

## **Antibodies and the Aberdeen typhoid outbreak of 1964**

### **II. Coombs', complement fixation and fimbrial agglutination tests**

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#### SUMMARY

In a previous paper (Brodie, 1977) the value of the Widal test in the diagnosis of typhoid fever was shown to be limited. Evaluation of possible alternative tests showed that:

(1) the sensitivity of the anti-human globulin (Coombs') test appeared greater than that of the agglutination test but the length of time (48 h) before results were available rendered it of little value in rapid diagnosis;

(2) the complement fixation test offered no particular help towards diagnosis;

(3) immunized and non-immunized typhoid fever patients developed fimbrial antibodies, as also did immunized healthy individuals. In this latter group, however, those immunized with alcoholized TAB vaccine had higher antibody titres to fimbrial antigen than those immunized with heat-killed phenolized vaccine.

#### INTRODUCTION

The limitations of the Widal reaction in the diagnosis of enteric fever during the Aberdeen typhoid outbreak of 1964 (Brodie, 1977) directed attention towards a comparison of methods for antibody titration to ascertain whether or not any method, not employed in the usual diagnostic range, would yield results which might be helpful in diagnosis.

Three tests were used, the anti-human globulin (Coombs') test, complement fixation tests using *S. typhi* 'O' agglutinable suspension as antigen, and fimbrial agglutination tests. In the work already discussed (Brodie, 1977) the antigen suspensions used in the Widal reactions had been supplied by the Standards Laboratory for Serological Reagents of the Central Public Health Laboratory, London. Agglutination tests were repeated using agglutinable suspensions obtained commercially from Burroughs Wellcome and Company. So that results in this further investigation might be correlated, all tests were carried out with antigens from this source, except the fimbrial agglutination assays, where the antigen was prepared and used as reported by Duguid & Campbell (1969). It was considered desirable to repeat the agglutination tests for *S. typhi* as the sera, having been subjected to deep-freeze storage, might have suffered some loss of antibody titre.

## MATERIALS AND METHODS

*Sera*

These further tests were done on 300 sera from ex-patients, on 40 sera from healthy nurses employed in King's Cross Hospital, Dundee, immunized with TAB and on 39 sera from healthy immunized laboratory technicians working in the Laboratory, City Hospital, Aberdeen, during the outbreak.

*Agglutinable suspensions*

All the antibody titrations against *S. typhi* 'H' were carried out using one batch of suspension obtained from Burroughs Wellcome. All the *S. typhi* 'O' antibody titrations, all complement fixation tests and all Coombs' tests were carried out using one batch only of *S. typhi* 'O' agglutinable suspension from Burroughs Wellcome.

*Widal tests*

The Widal reactions were carried out as described previously (Brodie, 1977).

*Anti-human globulin (Coombs') test technique*

Serum dilutions were prepared as for the Widal test and 0.5 ml of each dispensed into clean, round-bottomed tubes. *S. typhi* 'O' suspension (Burroughs Wellcome Agglutinable Suspension diluted 1/10 in saline) was added in 0.5 ml volumes to each tube giving final serum dilutions through the range of 1/25 to 1/800. After mixing, the tests were incubated overnight at 37 °C. The tubes were then centrifuged, the supernatant discarded and the deposit washed and recentrifuged three times before final re-suspension in nine drops of saline plus one drop of Coombs' reagent appropriately diluted according to titre. The results were read after further overnight incubation at 37 °C. Any degree of clearing of the suspension judged against the negative control tube was accepted as a positive reading and the titres calculated on this basis.

*Complement fixation tests*

These were carried out in round-bottomed tubes. A four volume system was used. *S. typhi* 'O' antigen (B.W. agglutinable suspension) was titrated at a dilution of 1/7.5. The serum was inactivated at 56 °C for 30 min then diluted with 0.85% saline to give a range of dilutions from 1/4 to 1/256, extending where necessary.

Using 0.4 ml volumes, 1 volume each of diluted serum, complement and antigen were added to each tube, mixed and incubated for 1 h at 37 °C in the water bath. The tubes were shaken every 15 min. To each tube 0.4 ml of sensitized sheep erythrocytes were added, the tubes shaken and incubated for a further 30 min at 37 °C. The tubes were given a shaking at the mid-period of incubation. The end-point of the reaction was taken as the last tube containing non-haemolysed red cells.

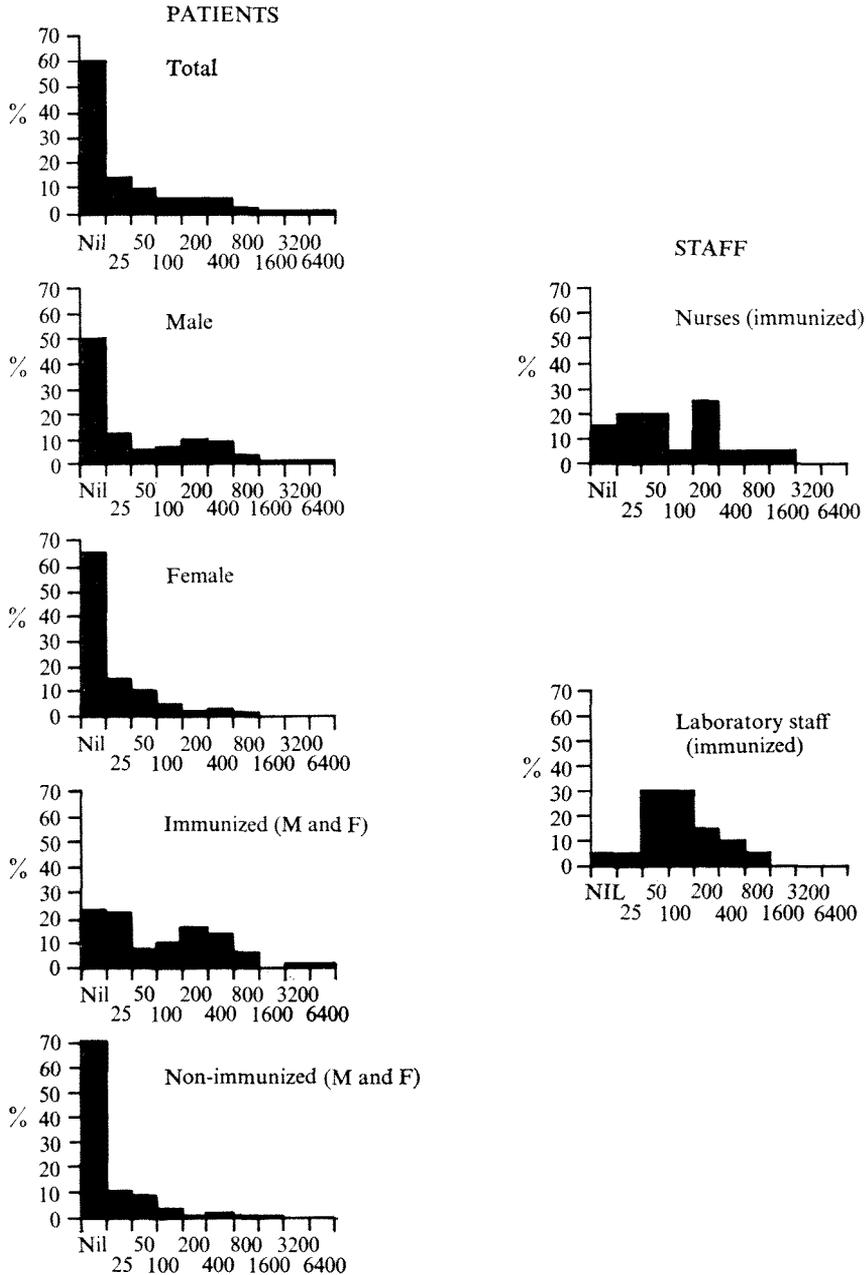


Fig. 1. Further investigations on the *S. typhi* 'H' agglutinin titres in 300 sera from typhoid patients, 40 sera from healthy TAB immunized nurses and 39 sera from healthy TAB immunized laboratory technicians. Results expressed as percentages of samples according to antibody titre present.

*Fimbrial agglutination tests*

A culture of *S. cubana* (211) was obtained, fimbrial suspensions made and the presence of fimbrial antibodies in patients' sera assayed according to the method of Duguid & Campbell (1969).

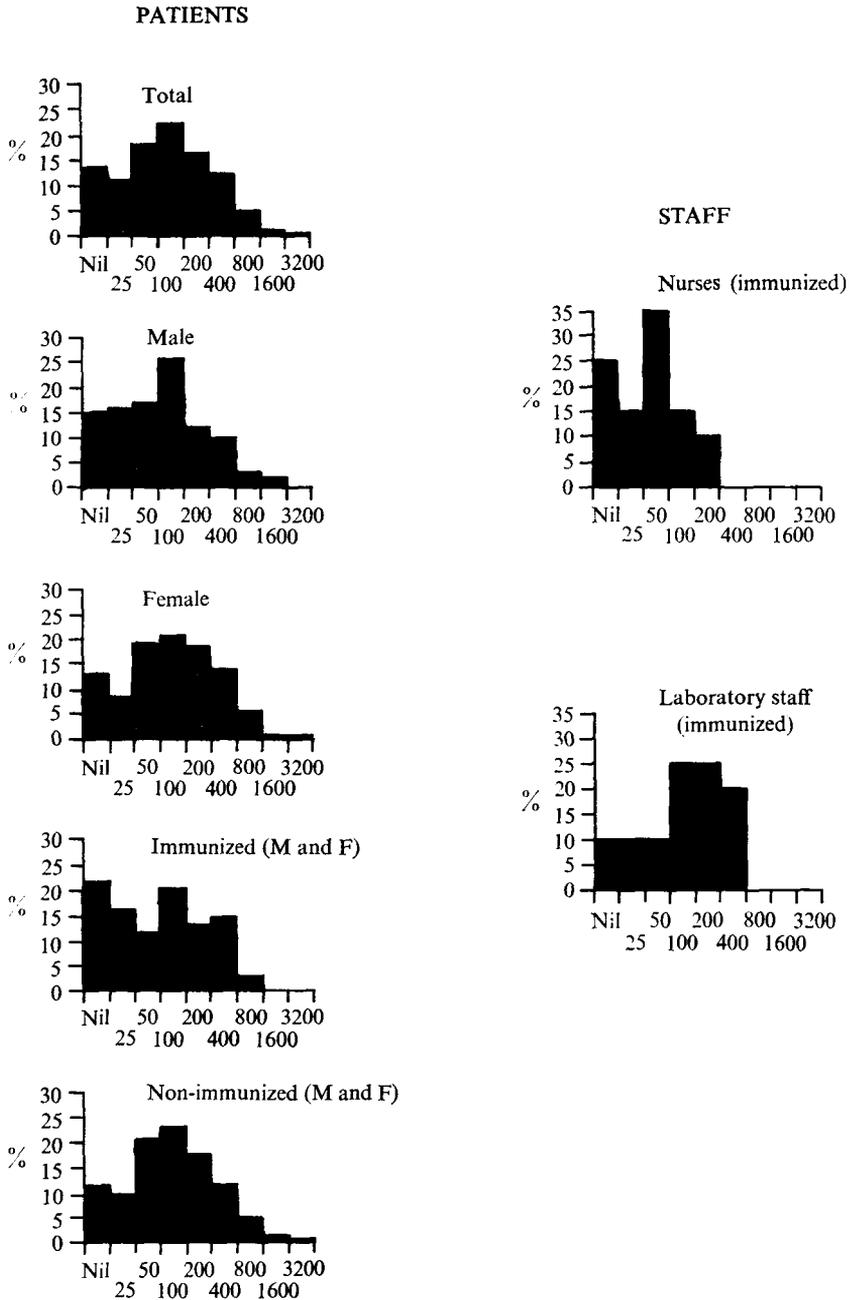
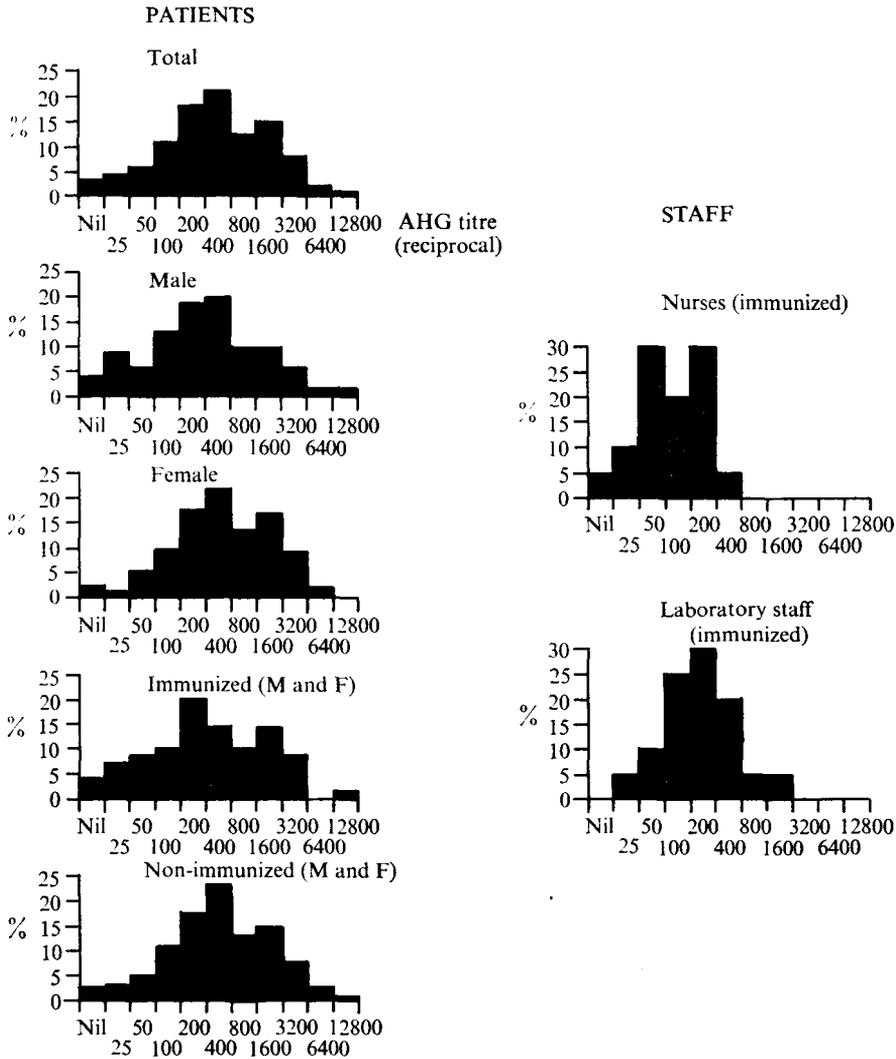


Fig. 2. Further investigations on the *S. typhi* 'O' agglutinin titres in 300 sera from typhoid patients, 40 sera from healthy TAB immunized nurses and 39 sera from healthy TAB immunized laboratory technicians. Results expressed as percentages of samples according to antibody titre present.



**Fig. 3.** Further investigations on agglutinin titres by AHG (Coombs') technique, in 300 sera from typhoid patients, 40 sera from healthy TAB immunized nurses and 39 sera from healthy TAB immunized laboratory technicians. Results expressed as percentages of samples according to antibody titre present.

**RESULTS**

The results obtained are shown graphically in Figs. 1-7. Each figure shows the results of examination of sera from cases of typhoid fever (patients) according to the antigen used, the sex of the patient and whether immunized or not. For further comparison each figure shows the results obtained in the examination of healthy immunized individuals. Figure 7 shows the findings on sera from patients taken at discharge from hospital and at 3 and 6 months thereafter. Because of shortage of sera at this point in the investigation the results should be compared vertically but not horizontally. The purpose of this layout is to facilitate comparison of the antibody titres obtained by the tests used, at three different times after

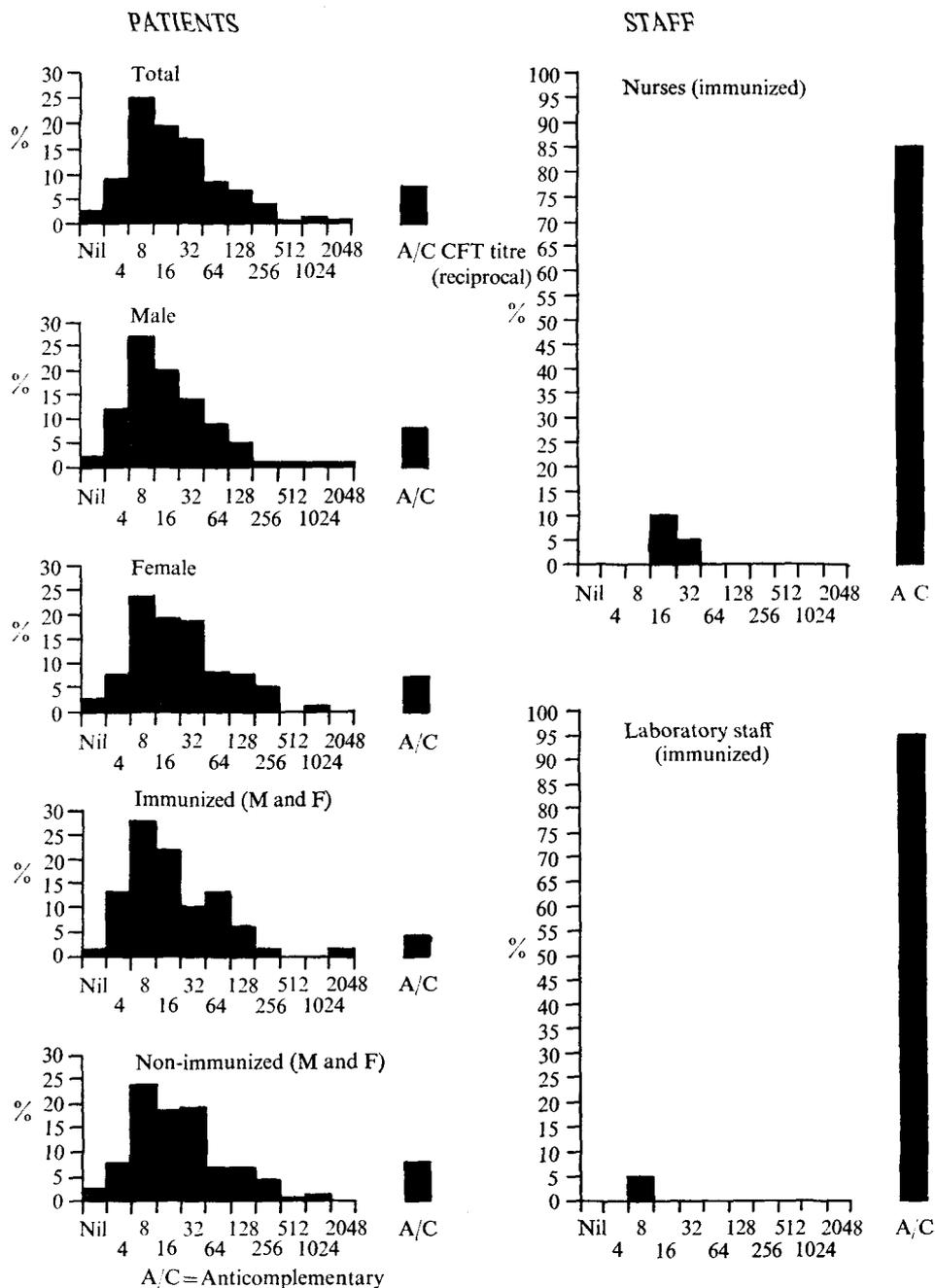


Fig. 4. Further investigations on the complement fixation test titres in 300 sera from typhoid patients, 40 sera from healthy TAB immunized nurses and 39 sera from healthy TAB immunized laboratory technicians. Results expressed as percentages of samples according to antibody level present.

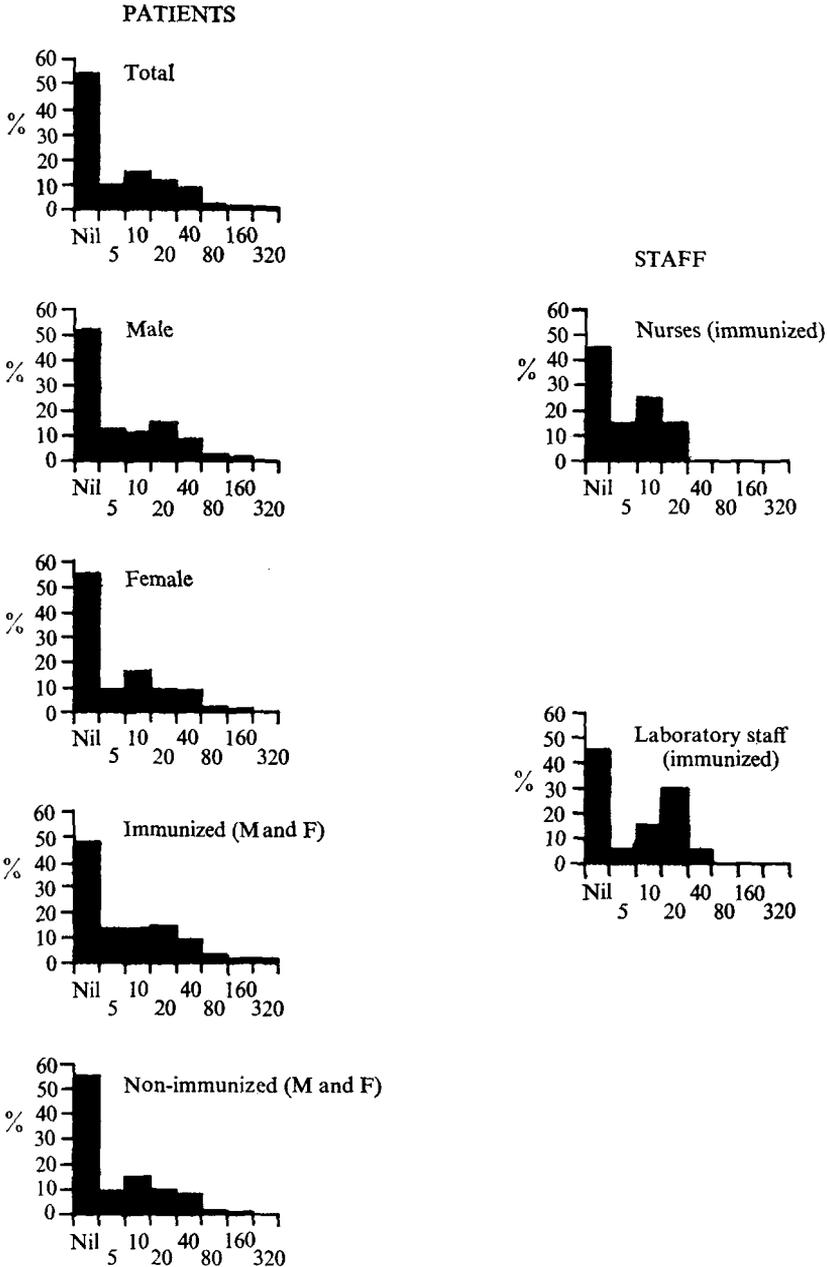


Fig. 5. Further investigations on the 'Vi' agglutinin titres in 300 sera from typhoid patients, 40 sera from healthy TAB immunized nurses and 39 sera from healthy TAB immunized laboratory technicians. Results expressed as percentages of samples according to antibody titre present.

infection, in order to determine whether any one test, in particular Coombs' test and the complement fixation test, neither of which is normally used in the diagnosis of typhoid fever, would prove of particular diagnostic value. However, the results in Figs. 1-6 should not be compared with the results obtained previously (Tables 2,

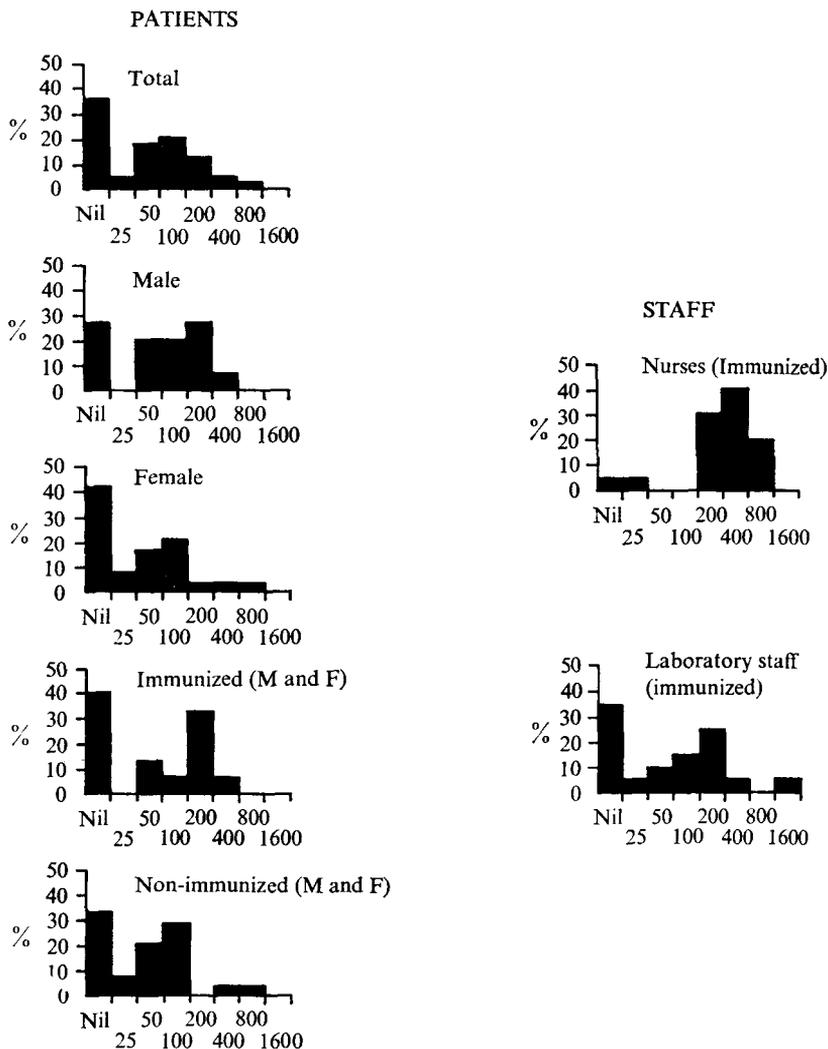


Fig. 6. Further investigations on the antibody titres to fimbrial antigen in 300 sera from typhoid patients, 40 sera from healthy TAB immunized nurses and 39 sera from healthy TAB immunized laboratory technicians. Results expressed as percentage of samples according to antibody titre present.

3 etc. Brodie, 1977) since it was impossible with the small amounts of sera remaining to arrange fully comprehensive investigations on individual patients.

#### DISCUSSION

The antibody responses to *S. typhi* 'H' antigen (Fig. 1) in male patients appeared to have been higher than in the female patients. When the results were subdivided into immunized and non-immunized this became still more apparent. These higher titres for males might be only a legacy from the immunization procedures required for all Service personnel in the 1939–45 World War. This legacy might have been instrumental in evoking higher antibody responses to *S. typhi*

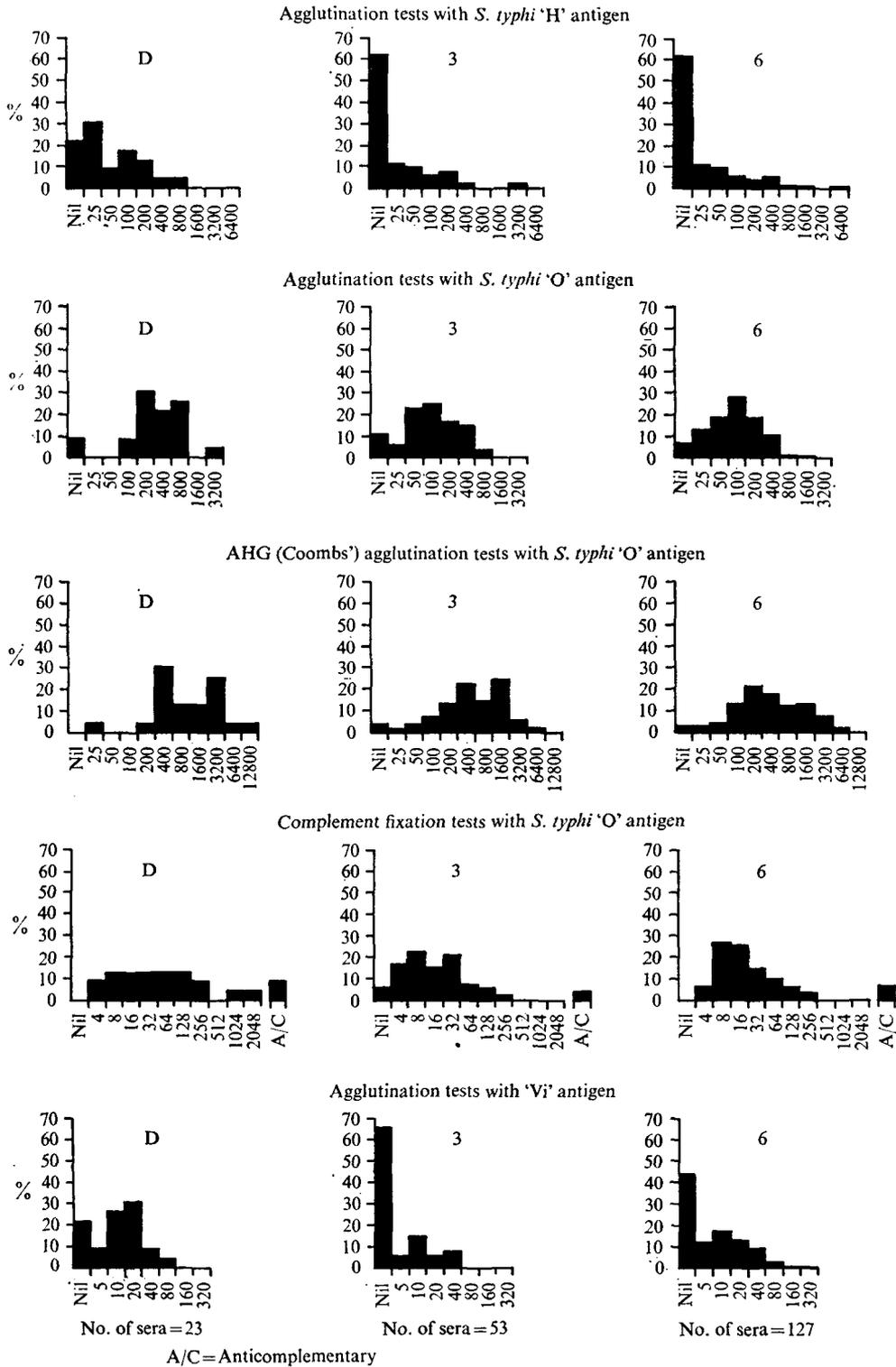


Fig. 7. Further investigations on the titres of antibodies in typhoid patients' sera on discharge from hospital (D), 3 months after discharge (3), and 6 months after discharge (6), according to antigen used and techniques applied. Titres expressed as reciprocals, e.g. nil, 25, 50, 100, etc. Results expressed as percentages of samples according to antibody titres present. For comparison purposes, tabulated results are to be read vertically and not horizontally (see text).

'H' antigen when prior immunization was followed by actual *S. typhi* infection than the lower responses engendered in the typhoid patient with antibody mechanisms without previous experience of *S. typhi* even in the form of a vaccine.

Recently TAB vaccinated non-typhoid individuals – nurses who had received alcoholized vaccine and laboratory staff given heat-killed phenolized vaccine – also produced anti-H levels often higher than those demonstrated in the 300 sera examined from ex-typhoid patients.

If the Widal test is to be of any diagnostic value or even to be of value as a screening test – and the anti-'H' titres can be assayed within a few hours of receipt of the blood specimen – then, in countries where enteric fever is not endemic, there should be a guarded use of prophylactic vaccination. Such immunization does not prevent typhoid fever although it may modify the severity of the infection. It does, however, rule out the usefulness of the quick Widal reaction assessment of anti-H seroconversion. Especially is this so when, in the face of a typhoid outbreak, panic measures are taken and vaccination is used indiscriminately.

When the antibody responses to *S. typhi* 'O' antigen were examined, the results of three different methods of assessment of antibody response required consideration.

The agglutinin responses to *S. typhi* 'O' are set forth in Fig. 2. There was little difference to be seen in any of the sets of results as the histograms indicate. Here again, prophylactic vaccination complicated the interpretations of the results. If laboratory workers are expected to interpret Widal findings, then it is essential that information is given on whether or not the patient has had previous inoculation. If this information is not given when the Widal is requested, a number of patients may find themselves in hospital with a tentative diagnosis of enteric fever and may suffer all the inconveniences attendant upon this. Tables 6 and 7 (Brodie, 1977) present evidence of possible such incidents where the diagnosis of typhoid fever had to be made on clinical grounds with whatever support the Widal reactions might have given towards this.

The second method of assessment of agglutinins to *S. typhi* 'O' involved the use of the anti-human globulin (Coombs') technique. The findings are shown in Fig. 3. The sensitivity of this technique appeared to be greater than the straight-forward agglutination test with *S. typhi* 'O' suspension. This is evident both with typhoid patients' sera and with immunized non-typhoid individuals when Fig. 2 is compared with Fig. 3.

Whether there is a need for AHG (Coombs') test in the diagnosis of typhoid fever is a moot point. The test is cumbersome to perform and no quick result is possible. Perhaps it may have some use in suspected typhoid fever cases where no isolation of *S. typhi* has been made from blood, faeces, urine, etc. It would seem to have no particular advantage to offer in suspect cases when it is known that they have been previously immunized – if the results set forth in Fig. 3 are any guide in this context.

The third method of assessment of antibody response made use of the complement fixation technique with *S. typhi* 'O' antigen. The results for typhoid patients showed little difference for males or females, immunized or non-immunized and

the titres were not high (Fig. 4). Unfortunately, the sera from immunized non-typhoid individuals showed such degrees of anti-complementary activities that the results were almost worthless, but they did at least demonstrate that complement fixing antibodies were present in the sera of immunized individuals and were not peculiar only to sera from proven typhoid fever cases. It was strange, however, to find such anti-complementary qualities in the sera of the immunized non-typhoid individuals while this property was not pronounced in the sera from immunized typhoid-infected patients. All sera had been subjected to the same conditions of storage, handling, etc. If the method of collection of the samples was in any way responsible, one would not have expected the specimens from the nurses to show the same characteristic as that also shown by the sera from the laboratory staff. The type of vaccine used also did not seem to have affected the results, the nurses received alcoholized vaccine whereas the laboratory personnel had been given the heat-killed phenolized vaccine.

Further investigations on the 'Vi' agglutinin titres were done on the 300 sera from typhoid patients, the 40 sera from the nurses and the 39 laboratory staff. The results are given in Fig. 5 which shows that there was little difference in titres in male or female, immunized or non-immunized ex-typhoid patients. The percentage of ex-patients showing 'Vi' titres seemed to suggest that a large number of typhoid carriers might have been expected after clinical recovery but only 6 of the cases in the outbreak became chronic carriers (Table 8, Brodie, 1977).

Among the 40 nurses and 39 laboratory technicians 55% showed 'Vi' antibody. The highest titre found in this nurse group was 1/20 shown by 15%. In the laboratory personnel group, 30% had a titre of 1/20 and a further 5% a titre of 1/40. The percentage of the 300 ex-typhoid patients with 'Vi' titres was 46%, which is 10% less than the immunized non-typhoid individuals. Almost 55% of all immunized ex-typhoid patients had demonstrable 'Vi' antibody. This 55% was exactly the same percentage as that obtained for those with 'Vi' antibody in the immunized non-typhoid individuals tested.

The findings in this further investigation again do not support the contention that examination for 'Vi' antibody is worth doing in field examinations for typhoid carriers, even as a screening test.

Tests for antibodies to fimbrial antigens were done according to Duguid & Campbell (1969) and the results given in Fig. 6. Of all the serum samples from ex-typhoid patients, 64% had demonstrable antibodies to this antigen. According to sex, 73% of the male and 58% of the female patients had fimbrial antibodies. Irrespective of sex but according to immunity status, 60% of immunized as against 67% of non-immunized patients showed fimbrial antibodies. The sera from laboratory staff showed 65% with antibodies but an astonishingly high percentage of 95% was obtained from the nurses. This remarkable difference may have been related to the nurses having received alcoholized vaccine as against the heat-killed phenolized vaccine given to the laboratory staff. Of the nurses, 90% had titres in excess of 1/100, made up of 30% with 1/200, 40% with 1/400 and the remaining 20% with 1/800.

The significance of fimbrial antibodies on the diagnosis, prognosis, etc., or even

prevention of typhoid fever, is so far unknown. Their demonstration also in immunized individuals rather suggests that, at the moment, the finding is of academic interest.

The final tests on the remnants of the sera were done for antibodies, other than fimbrial, to provide some comparisons of titres at different times of sampling. These were carried out on sera taken at discharge, at 3 and 6 months after discharge from hospital.

Only limited numbers of sera of adequate volume were available and, consequently it was not possible to use sera always from the same patients at the three sampling times covered. The results set forth in Fig. 7, therefore, must be compared vertically only. The titrations assessed agglutinin titres for *S. typhi* 'H' and *S. typhi* 'O' followed by the AHG (Coombs') results, the complement fixation titres and lastly the Vi titres all expressed as percentages for each titre. The results indicated that there was no particular period in time of sampling at which the patterns obtained by one test differed greatly from those obtained by any other. The AHG test results showed a shift to the right and, as before, showed higher titres than any of the other tests. Only one other feature of the results seemed notable and that was the results in the complement fixation tests. Where the *S. typhi* 'O' agglutinin responses were absent or of low titre, the lower dilutions of the sera tended to give positive reactions with the complement fixation test but here one must be careful for this observation may be more apparent than real since the graphs deal in percentages rather than individual results, and the numbers of sera were so much smaller than those available for study in the earlier stages of the investigations.

I am grateful to the Secretary of State for Scotland for the research grant which supported this and other work which followed on the Aberdeen typhoid outbreak of 1964. My thanks are due to the research assistants Dr Winifred McPherson and Avril J. C. Dawson, B.Sc., also to Dr W. M. Jamieson who supplied the sera from immunized nurses and to all others who assisted in any way during the investigations.

Anyone wishing to read the full report of the research is requested to apply to the Secretary, Biomedical Research Committee, Scottish Home and Health Department, St Andrew's House, Edinburgh.

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