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# The detection of the typhoid carrier state*

*Report of the P.H.L.S. Working Party on the Bacteriological Examination of Waterworks Employees*†

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## INTRODUCTION

Enteric fever is now a relatively rare disease in this country. We owe our freedom from it, however, neither to a diminution in the virulence of *Salmonella typhi*, nor to a rise in human resistance. As with so many other infectious diseases, the main safeguard against infection is to deny the organism access to the susceptible population. So far as concerns *Salm. typhi* and *Salm. paratyphi B*, this has been accomplished by improved sanitation—our water supplies and sewage disposal systems are on the whole of high quality, and anastomoses between the two are fortunately rare. As the incidence of enteric fever wanes, the carriers that follow in its wake diminish correspondingly in number. This contributes still further to the decrease in incidence of the disease, since the ultimate source of all infections with *Salm. typhi* and *Salm. paratyphi B* is the human carrier.

The present low level of enteric fever in this country will be appreciated when it is pointed out that, whereas only 737 cases of the disease were recorded in Great Britain between May 1958 and May 1959, 23,891 cases were reported from Italy, during the same period (Report, 1959). The populations at risk were approximately equal in the two countries. Our security is more apparent than real, however, and incidents occasionally occur which remind us that there are always sufficient carriers in the population to precipitate outbreaks when the conditions are favourable.

An outstanding demonstration of the need for constant vigilance was the waterborne outbreak of typhoid fever at Croydon in 1937, in which 341 cases occurred (Murphy, 1938; Report, 1938; Holden, 1939). This outbreak was finally attributed to the contamination of a well by a workman who was a chronic typhoid carrier. As the result of this misfortune, a memorandum, No. 221, was published by the Ministry of Health (Report, 1939) on the safeguards to be adopted by waterworks undertakings in order to minimize the possibility of their employing persons excreting

* The observations described in this report refer almost entirely to the detection of typhoid carriers. Investigations of a small number of paratyphoid B carriers yielded inconclusive results, and in view of the limited amount of material available it was not considered justifiable to include them.

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enteric pathogens. A section of this publication (para. 5) dealt with the medical examination of workmen to exclude the existence of the carrier state. Apart from determining the clinical history of each man in relation to previous enteric infection, recommendations were made that sera and excreta should be examined in order to detect infection with typhoid or paratyphoid bacilli.

The wording of the relevant paragraph is as follows:

> Careful discrimination should be exercised in the selection of workmen employed on waterworks other than works where no risk to the purity of the water suppy is likely to arise, and the clinical history of each such workman, particularly with reference to enteric infection, should be thoroughly investigated in order to determine his suitability for this kind of employment. Any man attacked by illness associated with looseness of the bowels should be suspended from work until his recovery is complete and medical examination shows that he is safe to return to work. Every new man proposed to be employed on any part of the works where there is risk of his contaminating the water should be examined by means of a Widal test of his blood in order to ascertain whether or not he is likely to be a typhoid carrier. If a positive result is obtained which is not attributable to preventive inoculation, he should not be employed unless bacteriological examination of his excreta on at least three occasions shows negative results as regards the presence of pathogenic bacteria.

A few years before Memorandum 221 was prepared, the Vi agglutination test had been suggested as a screen for the detection of typhoid carriers (Felix, Krikorian & Reitler, 1935). The bacterial suspension originally employed for this test was a Vi-positive strain of *Salmonella typhi*. Although this strain in the living state was relatively inagglutinable by a pure O antiserum, it possessed a full complement of O antigen and the Vi titre had to be assessed in terms of obvious agglutination above the known limit of O agglutinability of the suspension of *Salm. typhi* employed (Felix, 1938*b*).

This difficulty was overcome by the introduction by Bhatnagar, Speechley & Singh (1938) of strain ViI of *Salm. typhi*—a strain deficient in O and H antigens but rich in Vi antigen. It could therefore be used as a pure reagent for carrying out Vi agglutination tests. Moreover, it was found by Felix that when it was in the optimal state its sensitivity to agglutination by Vi antibody was of a suitable order to make it a satisfactory reagent for standardized Vi agglutination tests, of which more will be said later. Formolized suspensions of ViI are now in general use for the routine titration of Vi agglutinin.

Unfortunately, there are various drawbacks to the use of strain ViI. Primarily, it is unstable in agglutinability so that, while clones selected over a long period for the preparation of suspensions may prove to be satisfactory, lines not infrequently appear which are hyper- or hypo-agglutinable, and are therefore unsuitable for the standardized test. This applies even when freeze-dried cultures are used as the source of the antigen. Moreover, suspensions that are initially satisfactory eventually deteriorate and require replacement.

Although many workers have found the test satisfactory, others claim that it produces too high a percentage of false positive reactions. References to the use of the Vi agglutination test as a screen for typhoid carriers were published by Felix (1938*a*, 1944, 1950, 1951), and Lie Kian Joe, Wiratmadja, Hardjowardojo, Kartaenegara & Harmiati (1957). As the presence of Vi agglutinins is now accepted as an indication that the person concerned may be a typhoid carrier, various modified

versions of the Vi agglutination reaction have been suggested. Of these, the most promising are haemagglutination tests, in which use is made of erythrocytes to which purified Vi antigen is adsorbed (Spaun, 1952; Staak & Spaun, 1953; Landy & Lamb, 1953). More recently, a test was suggested in which crude Vi antigen, extracted from *Citrobacter ballerupensis* (*Salm. ballerup*) and adsorbed to sheep erythrocytes, was used as the reagent for the detection of Vi antibody (Schubert, Edwards & Ramsey, 1959), but this test was found to be unsatisfactory by Anderson (1960).

It has been felt recently that it would be profitable to review the procedures recommended in the 1939 memorandum for testing waterworks employees for the carrier state. Perhaps we may mention here that, since these examinations were instituted, no carriers of *Salm. typhi* or *Salm. paratyphi B* have been detected in England and Wales, among waterworks personnel, although a considerable proportion of the waterworks undertakings of the country screen their employees in accordance with the recommendations. We know that the proportion of typhoid carriers in the population of this country is very low, probably less than 1 per 100,000. Moreover, of 171 carriers in Great Britain between the ages of 18 and 65 years of whom records are kept in the Central Enteric Reference Laboratory and Bureau, 111 are women and 60 men. This suggests that only about one-third of chronic carriers are males. Thus, assuming an equal distribution of sexes in the general population, probably less than one man in 150,000 is a typhoid carrier in this country. The probability of a typhoid carrier applying for employment on a waterworks undertaking is therefore remote, so that the fact that no carriers have been detected during the years in which the tests have been used, gives no information on their reliability. Even if the tests are sensitive and specific, the results only confirm that the incidence of carriers is very low.

The wording of Memorandum 221 is rather vague in relation to the investigations to be performed, the blood test recommended being the Widal, without stressing any special aspects of this. When the memorandum was published the Vi agglutination test was not in general use, but within a few years it had become a standard part of the investigation and, indeed, is now regarded by some workers as being the most important facet of it. One of the largest undertakings using the Vi agglutination test is the Metropolitan Water Board (M.W.B.). Their present procedure is as follows:

All personnel likely to come in contact with the water supply are tested serologically for TVi, TO, TH, BH specific, non-specific salmonella and AH agglutinins. Excreta (faeces and urine) are examined only in persons giving a positive TVi reaction from 5* upwards, a positive TO reaction from 50 upwards, or showing the presence of a single H agglutinin in a person without a history of inoculation. When more than one H agglutinin is found, for example TH and BH specific, it is usually assumed that this is due to inoculation, and tests of excreta are not performed unless one H titre is very much higher than the others—a state of affairs which may possibly be due to infection with the organism concerned. When carried out, excretal examinations are performed three times at intervals of about 10–14 days.

Any person who is sick with gastro-intestinal symptoms is submitted to a single

* Dilutions and titres are given as reciprocals.

investigation of excreta before being permitted to resume work. If intestinal pathogens are isolated, the employee is kept from work until three negative specimens are obtained.

Mackenzie & Taylor (1945) found that 3·7 % of 1040 M.W.B. employees gave a positive Vi agglutination test. In a later publication (Mackenzie, 1954) the proportion of positive Vi reactors in the M.W.B. was given as 7·3 %. In no instance was it possible to isolate typhoid bacilli from positive Vi reactors. Similar findings, which have largely remained unpublished, have been communicated to one of us (E.S.A.) by a number of workers, and it has been confirmed that a small percentage of apparently normal persons have Vi agglutinin in their serum, although they show no bacteriological evidence of harbouring the typhoid bacillus. However, as long as it is considered advisable to screen waterworks employees for the chronic carrier state, and as long as the tests used are sensitive enough to detect most carriers, the disadvantage has to be accepted that a small proportion of false positive results will be encountered.

What is perhaps not generally appreciated is that the level of positivity of the TVi test in a given population seems to be controlled by the degree of endemicity of typhoid fever in the country concerned. For example, in Egypt, where the endemicity is high, a relatively high proportion of persons, not demonstrably suffering from the carrier state, show significant agglutinin titres with suspensions of *Salm. typhi* ViI (Miller, 1950; Anderson, unpublished). Conversely, in countries with low typhoid endemicity, such as Great Britain, few persons show significant levels. Thus, it is erroneous to use the findings in the United Kingdom as a basis for the interpretation of those in other countries, since a reassessment of the test is necessary in each country in which it is used.

When it was decided that a re-scrutiny of the procedures used for the routine screening of persons was advisable, two angles of approach suggested themselves. The first was to apply the tests to as many known chronic carriers of *Salm. typhi* as possible. The second was to screen a considerable number of 'normal' persons in order to explore the level of enteric antibodies in the population at large and thus to determine the proportion of false positive reactors.

## TECHNIQUES

### *Vi agglutinations*

Vi agglutinin titrations were performed in volumes of 1 ml. in 2 in. × ½ in. round-bottomed tubes. The dilutions of patients' serum were 5, 10, 20, 40, 80 and 200. A titration of the Provisional Standard Serum for Vi agglutination (Felix, 1938*b*) was included in each test of patients' sera. This standard serum, which is further discussed below, was titrated in the following dilutions: 800, 1000, 1200, 1400, 2000, 3000, 4000 and 6000. The TVi suspension (Standards Laboratory, Colindale) contained approximately $8 \times 10^9$ organisms per ml. in buffered formol saline and was added to all dilutions from a dropping pipette, one drop, approximately 0·05 ml., being added to each tube. The final concentration of organisms was thus about $4 \times 10^8$/ml.

The Vi suspension was tested for salt sensitivity by including in each series four tubes containing respectively distilled water, 0·85, 2·5 and 5·0 % of saline to which 0·05 ml. of the suspension was added. After adding the suspension the racks were agitated for a short time, transferred to the incubator at 37° for 2 hr., and then to the refrigerator at 4° C. overnight. Results were read 24 hr. after setting up the tests. The tubes were removed from the refrigerator and left at room temperature for 2 hr. before they were due to be read.

For reading, the tubes were illuminated from above with a 100 W. pearl lamp (an anglepoise bracket was used). A × 10 aplanat hand-lens was employed. Three criteria were used for assessing the degree of agglutination: the clarification of the supernatant; the pattern of dispersion of the deposit; and the coarseness of the granularity of the suspension after shaking the tube. The readings given were as follows:

+ + + = Maximum agglutination: clear supernatant, dense deposit, coarse granularity after flicking the tube.
+ + ± = Intermediate.
+ + = Intermediate.
+ ± = About 50 % point of agglutination. Moderately clear supernatant, moderately dense deposit, obvious residual agglutination.
\+ = Intermediate.
± = Last agglutination visible with the naked eye.
(±) = Trace.
((±)) = Faint trace.

The ± agglutination is the last in which the granules are visible with the naked eye. In saline control tubes the suspension settles as a clear-cut central button and the supernatant is opalescent; the button disperses smoothly when the tube is agitated. In tubes showing agglutination, the deposit is dispersed over the bottom of the tube as a pellicle, the density and tenacity of which are proportional to the degree of agglutination. The supernatant is macroscopically water-clear, although examination with the hand-lens naturally reveals fine granules in suspension. With a little practice, a reasonably constant amount of agitation can be attained, and it is then simple to compare varying degrees of agglutination. It must be emphasized that a final assessment of TVi agglutination cannot be arrived at without this agitation.

When determining the Vi titre of a serum it is always titrated in parallel with the Provisional Standard Serum for Vi agglutination (P.S.S.). This, a horse serum, was the third standard used for this purpose. The first was prepared in 1935 and, as the result of comparison with the sera of a number of typhoid carriers and of normal persons, was allotted a 'standard' titre, which was the dilution found to produce a degree of agglutination equivalent to that at the lower limit of significance in the carriers' sera. In the original P.S.S., this dilution was 600. In the current P.S.S. it is 1400. The 'standard' titre of a serum under test is that dilution which gives agglutination equal in degree to the 1400 dilution of the P.S.S. It is to be noted, therefore, that this is not an end-point reading.

TVi agglutinins appear late in typhoid fever and usually disappear in convalescence. They rarely reach high titres, 200 being exceptional, and frequently do not appear at all during the active disease. The persistence and stabilization of TVi agglutinins after typhoid fever are regarded as indicating that the patient concerned is still infected with the typhoid bacillus and may be a candidate for the chronic carrier state.

*O agglutinations*

The technique of performing these is in general similar to that of the TVi titration. Round-bottomed tubes are much more satisfactory than Dreyer's tubes for this work, since it is impossible to shake the latter satisfactorily in order to examine the texture of the clumps—the third criterion used in reading the tests. In the Central Enteric Reference Laboratory, screening titrations are carried out in dilutions of 50, 100 and 200. 1 ml. volumes of serum dilution are used and 0·05 ml. of TO suspension, containing $8 \times 10^9$ organisms/ml. killed with alcohol and preserved in formol saline, is added to each tube. Incubation, storage up to the time of reading, and the method of reading are also identical with those of Vi agglutination.

O agglutination is finer than Vi agglutination and the readings are a step higher than the latter for a given coarseness of granularity after shaking. Thus, a $+\pm$ reading with Vi agglutination is recorded as $++$ for O, a $++$ with Vi is $++\pm$ for O, and so on. The O titration is not yet a standardized test, although a move was made in that direction by Felix (1950) and Felix & Bensted (1954). At present, results are reported as macroscopic end points, the lowest level reported being $+$.

As regards significance, the late Dr Felix, in numerous discussions with one of us (E.S.A.), claimed that anything below 100 was insignificant and that 100 was only slightly suspicious. He regarded 200 as being the upper limit of the so-called 'normal' TO titre.

*H agglutinations*

H agglutinations were performed in Dreyer's tubes, the serum dilutions being prepared in volumes of 0·5 ml. The suspensions used, which were prepared by the Standards Laboratory, were typhoid H, paratyphoid BH specific, non-specific salmonella H and paratyphoid AH; 0·5 ml. of suspension was added to each tube.

When non-specific salmonella alone gave a positive reaction, the serum concerned was titrated with *Salm. typhimurium* specific H suspension, agglutination of which was accepted as an indication of infection at some time with *Salm. typhimurium*. The final serum dilutions used for flagellar agglutination screening tests were 25, 100 and 200. When the titrations were complete the tubes were heated in a water bath at 50° C. for 2 hr. and then left at room temperature for a further 2 hr. Readings were carried out with the same illumination as in Vi and O titrations, but the assessment of agglutination was based entirely on the degree of flocculation of the suspension without agitation. The serum of man does not appear to contain H agglutinins except in response to specific stimuli such as infection or inoculation, and any degree of agglutination down to trace readings was regarded as significant.

## RESULTS

### *Serological*

The material examined was divided into two groups: the sera of known chronic typhoid carriers, and the sera of persons not suspected to have been infected with the typhoid bacillus.

(1) *Sera of known chronic typhoid carriers*

Sixty-seven chronic carriers were examined, of whom 55 were female and 12 male. Most of the females were mental hospital patients of middle age or later life.

The findings on these sera are summarized in Tables 1 and 2.

Table 1. *Serological tests of known typhoid carriers*

| Vi | O | H | No. | % |
|---|---|---|---|---|
| | A. O titres under 200 ignored | | | |
| + | + | + | 9 | 13·4 |
| + | + | − | 3 | 4·5 |
| + | − | + | 24 | 35·8 |
| + | − | − | 12 | 17·9 |
| − | + | + | 0 | 0 |
| − | + | − | 0 | 0 |
| − | − | + | 13 | 19·4 |
| − | − | − | 6 | 9·0 |
| | | Total | 67 | 100·0 |

Summary: 91 % gave positive results; 9 % gave negative results.

| | | | | |
|---|---|---|---|---|
| | B. O titres of 50 or over regarded as significant | | | |
| + | + | + | 27 | 40·3 |
| + | + | − | 13 | 19·4 |
| + | − | + | 5 | 7·5 |
| + | − | − | 3 | 4·5 |
| − | + | + | 9 | 13·4 |
| − | + | − | 5 | 7·5 |
| − | − | + | 3 | 4·5 |
| − | − | − | 2 | 3·0 |
| | | Total | 67 | 100·1 |

Summary: 97 % gave positive results; 3 % gave negative results.

When serological evidence of T.A.B. inoculation was present (positive results with more than one H suspension) the TH reading was not regarded as significant and was therefore recorded as negative for the purpose of the present investigation.

In Table 1 the results are examined in two ways: in section A on the assumption that O titres less than 200 are insignificant; in section B on the assumption that an O titre of 50 upwards is significant. A reading of + was arbitrarily established as the end point.

In Table 1A, the majority of positive findings (73 %) are contributed by TVi and TH agglutinins alone or together but without significant O agglutinins. In fact, with the level of significance demanded for the TO agglutinin in this section,

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a total of only 18 % of sera were positive for it and none was positive for TO alone. Only 13·4 % of sera gave positive results for all three agglutinins. When 50 was accepted as the significant level for O agglutination, the proportion of positive TO titrations alone or in combination with positive TVi and/or TH titrations rose to 80·6 %, while the positives contributed by TVi and TH alone or together, to the exclusion of TO, fell to 16·5 %. The percentage of sera that could be diagnosed as positive with all three typhoid agglutinins simultaneously in the analysis in Table 1 B was 40·3 as against 13·4 % in Table 1 A. In Table 1 A, a total of 91 % yielded positive results with one or more agglutinins, and 9 % negative results. In 1 B, only 3 % of sera are shown as negative with all three suspensions.

When the findings were analysed on the basis of single agglutinins, the results shown in Table 2 were obtained.

Table 2. *Serological tests of known typhoid carriers*

Analysis by separate agglutinins

| A. O titres under 200 ignored | | B. O titres of 50 or over regarded as significant | |
|---|---|---|---|
| | % | | % |
| Vi+ 48/67 | 71·6 | . | . |
| Vi− 19/67 | 28·5 | . | . |
| O+ 12/67 | 17·9 | O+ 54/67 | 80·6 |
| O− 55/67 | 82·1 | O− 13/67 | 19·4 |
| H+ 46/67 | 68·7 | . | . |
| H− 21/67 | 31·3 | . | . |
| Vi+ only 12/67 | 17·9 | Vi+ only 3/67 | 4·5 |
| O+ only 0 | 0 | O+ only 5/67 | 7·5 |
| H+ only 13/67 | 19·4 | H+ only 3/67 | 4·5 |

In Table 2, section A, a total of 71·6 % of sera were positive for the TVi agglutinin. This figure is lower by about 20 % than that quoted by Felix (1944, 1951) but still lies at a very useful level. The sera positive only for TVi agglutinin constituted 17·9 %. In contrast, in the same part of this table, it will be noted that a total of only 17·9 % were positive for TO agglutinin, none showing this antibody alone. In section B of Table 2 analysis was based on the acceptance of 50 as the significance level for O agglutination. On this basis, as we have already indicated, 80·6 % of sera were positive with TO suspension, and 7·5 % with this suspension alone.

It was evident from these results that a determination of the significance level of TO agglutinins was desirable, and it was hoped to obtain this information from the examination of the sera of normal persons.

The carriers in this series were between the ages of 12 and 85, but age did not appear to influence the agglutinin titres.

(2) *Sera from normal persons*

Sera were obtained from three sources:

Colonel M. H. P. Sayers, O.B.E., of the David Bruce Laboratories, Everleigh, kindly provided 112 sera from uninoculated young adult males (Everleigh Sera).

The Public Health Laboratory, Worcester, supplied 136 sera from ante-natal and routine hospital sources. Of these sera, 109 were from pregnant females and 27 from males ranging from 1 to 61 years (Worcester Sera).

The Metropolitan Water Board supplied 95 sera from adult males between 18 and 65 years. Sixty-nine of these were known to have been inoculated (M.W.B. Sera).

*Everleigh Sera.* All 112 of these sera were tested with TVi and TO and the various H suspensions. None gave a significant titre with TVi suspension and they were also all negative with human erythrocytes sensitized with purified Vi antigen. Seven (6·3 %) agglutinated TO suspension at 50, four of these (3·6 %) to 100.

Only one (0·9 %) of these sera agglutinated TH suspension, but six (5·4 %) were positive with non-specific salmonella H suspension alone, and one of these agglutinated *Salm. typhimurium* specific suspension.

*Worcester Sera.* One out of 136 sera (0·7 %) gave a significant reading with TVi suspension to a dilution of 20. This serum was one of 130 from uninoculated persons and did not agglutinate Vi-sensitized erythrocytes. One hundred and thirty sera were tested with TO suspension; of these, 124 were from uninoculated and the remaining six from inoculated subjects. Thirteen (10·5 %) of the uninoculated and two of the inoculated group were positive at 50. Four of the uninoculated group (3·2 %) were positive at 100; in neither of the positive sera from the inoculated group did the titre exceed 50.

Seven of 128 sera (5·5 %) gave a positive reaction with TH suspension and five of these (3·9 %) showed evidence of T.A.B. inoculations. Thus, the TH agglutinin was present without obvious cause in only two of 123 sera (1·6 %) from uninoculated persons.

Tests with BH specific, non-specific salmonella and AH suspensions were performed in 120 sera, of which 116 came from uninoculated subjects. Four (3·4 %) of these showed the presence of non-specific salmonella agglutinins, and three of the four, all from uninoculated persons, were found also to contain 'i' agglutinin which suggested that the persons concerned had been infected at some time with *Salm. typhimurium.*

*M.W.B. Sera.* Of the 95 sera coming from this source, sixty-nine (72·6 %) came from subjects known to have been inoculated with T.A.B. vaccine; the remaining persons presented no history of inoculation. Three (3·2 %) of the 95 sera gave positive (standard) readings with TVi suspension, one at 5 and two at 10. Two of these positive reactors also agglutinated Vi-sensitized erythrocytes. Five further sera (5·3 %) reacted with the sensitized erythrocytes but not with the bacterial Vi suspension. None of the three subjects who reacted with TVi suspension had been inoculated. Four of the five persons giving positive Vi haemagglutination only had been inoculated. In one of these patients, tests of excreta were carried out, with negative results.

Table 3. *Summarized positive results with normal sera examined for TVi, TO and TH agglutinins*

| Sera | TVi | | | TO | | | TH | | |
|---|---|---|---|---|---|---|---|---|---|
| | Inoculated | Uninoculated | Total | Inoculated | Uninoculated | Total | Inoculated | Uninoculated | Total |
| Everleigh, 112 sera | 0 | 0/112* | 0/112 | | 7/112 = 6·3% | 7/112 = 6·3% | . | 1/112 = 0·9% | 1/112 = 0·9% |
| Worcester, 136 sera | 0/6 | 1/130 = 0·77% | 1/136 = 0·74% | 2/6 | 13/124 = 10·5% | 15/130 = 11·5% | 5/5 = 100% | 2/123 = 1·6% | 7/128 = 5·5% |
| Metropolitan Water Board, 95 sera | 0/69 | 3/26 = 11·5% | 3/95 = 3·2% | 17/69 = 24·6% | 3/26 = 11·5% | 20/95 = 21% | 45/65 = 69% | 4/25 = 16% | 49/95 = 51·6% |
| Totals | 0/75 | 4/268 = 1·5% | 4/343 = 1·17% | 19/75 = 25·3% | 23/262 = 8·8% | 42/337 = 12·5% | 50/70 = 71·4% | 7/260 = 2·7% | 57/330 = 17·3% |

* Numerator = number of sera showing positive results. Denominator = total number of sera.
Significance level for TO = 50. For TVi and TH significance levels see text.

Table 4. *Comparison of findings on sera of normal individuals with those on sera of known typhoid carriers*

| Sera | | TVi | | TO | | TH | |
|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | No. | % |
| Normal sera | I* | 0/75† | 0 | 19/75 | 25·3 | 50/70 | 71·4 |
| | U* | 4/268 | 1·5 | 23/262 | 8·8 | 7/260 | 2·7 |
| | T* | 4/343 | 1·17 | 42/337 | 12·5 | 57/330 | 17·3 |
| Sera from known carriers | | 48/67 | 71·5 | 54/67 | 80·5 | 46/67 | 68·7 |

* I = inoculated; U = uninoculated; T = total.

† Numerator = number of sera showing positive reactions. Denominator = total number of sera.

For significance levels see text.

Twenty of the 95 sera tested (21 %) gave positive results with TO suspension. Of these, 3 were from the 26 uninoculated persons (11·5 %) and 17 from the 69 inoculated subjects (24·6 %).

Ninety of the sera, 65 from inoculated and 25 from uninoculated subjects, were tested with all the routine H suspensions. In the inoculated group, 55 (84·6 %) gave a positive reaction with at least one H suspension, 21 (32·3 %) being positive with all and an equal percentage positive with three of the four suspensions. A total of 45 (69 %) were positive with TH, and only one of these showed no other H agglutinins. AH agglutinins were found in a total of 41 sera (63 %).

Of the 25 reputedly uninoculated subjects, nine (36 %) showed the presence of some H agglutinins. Four showed TH agglutinins only, and one both TH and BH. Four of this group were positive with non-specific salmonella suspension only, and two of these sera also contained 'i' agglutinin. None of the sera from uninoculated persons agglutinated AH suspension. As would be expected, the difference between the proportion of inoculated and uninoculated persons showing H agglutinins is highly significant.

Table 3 summarizes the results with the three types of agglutinin investigated. The TVi bacterial agglutination test was positive in only four of the 343 persons tested (1·17 %), and none of the four had a history of T.A.B. inoculation. In the totals at the foot of the table, the difference between the proportion of positive TO reactions in the uninoculated and inoculated groups is highly significant. In Table 4 the findings on the normal sera are compared with those on the sera of the chronic carriers.

This table shows that the incidence of TVi, TO and TH agglutinins was lowest in uninfected and uninoculated persons. Inoculation raised the frequency of TO agglutinins about threefold, at a titre of 50, and increased that of the TH agglutinin to a point where it became useless as an indicator of infection. The incidence of TVi agglutinin, on the other hand, remained low even after antityphoid inoculation.

In contrast, a high proportion of chronic carriers exhibited TVi, TO and TH agglutinins, either separately or in combination. It is thus evident that the serological test constitutes a useful screening method for the detection of typhoid carriers. TVi agglutination is valuable in both uninoculated and inoculated subjects, and the TO and TH tests are also useful for this purpose, especially in the uninoculated.

### *The bacteriological examination of chronic typhoid carriers*

Unfortunately, it was impossible for a number of reasons to carry out long-term surveys of excreta of the carriers on which the serological examinations had been performed. However, a few surveys of this type had been done by other members of the P.H.L.S. who kindly made their results available to us for analysis. We are greatly indebted to Drs Betty C. Hobbs (series I), J. H. McCoy (series II), J. H. C. Walker (series III) and Lynette M. Dowsett (series IV) for supplying us with the results of their investigations. Enrichment and selective media were employed in all the bacteriological examinations of these carriers.

In series I, 10 chronic carriers, all female mental patients and mostly middle-

aged, were investigated. Specimens of excreta were taken weekly, over a maximum period of 77 weeks. TVi titrations were carried out on all patients. Series II also comprised 10 carriers, two of whom were males and eight females, mostly middle-aged. Four were mentally normal and the remainder abnormal. These patients were also bacteriologically examined weekly, in some cases for as long as 173 weeks.

Table 5. *The bacteriological examination of chronic typhoid carriers*

| Series no. | Carrier no. | Positive/total specimens | | TVi titre | P 3– |
|---|---|---|---|---|---|
| | | No. | % | | |
| I | 1 | 74/77 | 96 | 10 | . |
| | 2 | 10/76 | 13 | 5 | 0·65 |
| | 3 | 66/67 | 86 | 5 | . |
| | 4 | 33/77 | 43 | 20 | . |
| | 5 | 63/69 | 91 | 10 | . |
| | 6 | 68/69 | 98·5 | 20 | . |
| | 7 | 67/77 | 87 | 10 | . |
| | 8 | 59/69 | 86 | 40 | . |
| | 9 | 65/69 | 94 | 160 | . |
| | 10 | 54/69 | 73 | 80 | . |
| II | 1 | 11/34 | 32 | 15 | . |
| | 2 | 31/39 | 80 | 20 | . |
| | 3 | 117/122 | 96 | 10 | . |
| | 4 | 144/173 | 83·5 | 10 | . |
| | 5 | 28/173 | 16·2 | 12·5 | 0·6 |
| | 6 | 74/80 | 92·5 | N.D. | . |
| | 7 | 1/106 | 0·95 | N.D. | 0·99 |
| | 8 | 51/123 | 41·5 | 20 | . |
| | 9 | 7/32 | 22·0 | 40 | . |
| | 10 | 3/35 | 86·0 | < 5 | 0·76 |
| III | 1 | 4/17 | 23·0 | N.D. | . |
| | 2 | 6/7 | 0·86 | N.D. | . |
| | 3 | 36/47 | 77·0 | N.D. | . |
| IV | 1 | 1/32 | 3·1 | 80 | 0·91 |
| | 2 | 1/29 | 3·4 | < 5 | 0·9 |
| | 3 | 27/30 | 90·0 | 80 | . |
| | 4 | 1/29 | 3·4 | 20 | 0·9 |
| | 5 | 3/30 | 10·0 | 40 | 0·73 |
| | 6 | 28/29 | 96·5 | 40 | . |

N.D. = not done.

There were three carriers in series III, all elderly women and all mentally normal. All three had infected other members of the family. These carriers were also examined weekly. Series IV comprised six carriers, all of whom were middle-aged female mental patients. Bacteriological examinations were carried out at monthly intervals, the longest period being 30 months.

The findings on the four groups of carriers are combined in Table 5. Vi agglutination readings were available in series I, II and IV.

It is apparent from this table that intermittency of excretion was the rule. No

carrier gave 100 % of positive specimens. It is of course possible that the organism was present in small numbers in the specimens found to be negative, in spite of the fact that the cultural methods used were those now accepted as being of maximal sensitivity for this work. Even if this were so, however, the results would still establish that in carriers there is a great degree of variation from time to time in the abundance of excretion of *Salm. typhi*, and that the infecting organism can easily be missed. This indicates that the examination of a single specimen of excreta as a screening test for the chronic carrier state is unreliable.

From the proportion of negative stools obtained during the survey period, a rough estimate was made for each carrier of the likelihood of three stools randomly examined yielding negative results. The figures arrived at are listed in the right-hand column (P 3–) of Table 5 and include only those in which this probability was 0·5 or higher, that is, where there was a 50 % or better chance that in these known carriers the examination of three specimens of faeces would not detect *Salm. typhi*. Eight of the 29 carriers investigated (28 %) showed a P 3– of this magnitude. Thus, twenty of the 29 carriers (72 %) had a better than even chance of being detected by the examination of the three samples of faeces. It may be added that 15 carriers (52 %) had a higher than 99 % probability of detection when three samples were examined.

It is worth while noting that, of the 24 carriers in whom the TVi agglutination test was recorded, twenty-two (92 %) gave positive results, a higher level of Vi positivity than that observed in the series of 67 carriers discussed earlier.

## DISCUSSION

It was claimed by Felix (1944, 1951) that nine out of 10 carriers gave a positive bacterial Vi agglutination test. The findings in our series of 67 carriers did not yield such a high figure, but the 71·5 % of positive results arrived at still makes the test a very useful one, assuming that its specificity is adequate. In the comparison of the results in 'carrier' and 'normal' sera shown in Table 3, only 1·17 % of the 343 normal sera gave positive results in the TVi agglutination test. This compares favourably with the percentage of 3·7 reported by Mackenzie & Taylor in 1945 and 7·3 by Mackenzie in 1954.

The usual complaint raised against the Vi agglutination test is that it produces a high proportion of false positives. We believe that such errors can be considerably reduced if the technique outlined earlier in this paper is closely followed. It seems probable that the proportion of positive Vi agglutination tests in this country would then be of a similar order to that found in our normal sera. We have pointed out earlier the need of reassessing the significance of the Vi agglutination test in other countries in terms of the local endemicity of typhoid fever. It has to be accepted, however, that, with any test of reasonable sensitivity, a proportion of positive results without ascertainable cause may be encountered. The explanation for some false positive Vi reactions in a small proportion of cases may be that the persons concerned are harbouring the typhoid bacillus in some closed focus without excreting it (Felix, 1938*a*; Pijper & Crocker, 1943) or may be carrying

Vi-containing strains of salmonella or *Escherichia coli*, although examples of the latter have been difficult to find in man.

TO agglutinin must be expected more frequently than Vi agglutinin in uninfected persons, and its incidence at low titres is significantly raised by T.A.B. inoculation. For example, the sera of 8·8 % of uninoculated and 25 % of inoculated subjects showed a TO titre of 50. When the significance level was raised to 200, the proportion of normal sera with a positive titre was negligible, but as Table 1A and section B of Table 2 show, the frequency of TO positivity in known carriers sank to a uselessly low figure. Our titrations showed, however, that at 100, only about 3·0 % of both inoculated and uninoculated subjects gave a positive result for TO agglutinin. As far as carriers were concerned, 81 % were positive for TO at 50 and 37 % at 100, a considerable reduction in sensitivity. The decision concerning significance thus had to be made between a TO agglutinin level which would detect a high proportion of carriers but would also produce a false positive return as high as 25 % in inoculated persons, and one which had a lower sensitivity towards carrier detection but was much less misleading in relation to false positives. The absolute chances of carrier detection in any survey of the general population are very small, not because of insensitivity of the tests but because of the extreme rarity of carriers. Moreover, even if the results of TO and TH agglutination were ignored altogether, the TVi titration would still detect over 70 % of carriers. It was therefore felt justifiable to recommend the use of 100 as the TO level of significance in order to reduce the extra work that would be required to check false positive results.

Concerning the H titres, it is apparent that the level of TH agglutinins in the sera of uninfected and uninoculated persons is very low—probably less than 3 % at a dilution of 25. In the sera of carriers, on the other hand, TH agglutinins were found in 69 %, a percentage similar to that of TVi agglutinins, and reference to Table 1B will show that TH agglutinins were found in 18 % of subjects who did not exhibit Vi agglutinin.

In inoculated subjects, H agglutination is virtually valueless as a screening test because the proportion of positive reactors is as high as that found in chronic carriers.

From a comparison between results with sera from normal persons and carriers, it can be concluded that serological examination offers a good probability of detecting the typhoid carrier state, and that the inconvenience likely to spring from false positive reactions in normal persons is not high enough seriously to impair the reliability of the test.

The object of this survey was to review the carrier screening tests as they now stand, and to consider the advisability of revising the terms in which these are dealt with in Ministry of Health Memorandum 221 mentioned earlier. One aspect of the investigation was to be the bacteriological examination of carriers, with a view to comparing the reliability of excretal and serological examinations in the detection of the chronic carrier state. The important drawback to the examination of faeces is the occurrence of intermittent excretion and, although modern enrichment and selective media have facilitated the task of isolating intestinal pathogens,

intermittency of excretion is still an indubitable fact. Indeed, it is logical enough to expect it in the intestinal excreter, especially when the residual focus of typhoid infection, which is usually in the gall-bladder, is small, for the typhoid bacillus has increasing difficulty in manifesting its presence in competition with the other intestinal flora in its journey from the ampulla of Vater to the rectum. If intestinal stasis is present, it may disappear altogether or be present so sparsely as to be missed by the time excretion occurs. The analysis of the results of the long-term faecal examination of chronic carriers, presented earlier in this paper, shows that the investigation of single samples of stools is not a reliable method of detecting the carrier state. However, the examination of three or more specimens raises the probability of detection to a high level.

In the light of our findings in carriers and in the sera of normal persons, it was felt that certain modifications in detail were advisable in paragraph 5 of Memorandum 221. We propose the following as a substitute for the original wording of this paragraph:

Careful discrimination should be exercised in the selection of workmen employed on waterworks other than works where no risk to the purity of the water is likely to arise, and the clinical history of each such workman, particularly with reference to any infection liable to be water-borne, should be thoroughly investigated in order to determine his suitability for this kind of employment.

Each new man proposed for employment on any part of the works where there is risk of his contaminating the water should be examined by testing his blood to determine whether or not he is likely to be a typhoid carrier. When a positive result is obtained from blood tests* which is not attributable to preventive inoculation, he should not be employed unless repeated examination of his stools and urine fails to show the presence of pathogenic bacteria.

If preliminary blood tests are not used, bacteriological examination of stools and urine must be carried out on at least three occasions at weekly intervals in all cases. This should reveal 70–80 % of chronic carriers.

Any man attacked by illness associated with looseness of the bowels, or by an undiagnosed pyrexia of more than five days' duration, should be suspended from work until his recovery is complete and examination on the lines indicated above shows that he is safe to return to work.

* The blood tests are aimed at the detection of typhoid Vi, O and H agglutinins. It is worth while also to include *Salm. paratyphi B*H specific, non-specific salmonella H and *Salm. paratyphi A*H suspensions. Apart from the possibility that the *Salm. paratyphi B* specific and the non-specific salmonella suspensions may detect agglutinins which could indicate the carriage of *Salm. paratyphi B*, the presence of both TH and BH agglutinins usually suggests that the subject has been inoculated, a fact which cannot always be determined from his history. Similarly, the presence of AH agglutinins, alone or in association with TH or BH agglutinins, is indicative of previous inoculation with T.A.B. vaccine, since paratyphoid A fever does not occur in this country

## SUMMARY

1. The existing procedures recommended for the detection of carriers of enteric pathogens are reviewed.

2. The techniques employed in this work at present were re-examined.

3. An investigation of the sera of 67 chronic typhoid carriers and 343 normal persons, suggested that titrations for typhoid Vi, O and H agglutinins are useful screening tests for the detection of typhoid carriers. The examination may be usefully extended to the titration of H agglutinins against paratyphoid B and

paratyphoid A, the former as a possible indicator of the carriage of *Salm. paratyphi B*, the latter as an indicator of previous inoculation with T.A.B. vaccine.

4. Inoculation did not appear to increase the proportion of positive reactions with TVi bacterial suspension, which in uninfected persons was about 1%. In contrast, the proportion of positive reactors with TO suspension rose from 8·8% in uninoculated to 25·3% in inoculated persons, at a serum dilution of 50; and with TH suspension the positive figure rose from 2·7% in uninoculated to 71·4% in inoculated persons at a serum dilution of 25. Inoculation thus impairs the value of TO agglutination and destroys that of TH agglutination as indicators of infection.

5. The investigation of 29 chronic carriers showed that a single bacteriological examination of excreta for the presence of *Salm. typhi* is not a reliable method of carrier detection. Increasing the number of specimens to three greatly raises the probability of detection.

6. The findings are discussed and a revised form of paragraph 5 of the Ministry of Health Memorandum on the safeguards recommended for adoption by water undertakings is suggested.

The Working Party is grateful to a number of pathologists for the supply of sera from chronic carriers and we should also like to thank Mr F. J. Flyn of the Central Enteric Reference Laboratory for his great technical help.

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