal sera when compared with those of H and O agglutinins, and of the frequency with which these agglutinins occur in the sera of chronic typhoid carriers, it is considered that the routine examination of suspected carriers for these antibodies does afford a useful adjunct to existing technical methods. The instance of the carrier Kachamba is a case in point, for if attention had not been focused upon him by the presence of low-titre Vi agglutinins he would probably have escaped detection.

Summary

1. 656 samples of sera taken at random from natives in Southern Rhodesia were examined for the presence of typhoid Vi agglutinins.

2. Forty-nine or 7·47% were shown to contain Vi agglutinins in titres of 1 in 5 to 1 in 12·5.

3. Samples of faeces and urine from twenty-six of the positive Vi reactors were cultured for *Bact. typhosum*, which was isolated from only one individual who was shown to be a chronic urinary carrier.

4. The significance of these findings is discussed.

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Note. Since this paper was submitted for publication the writer's attention has been drawn to a paper by C. P. Eliot (*Am. J. Hyg.* 1940, **31**, B 8) which records the presence of typhoid Vi agglutinins in 43 of 45 proved typhoid carriers, but in only 4 of 219 control sera from presumed normal individuals. The lowest titres observed however were 1 in 20. This author used the absorption method and employed living suspensions of the "Watson" strain as the testing antigen, direct agglutination with the "Bhatnagar" strain being found unsatisfactory as the cultures had acquired sensitivity to "O" agglutinins.

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