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Antibodies and the Aberdeen typhoid outbreak of 1964

I. The Widal reaction

By J. BRODIE
The Laboratory, City Hospital, Aberdeen

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SUMMARY

The outbreak of typhoid fever in Aberdeen during 1964 (Walker, 1965) presented an opportunity to study the antibody titres of typhoid fever patients and of TAB immunized individuals to obtain further knowledge concerning the behaviour of these titres with the passage of time.

This paper gives an abbreviated version of part of a research programme which followed the Aberdeen typhoid outbreak of 1964.

The antibody titres of patients were followed up for a period of 2 years after discharge from hospital and the findings have been compared with those in TAB immunized healthy individuals. The following points emerged:

(1) The value of the Widal test as an aid to diagnosis was limited;

(2) the flagellar antibody titre in patients' sera provided a more reliable aid towards diagnosis than did the somatic antibody titre;

(3) the response of immunized and non-immunized patients to the somatic antigens was poor and often delayed well into the period following discharge from hospital;

(4) titres of 1/40 and over for Vi agglutinins were present in immunized and non-immunized patients for at least 12 months after discharge without their being *S. typhi* excretors;

(5) Vi agglutinin titres as high as 1/40 were present in TAB immunized healthy individuals and also in members of the general public;

(6) the presence of *S. typhi* septicaemia need not result in a high antibody titre;

(7) patients who relapse, may do so without enhancement of previous antibody titres and may relapse even in the presence of earlier appreciable titres.

INTRODUCTION

If one is to presume that the Widal reaction findings recorded in Tables 2–5 below render such investigations unreliable as diagnostic aids then the Widal reaction would appear to be not worth the time and labour spent on its performance. If, however, we pause to consider the Widal test applied for diagnosis during a typhoid fever outbreak, there is a wealth of background data which must be considered before the test is condemned.

The agglutination tests set out to show whether or not there has been produced in the blood of the patient suspected of suffering from typhoid fever, antibodies

Table 1. *Salmonella serotypes antigenically related to* Salmonella typhi

S. typhi	9, 12 ('Vi')	d: —
S. ndolo	**1**, 9, 12	d: 1, 5
S. tarshyne	9, 12	d: 1, 6
*S. rhodesiense**	9, 12	d: e, n, x
S. zega	9, 12	d: z_6
S. jaffna	**1**, 9, 12	d: z_{35}
S. strasbourg	9, 46	d: 1, 7
S. plymouth	9, 46	d: z_6

Somatic symbols in bold (e.g. **1**) = phage determined.
* Subgenus II.

to the flagellar 'd' antigen and/or to the somatic 9 (and 12) antigens possessed by *S. typhi*. Somatic antigen 12 has been purposely placed in parentheses and will be considered after the 'd' and '9' antigens have been discussed.

In the test, suspensions of killed *S. typhi* specially prepared for (1) the detection of antibody reacting with the flagellar ('H') antigen 'd' and (2) the somatic ('O') antigens 9 (and 12), are used.

Any organism in the *genus Salmonella* possessing H antigen 'd' or 'O' antigens 9 (and 12) is capable of stimulating the immunity mechanisms of its host to produce antibodies against these antigens in addition to antibodies against any other antigens it may possess. In 1969, the Kauffmann–White scheme which classifies salmonellas by their somatic and flagellar antigens included 97 salmonellas possessing flagellar antigen 'd'. Of these, however, only 8 including *S. typhi* possessed somatic antigen 9. Of these only 6, including *S. typhi*, possessed somatic antigen 12.

The complete antigenic formulae for each of these 8 antigenically related salmonellas are shown in Table 1.

S. rhodesiense belonging to subgenus II is common in reptiles and rarely causes infection in man. In countries where these serotypes are common, infection due to any one of them may produce a Widal result likely to be mistaken for infection with *S. typhi*. However, one would expect laboratory personnel in any such country to be aware of this and to be cautious in the interpretation of Widal results, especially where no clinical details or other confirmatory evidence was available.

Somatic antigen 12

Some 177 serotypes of *Salmonella*, including those mentioned above possessing flagellar antigen 'd' and somatic antigen 9, possess somatic antigen 12. Infection with any of these may result in the serum of patients giving agglutination when titrated against *S. typhi*, *S. paratyphi B*, or *S. paratyphi A* 'O' suspensions. Except for the 8 serotypes listed in Table 1, infection with any of these 117 serotypes should not give agglutination with *S. typhi* 'H' suspension.

In the light of the above facts, the demonstration in a patient's serum of agglutinins reacting with *S. typhi* 'H' and 'O' suspensions constitutes strong presumptive evidence of typhoid fever especially if the patient has never been inoculated with TAB vaccine. The further evidence of rising titres in the patient's

blood over a few days following the first sample provides additional support for suspecting active infection in both the non-immunized and the immunized patient. Further investigations to confirm the diagnosis by the isolation of *S. typhi* from blood, faeces, urine, etc., are then essential.

In Great Britain typhoid fever is not endemic, the seven serotypes closely related to *S. typhi* are uncommon, and prophylactic vaccination is not a routine procedure in the general population. Given this background the demonstration of antibodies to *S. typhi* in a patient's blood carries greater significance in Great Britain than it might in any country where typhoid fever is endemic, or where immunization is practised on a wide scale. However, even now in Great Britain, TAB vaccination is practised on a wider basis than previously as, apart from personnel in the Armed Forces, increasing numbers of the general population are vaccinated before going abroad on business or vacation.

In the Aberdeen typhoid outbreak of 1964 (Walker, 1965; Russell, 1965) there were 507 cases of infection with *S. typhi* phage type 34.

Of the cases 403 were confirmed by isolation of the organism. Sixty-six were diagnosed on clinical grounds supported by serological evidence of infection. In the remaining 38 patients a diagnosis of typhoid fever, though not confirmed bacteriologically or serologically, could not be excluded. These figures do not include eight patients who contracted typhoid fever whilst visiting Aberdeen but were treated elsewhere.

At the beginning of the outbreak the Widal test used consisted of agglutinations against *S. typhi* 'H' and 'O', *S. paratyphi A* 'H' and *S. paratyphi B* 'H' and 'O' suspensions. As the samples came flooding in, however (at the height of the outbreak between 20th May and 30th June, over 10,000 samples of clotted blood were submitted for examination), it became necessary to reduce the tests to agglutination of *S. typhi* 'H' suspension over a range of twofold dilutions from 1/25 to 1/400, read after 4 h incubation in the water bath at 56 °C. This abbreviation made it possible to examine and report three sets of tests daily, but raised difficulties in the interpretation of positive results, especially in individuals immunized with TAB. Positive results were, however, communicated by telephone to the doctors concerned, the interpretation was discussed and repeat specimens for evidence of rising titres requested.

Clot cultures were carried out on all bloods received and in addition three specimens each of faeces and urine from patients were examined. During the outbreak 11,043 clots were cultured and 122 cases received their first bacteriological confirmation from this procedure.

When patients were admitted to hospital, a full Widal test was applied where possible.

MATERIALS AND METHODS

Blood specimens

Patients

It was arranged that follow-up blood specimens would be taken from patients just before discharge from hospital and then at intervals of 3, 6, 12, and 24 months after discharge. All sera were examined for antibodies and the results recorded.

As far as possible the earlier diagnostic sera were also re-examined and recorded.

The reason for this follow-up study was explained to patients, and letters were sent to those who agreed to cooperate indicating the times of their attendances at the laboratory for the collection of further specimens. Most patients attended well but some failed to honour the arrangement and in consequence samples from some patients did not cover the full range which was planned. The samples were identified according to the times of collection in relation to illness, thus: A, on admission; D, immediately before discharge; 3, 6, 12, 24 months after discharge.

Immunized nurses

In the earlier stages of this investigation, samples of blood from 86 of the nursing staff of King's Cross Hospital, Dundee, were examined. All had been inoculated with alcoholized TAB vaccine and had received booster doses at regular intervals.

Normal population

To ascertain the titres of antibodies in the general population, 165 sera were examined, received through general practitioners, from patients with pyrexia of unknown origin but from whom *S. typhi* or *S. paratyphi B* were not isolated.

Storage of sera

All sera were examined as soon after collection as possible. Where delay occurred, the sera were kept in deep freeze and, after examination, were returned to deep freeze to await subsequent investigation.

Widal tests

Tests were carried out using suspensions of *S. typhi* 'H' and 'O', and *S. paratyphi B* 'H' and 'O', obtained from the Standards Laboratory for Serological Reagents, Central Public Health Laboratory, Colindale. Doubling dilutions of patients' serum were made in 0·85% saline starting at 1/12·5. Volumes of 0·25 ml of each dilution were placed in round-bottomed tubes for 'O' antigen suspensions and in Dreyer tubes for 'H' antigen suspensions. An equal volume of the appropriate antigen suspension was added to each tube to give final serum dilutions from 1/25 upwards. All tubes were incubated in a water bath at 56 °C for 4 h, removed and left on the bench at room temperature overnight before the final readings were taken.

The *S. paratyphi A* 'H' suspension which is included in the Widal test under normal laboratory conditions was omitted from tests done on patients and immunized nurses, but was included in the examination of sera received from practitioners from patients with pyrexia of unknown origin.

Vi antibody tests

The test consisted of preparing, in 0·85% saline, 1 ml volumes of the patient's serum through a range of twofold dilutions from 1/5 to 1/320 (extended where necessary). To each dilution 0·05 ml of 'Vi' antigen was added.

A control with a standardized anti-'Vi' serum was done over a range of dilutions according to the titre of the standard. Further tubes were set up but with distilled

water, 5% saline, 2·5% saline, and 0·85% saline replacing the serum to act as control of the stability of the antigen suspension.

All tubes used were round-bottomed, clean and without scratches. The tests and controls were incubated at 37 °C for 2 h before being refrigerated at 4 °C for 20 h. After refrigeration, the tubes were left on the bench for 2 h at room temperature before readings were made.

Antibody titres in repeated tests

The sera collected from patients up to and including the 12 months after discharge samples were examined as soon after collection as possible using as antigens the batches of suspensions available through normal channels at the time of testing. In the period between the 12-month and 24-month sampling, the sera already tested were again assayed but this time only one batch of each of the antigens was used to reveal, as far as possible, any variation which might be produced by using various batches of antigens, and the degree of technical error one might expect to find in carrying out the tests. In brief, these two sets of antibody titrations would give a measure of the reproducibility of agglutination results, and give some indication as to the degree of validity of antigen–antibody reactions during illness, in convalescence, and for a period of 24 months after illness.

RESULTS

Reproducibility of the antigen–antibody reactions

Sera in the range D, 3, 6 and 12, titrated with varying batches of antigen and then with only one batch of each antigen showed a maximum discrepancy of not more than 2 dilutions – a 2-tube difference.

The findings in the sera of typhoid patients with no history of previous TAB inoculation and with a history of previous TAB inoculation are given in Table 2. The general pattern was the same for non-immunized and immunized patients, namely that there was greater correlation and accuracy in the anti-'H' than in either the anti-'O' or the anti-'Vi' titres. This seemed to be related to the greater ease in reading the floccular 'H' agglutination as against the difficulty in assessing a clear-cut end-point of the granular 'O' agglutination and of the agglutination pattern peculiar to the 'Vi' agglutination reaction rather than to technical errors in carrying out the tests.

Patients' antibody titres from admission to hospital to 24 *months after discharge inclusive*

With the range of sera available it was possible to follow the behaviour of antibody titres in the sera from individual hospital patients from admission to 24 months after discharge. Certain patterns of response presented, and allowed the results to be grouped according to the behaviour of the antibodies in relation to the first titre available. This first titre was that on admission to hospital (A) except where the earliest sample was that taken on discharge (D). Sera failing to give agglutination in the first tube, i.e. the highest concentration (1/25) of serum

Table 2. *Comparisons of repeated agglutination results in TAB immunized and non-immunized typhoid patients*

		TAB immunized patients		Non-immunized patients	
Time of sample	Antigen	No. of sera	% with no or only 1 tube difference	No. of sera	% with no or only 1 tube difference
Discharge	*S. typhi* 'H'	47	91·5	186	90·9
3 months		47	91·5	185	92·9
6 months		49	95·9	188	96·3
12 months		61	100	249	100
Discharge	*S. typhi* 'O'	47	63·8	186	75·8
3 months		47	72·3	185	69·7
6 months		49	71·4	188	61·7
12 months		61	96·7	249	92·8
Discharge	*S. paratyphi B* 'H'	47	91·5	186	99·4
3 months		47	93·6	185	100
6 months		49	89·8	188	100
12 months		61	100	249	100
Discharge	*S. paratyphi B* 'O'	47	63·8	186	68·3
3 months		47	55·3	185	62·7
6 months		49	59·2	188	57·9
12 months		61	85·3	249	88·3
Discharge	'Vi'	17	70·6	100	74·0
3 months		29	55·2	126	61·1
6 months		17	58·8	77	61·0
12 months		59	79·7	244	89·3

tested were recorded as 'Nil'. Where agglutination did occur the result was considered as a 'Readable titre' to avoid confusion with the 'Nil' results.

The patterns of antibody behaviour against each of the different antigens have been set forth as:

(1) All results nil = all antibody titres < 1/25.
(2) Fall to nil = fall from an initial readable titre to < 1/25.
(3) Rise from readable titre = titres on the rise from 1/25, 1/50, etc.
(4) Mid range fall = readable titres higher in early and late specimens.
(5) Fall to readable titre = falling from higher titre but not to nil.
(6) Static = readable titres neither rising nor falling.
(7) Mid range rise = initial and late titres lower than mid-way titres.
(8) Rise from nil = titres initially < 1/25 then showing antibody development.

'H', 'O' and 'Vi' antibody responses

Tables 3 and 4 give the behaviour pattern of the agglutinin contents of the patients' sera on the above basis for *S. typhi* and *S. paratyphi B* respectively together with the numbers and percentages of patients in each pattern, except for

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Table 3. *Agglutinin behaviour patterns in non-immunized and immunized patients to* S. typhi *suspensions*

Behaviour pattern	*S. typhi* suspension	Non-immunized: No. of patients giving pattern	Non-immunized: Bacterio-logically confirmed	Immunized: No. of patients giving pattern	Immunized: Bacterio-logically confirmed
All results nil	H	63	53 (84·1)	5	4 (80)
	O	128	120 (93·7)	51	21 (41·2)
	Vi	31		11	
Fall to nil	H	159	143 (90)	22	19 (86·4)
	O	14	13 (93)	9	9 (100)
	Vi	107		38	
Rise from readable titre	H	5	5 (100)	30	11 (36·7)
	O	7	7 (100)	0	0
	Vi	37		13	
Mid range fall	H	5	5 (100)	3	0
	O	4	4 (100)	0	0
	Vi	31		6	
Fall to readable titre	H	27	25 (92·6)	22	5 (22·7)
	O	0	0	1	1 (100)
	Vi	55		18	
Static	H	4	4 (100)	6	2 (33·3)
	O	0	0	0	0
	Vi	13		3	
Mid range rise	H	71	59 (83·1)	14	9 (64·3)
	O	116	113 (97·4)	27	18 (66·7)
	Vi	39		11	
Rise from nil	H	17	14 (82·3)	3	2 (66·7)
	O	60	57 (95)	23	13 (56·5)
	Vi	21		6	
Totals: all patients					
(*a*) *S. typhi* 'H'		351	308 (87·8)	105	52 (49·5)
(*b*) *S. typhi* 'O'		329	314 (95·4)	111	62 (55·8)
(*c*) 'Vi'		334		106	

Figures in parentheses are percentages.

'Vi', from whom *S. typhi* was isolated. Both tables differentiate between non-immunized and immunized patients as far as was possible from the clinical histories.

S. typhi '*H*' *agglutinins*

Of the 456 patients examined (Table 3), 68 (15%) showed 'All results nil' over the 2-year period. Of these 68 patients, 57 (83·3%) were bacteriologically confirmed, i.e. *S. typhi* was isolated from specimens of blood, faeces, etc. A total of 2180 sera were examined to obtain these figures and from 375 of the 456 patients at least 4 serial samples were examined.

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Table 4. *Agglutinin behaviour patterns in non-immunized and immunized patients to* S. paratyphi B *suspensions*

Behaviour pattern	*S. paratyphi B* suspension	Non-immunized: No. of patients giving pattern	Non-immunized: Bacteriologically confirmed	Immunized: No. of patients giving pattern	Immunized: Bacteriologically confirmed
All results nil	H	329	312 (94·8)	36	26 (72·2)
	O	205	193 (98)	61	26 (42·6)
Fall to nil	H	6	5 (82·7)	10	7 (70)
	O	31	29 (93·5)	8	7 (87·5)
Rise from readable titre	H	3	1 (33·3)	16	5 (31·2)
	O	0	0	2	0
Mid range fall	H	0	0	1	1 (100)
	O	5	(100)	1	0
Fall to readable titre	H	2	1 (50)	13	5 (38·5)
	O	2	1 (50)	0	0
Static	H	3	2 (66·7)	6	4 (66·7)
	O	0	0	0	0
Mid range rise	H	9	6 (66·7)	10	6 (60)
	O	2	2 (100)	17	14 (82·3)
Rise from nil	H	2	2 (100)	9	0
	O	24	22 (91·7)	13	11 (84·6)
Totals: all patients					
(*a*) *S. paratyphi* 'H'		354	329 (92·9)	101	54 (53·5)
(*b*) *S. paratyphi* 'O'		269	252 (93·3)	102	58 (56·9)

Figures in parentheses are percentages.

S. typhi '*O*' *agglutinins*

The behaviour of the antibody titres to *S. typhi* 'O' antigen over the same period indicated that 179 (40·7%) of 440 patients had the 'All results nil' pattern. Of these 179 patients, 141 (78·8%) were bacteriologically confirmed. A total of 2154 sera were tested to produce these results and of the 440 patients from whom the sera were obtained 329 had four or more serial samples examined.

S. typhi '*Vi*' *agglutinins*

Of the sera from 334 non-immunized typhoid patients, 282 (84·4%) showed some antibody content at the time of discharge from hospital. Of the 52 who did not, 31 (9·3%) gave 'All results nil': the remaining 21 (6·3%) yielded the 'Rise from nil' pattern. Of the 106 immunized patients, 89 (84%) had produced 'Vi' antibody by the time of discharge from hospital. Of the 17 who had not 11 (10·4%) gave 'All results nil'. The remaining 6 (5·6%) showed the 'Rise from nil' pattern.

Antibody responses to the 'Vi' antigen were done on the sera from 440 patients and at least 4 sera were examined from 394 of these. The 'All results nil' pattern pertained to 42 (9·5%).

With regard to the development of 'O' agglutinins, 128 (37%) of 329 non-

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immunized patients gave the 'All results nil' pattern whereas only 63 (17·9%) of 351 patients yielded the same pattern to the 'H' antigen.

In the patients who gave histories of TAB vaccination, 51 (45·9%) of 111 patients gave 'All results nil' for *S. typhi* 'O' agglutinins, but only 5 (4·7%) of 105 patients examined for *S. typhi* 'H' agglutinin.

These results indicated that in both the non-immunized and immunized typhoid patients the 'O' agglutinin responses were decidedly poor. It was even more surprising to find that the percentage of immunized patients with no 'O' agglutinin response was higher, 51/111 (46%), than that found in the non-immunized patients (128/329, 39%). Of the 128 non-immunized patients 120 (93·7%) were confirmed bacteriologically: of the 51 immunized patients 21 (41·2%).

In the patients with no 'H' agglutinins, of 63 non-immunized patients 53 (84·1%) were confirmed bacteriologically: of 5 immunized patients, 4 (80%) were confirmed bacteriologically.

S. paratyphi B *'H' agglutinins*

Of the 354 non-immunized typhoid patients tested for *S. paratyphi B* 'H' agglutinins 329 (92·9%) yielded the 'All results nil' pattern and of the 329, 312 (94·8%) were confirmed bacteriologically.

Of 101 immunized patients, 36 (35·6%) yielded 'All results nil' for the same 'H' agglutinins. Of these 36, 26 (72·2%) were confirmed bacteriologically.

One does not expect *S. typhi* infection to elicit an antibody response to the 'b' flagellar antigen of *S. paratyphi B* and, in the main, the results confirm this. Although patients were very carefully questioned regarding previous TAB inoculation, no guarantee can be given that the figures quoted are fully accurate. Some patients were indeed very hazy as to what TAB vaccination was and as to whether or not they had received it.

S. paratyphi B *'O' agglutinins*

In so far as *S. typhi* possesses, in common with *S. paratyphi B*, the somatic 'O' 12 antigen some cross-agglutination with the 'O' suspension of *S. paratyphi B* might be expected to occur with sera from typhoid fever patients. In 205 (71·7%) of the 269 non-immunized typhoid patients, the 'All results nil' pattern for *S. paratyphi B* 'O' agglutinins was obtained despite the fact that of the 205, 193 (98%) were confirmed bacteriologically. Of the immunized patients, 61 of 102 (60·4%) so tested also gave 'All results nil' although 26 (42·6%) of the 61 were confirmed bacteriologically. One is left again with poor serological responses to the common '12' somatic ('O') antigen in both non-immunized and immunized patients even although the latter may be presumed to have had previous experience of this antigen at the time of inoculation and accordingly should have had a further stimulus, as a result of infection with *S. typhi*, towards a boosting of any existing antibody mechanism for the production of 'O' agglutinins for somatic '12' antigen.

Table 5. *Agglutinin content of sera from immunized and non-immunized normal persons*

(Percentages in parentheses.)

S. typhi			*S. paratyphi B*		Immunized		Non-immunized
'Vi'	'H'	'O'	'H'	'O'	Nurses	General population*	General population†
+	+	+	+	+	.	1 (4)	.
+	+	+	+	−	.	4 (16)	.
+	+	+	−	+	.	.	2 (1·4)
+	+	−	+	+	2 (2·3)	.	.
+	+	−	+	−	36 (41·9)	9 (36)	5 (3·6)
+	+	−	−	−	4 (4·6)	.	2 (1·4)
+	−	+	+	−	.	1 (4)	.
+	−	−	+	−	1 (1·2)	1 (4)	.
−	+	+	+	+	1 (1·2)	1 (4)	.
−	+	−	+	−	34 (39·5)	8 (32)	1 (0·7)
−	−	+	−	+	.	.	1 (0·7)
+	−	−	−	−	4 (4·6)	.	27 (19·3)
−	+	−	−	−	.	.	5 (3·6)
−	−	+	−	−	.	.	2 (1·4)
−	−	−	+	−	.	.	2 (1·4)
−	−	−	−	+	.	.	2 (1·4)
−	−	−	−	−	4 (4·6)	.	91 (65·0)
Total					86	25	140

* Agglutinins to *S. paratyphi A* 'H' present.
† Agglutinins to *S. paratyphi A* 'H' absent.

Antibody titres in immunized nurses and non-enteric fever patients

Table 5 presents the results of examination of the sera of 86 immunized nurses and of 165 sera received from general practitioners for the investigation of pyrexia of unknown origin. None of these patients were found to be suffering from either typhoid or paratyphoid fever. All sera were examined for *S. typhi* 'H', 'O' and 'Vi' antibodies. In addition the 165 sera received from general practitioners were examined for the presence of antibodies against *S. paratyphi A* 'H'.

Sera from immunized nurses

Of the 86 sera, 77 (89·5%) had antibodies to *S. typhi* 'H'; 74 (86%) antibodies to *S. paratyphi B* 'H', but only (1·2%) had antibodies to *S. typhi* 'O' antigen and only 2 (2·4%) to *S. paratyphi B* 'O' antigen.

The most common patterns of antibody response were those which showed agglutinins against the 'H' antigens of *S. typhi* and *S. paratyphi B*, namely 73 (84·9%) of the total 86 sera.

Thus even in healthy TAB vaccinated individuals there was a paucity of 'O' agglutinin production not only to the '12' antigen common to both *S. typhi* and *S. paratyphi B* but also to the somatic '9' antigen of *S. typhi* and the '4' antigen of *S. paratyphi B*. The 'H' antigens produced the best responses and these

seemed to persist for longer than any which might have been evoked by the 'O' antigens.

Sera from patients suffering from P.U.O.

Presumed inoculated with TAB. The sera from 25 of these patients contained antibodies to the 'H' antigen of *S. paratyphi A*, indicating inoculation with TAB vaccine.

Of the 25 sera 23 (92%) had antibodies to *S. typhi* 'H', 25 (100%) antibodies to *S. paratyphi B* 'H', but only 7 (28%) had antibodies to *S. typhi* 'O' antigen and only 2 (10%) to *S. paratyphi B* 'O' antigen.

The most common patterns of antibody response were again those which showed agglutinins against the 'H' antigens of *S. typhi* and *S. paratyphi B*, i.e. 23 (92%) of the 25 sera.

Presumed not inoculated with TAB. The sera from the remaining 140 general practitioner patients failed to agglutinate the 'H' antigen of *S. paratyphi A* and are therefore presumed not to have been inoculated.

A total of 91 of the 140 (65·0%) failed to show any agglutinins, 15 (10·7%) reacted with *S. typhi* 'H' antigen, 5 (3·6%) with *S. typhi* 'O' antigen, 8 (5·7%) with *S. paratyphi B* 'H' antigen, 5 (3·6%) with *S. paratyphi B* 'O' antigen. Six sera (4·3%) reacted with at least 2 of the 'H' antigen suspensions.

'Vi' antibody response

From Table 3 it is evident that the sera of 303 (90·7%) of the 334 non-immunized patients had 'Vi' antibodies at one time or another during the two year period of surveillance, a percentage higher than that obtained for either *S. typhi* 'H' or 'O' antibody contents. Of the 106 immunized patients 95 (89·6%) had 'Vi' antibodies, which would seem to indicate the 'Vi' antibodies are developed in the same proportions of typhoid cases irrespective of whether previously immunized or not.

Before accepting this premise at its face value one would require to have further information on its validity. To this end, the results were examined in greater depth as from the figures given above some patients must have had antibodies to the 'Vi' antigen but no antibodies to *S. typhi* 'H' and 'O'. Searching of the records revealed 33 such patients. Of these 33 patients 30 (91%) were confirmed bacteriologically (19 had at least one positive blood culture). Two of the 33 suffered clinical relapse despite which antibodies to the 'H' and 'O' antigens did not develop. Four patients failed to develop 'Vi' antibodies although all 4 were confirmed bacteriologically – 2 by blood culture.

From Table 5, 47 (54·6%) of 86 healthy nurses immunized with alcoholized TAB had 'Vi' antibodies. Four (4·6%) possessed 'Vi' antibodies alone. A further 4 (4·6%) had no demonstrable antibodies to any of the antigens tested. Thus it is possible to demonstrate in healthy immunized individuals a similar lack of antibody responses in the presence of 'Vi' antibodies alone.

From Table 5, however, 16 (64%) of 25 patients whose bloods were submitted for agglutinin content because of pyrexia of unknown origin (considered to have been inoculated with TAB vaccine because of the presence of antibodies against

paratyphi A 'H') contained 'Vi' antibodies and all reacted with at least 2 antigens.

Again from Table 5, of the 140 patients not considered to have been inoculated because of the absence of antibodies to *S. paratyphi A* 'H', completely negative results were obtained in 91 (65%), and 27 (19·3%) contained 'Vi' antibodies alone. This percentage of 19·3% in this group is noteworthy since it applies to 140 individuals who were not suffering from typhoid or paratyphoid fever.

DISCUSSION

S. typhi *'H' and 'O' antibodies*

The outstanding differences between the conclusions of Huckstep (1962) and those to be drawn from the typhoid outbreak in Aberdeen concern the findings regarding anti-'H' and anti-'O' agglutinins. Throughout the investigations the *S. typhi* anti-'H' agglutinin titres were infinitely more helpful than the anti-'O'. These findings are a reversal of the opinions of Huckstep. McKendrick (1973) stated that, in the uninoculated and in those who have not previously suffered from typhoid, 'O' agglutinins appear first, that most typhoid patients show a four-fold rise of titre within 4 or 5 days of 'O' agglutinins being detected, that in patients without previous experience of *S. typhi* antigens a titre of 1200 for 'O' agglutinins is highly suggestive of typhoid fever, that it is quite usual for *S. typhi* 'O' titres to reach 1/1000 or more and that in a few patients the titre of 'O' antibodies is slow to rise and in others it may never reach a 'diagnostic' level. Of the 'H' agglutinins, McKendrick states that these appear more slowly but tend to persist for longer than the 'O' agglutinins both after the disease and after inoculation.

The antibody studies of the 1964 typhoid outbreak in Aberdeen do not uphold the claims made for the 'O' agglutinin titres. Almost the reverse was found, namely that the 'H' agglutinin titres were more helpful than the 'O' agglutinin titres in early diagnosis. Thus 37% of non-immunized patients gave the 'All results nil' pattern with regard to *S. typhi* 'O' agglutinins whereas to the 'H' antigen, only 17·9% of patients yielded the same pattern.

In the patients who gave histories of TAB vaccination 45·9% of patients gave 'All results nil' for *S. typhi* 'O' agglutinins but only 4·7% for 'H' agglutinins (Table 2).

The same finding was also found in the examination of sera from 86 immunized nurses. Of these only 1·2% possessed antibodies to the 'O' antigen of *S. typhi* but 89·5% possessed antibodies against the 'H' antigen (Table 5).

As *S. typhi* and *S. paratyphi B* possess the somatic antigen 12 in common, some cross agglutination with the 'O' suspension of *S. paratyphi B* might be expected to occur with sera from typhoid fever patients. However, 71·7% of non-immunized typhoid patients failed to develop agglutinins to *S. paratyphi B* 'O' antigen, as also did 60·4% of immunized patients.

The outstanding feature so far has been the poor agglutinin response to the somatic 'O' antigens. This same feature was noted during a paratyphoid B outbreak in 1941 (Brodie, 1942) in which there were 331 cases bacteriologically con-

Table 6. *'Vi' antibody titres in hospitalized patients, immunized nurses, and the general population*

'Vi' antibody titre	Hospitalized patients*	Immunized nurses†	General population
1/160	1	.	.
1/80	2	.	.
1/40	6	2	4 (2)*
1/20	24	11	10 (5)
1/10		22	17 (10)
1/5		12	21 (10)
<1/5		39	113

* *S. typhi* 'H' and 'O' antibodies absent.
† Immunized with alcoholized TAB.

firmed. In the conclusions drawn from the investigations regarding diagnostic and discharge Widal tests, it was noted that the responses of patients to the somatic ('O') antigen of *S. paratyphi B* were poor and that the demonstration of flagellar ('H') antibody proved a more reliable aid to diagnosis (Davidson & Brodie, 1944). During this outbreak there were also cases confirmed bacteriologically but with negative serological findings.

'Vi' antibodies

Presence in blood

Of the 86 healthy nurses who had received alcoholized TAB vaccine, 47 (54·6%) had developed 'Vi' antibodies; 52 (31·5%) of the non-enteric general practice patients also possessed 'Vi' antibodies (Table 5). However, since 1948 the TAB vaccine issued from this laboratory to general practitioners in the North-East Region of Scotland has been the heat-killed phenolized vaccine, which does not usually provoke the appearance of circulating 'Vi' antibody when given subcutaneously whereas the alcoholized vaccine does (Wilson & Miles, 1964*a*). Twenty-five of these patients showed antibodies to *S. paratyphi A* 'H', conclusive evidence of previous inoculation. Of these 16 (64%) had circulating 'Vi' antibody. Of the remaining 140 patients, if we accept evidence of reaction with at least one of each of the *S. typhi* and *S. paratyphi B* antigen suspensions as indicative of previous inoculation then 9 would appear to have received heat-killed phenolized vaccine and of these 7 to have demonstrable 'Vi' antibodies. It is more difficult to explain the significance of the 'Vi' antibodies in 27 (16·4%) of the 165 patients in the community when all other 'H' and 'O' antibody titres were negative. However, 'Vi' antigens identical with or closely related to that of the typhoid bacillus have been demonstrated in *Citrobacter* spp., *Escherichia* spp. and other micro-organisms (Wilson & Miles, 1964*b*). Some of the coliform bacilli contain more 'Vi' antigen than *the average strain of S. typhi and a specially selected strain of Esch. coli is now used to prepare the 'Vi' antigen suspension. The demonstration of 'Vi' antibody in* a serum is not irrefutable proof of infection with *S. typhi* but only indicates past or present infection with an organism possessed of an identical or closely related 'Vi' antigen.

'Vi' antibody titres

If the presence of 'Vi' antibody is insufficient proof of typhoid infection then perhaps the antibody titre might be a better guide. Table 6 shows the titres of 'Vi' antibodies in 33 hospitalized patients who had no antibodies to *S. typhi* 'H' and 'O' antigens, in 86 healthy nurses immunized with alcoholized TAB and in 165 patients in the general population.

In the hospitalized patients, a titre of 1/160 was found only once, in the admission specimen from a female aged 60. Her subsequent titres fell to 1/40 at the time of discharge, to 1/10 at 3 months and to < 1/5 at 6 months after discharge. Two further cases gave titres of 1/80. In one patient the titres fell subsequently: the other showed the 'Mid range rise' pattern, with the titre falling to the initial level 12 months after discharge. In the healthy immunized nurses and in patients in the general community, none of the titres exceeded 1/40.

In a study of the 'Vi' reaction Forrest, Matthews, Robertson & Hanley (1967) chose a titre of 1/32 as the upper limit of normal for the population of Hong Kong. This level is much higher than the 1/5 cited as the British level (PHLS Report, 1961). In view of the findings in the present study regarding the 'Vi' antibody titres of healthy immunized nurses and patients in general practice, an arbitrary titre of 1/40 was chosen as the point at which some further scrutiny of the positive 'Vi' antibody responses of the typhoid patients should be made.

'Vi' antibody titres < 1/5. Bhatnagar (1944) stated that the presence of 'Vi' agglutinin was invariably associated with the presence of the typhoid bacillus within the body. The data on 42 patients with no demonstrable 'Vi' antibodies (titre < 1/5) from admission until 24 months after discharge were reviewed. Of these, 31 patients had not been immunized. In 30 of these patients the diagnosis of typhoid fever was confirmed bacteriologically (19 by blood or clot culture). Six patients suffered relapses. Four patients (males aged 1½ and 57, females aged 17 and 35) had complete absence of antibodies to *S. typhi* 'H' and 'O' antigens throughout. Of 10 patients exhibiting antibodies to *S. typhi* 'O', 7 failed to develop antibodies until 6 months after discharge. Only 6 of these 30 confirmed cases failed to develop 'H' antibodies. Eleven patients had been immunized. Only 2 were confirmed bacteriologically (1 by clot culture). Four patients were accepted as typhoid infections on clinical or serological grounds. Four were not confirmed clinically, serologically, or bacteriologically. The remaining patient who had recently received TAB inoculation was diagnosed as a TAB reaction. Only one of the 11 showed any demonstrable *S. typhi* 'O' agglutinins which appeared as a 'Mid range rise' but was not accepted clinically on this evidence as a case of typhoid fever.

'Vi' antibody titres > 1/40. Anderson & Richards (1948), reporting on an outbreak of typhoid fever in the Middle East, considered that an interval of 6 weeks from discharge of the average case was not long enough to allow the reactive 'Vi' titres to fall to zero.

Since Forrest *et al.* (1967) suggested a 'Vi' titre of 1/32 as the upper limit of normal for the population of Hong Kong, and in view of the upper limit of 1/40 found

Table 7. *Details of non-immunized patients with 'Vi' agglutinin titres of 1/40 or over, 12 months after discharge from hospital*

Age	Sex	'Vi' agglutinin titres at					Reciprocals of *S. typhi* 'H' agglutinin titres at						*S. typhi* 'O' agglutinin titres at						Specimens positive for *S. typhi*				Basis for diagnosis
		D	3	6	12	24	H	D	3	6	12	24	H	D	3	6	12	24	B.C	C.C.	F.	U.	
10	M	40	10	.	40	40	25	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	Serological
6	F	80	.	.	40	.	200	.	.	.	.	.	.	.	.	.	50	.	1	1	3	.	
9	F	20	.	20	40	10	50	.	.	.	.	.	.	.	.	.	50	.	1	1	.	.	
12	F	40	80	320	640	40	50	100	50	25	.	.	.	.	.	100	.	.	1	1	2	.	
13	F	10	20	160	40	40	400	100	1600	200	200	50	.	.	.	200	400	.	.	1	1	.	
14	F	40	80	.	40	20	25	50	.	.	.	.	.	.	.	.	.	.	.	.	1	.	
15	F	20	.	10	40	.	400	100	.	.	.	.	.	.	.	.	100	.	.	.	2	.	
16	F	80	10	10	40	.	25	.	.	.	.	.	.	.	.	100	400	.	.	.	.	.	Clinical
16	F	40	5	40	40	20	100	50	.	.	.	.	.	.	.	.	50	.	.	1	.	.	
16	F	40	.	20	40	20	400	100	.	.	.	.	.	.	.	400	800	.	.	.	2	.	
18	F	40	.	20	40	.	5120	400	50	.	25	.	.	.	.	.	50	.	2	.	1	.	
18	F	20	.	.	40	20	1600	50	.	.	.	.	.	.	.	.	200	50	1	1	.	.	
20	F	10	.	20	40	20	50	.	.	.	.	.	.	.	.	.	50	.	1	.	.	.	
23	F	80	40	160	40	.	.	50	.	.	.	.	.	400	200	400	200	.	2	.	1	.	
25	F	10	20	20	40	.	100	100	400	100	25	.	.	.	.	25	100	.	.	.	4	1	
26	F	20	40	.	40	.	25	.	.	.	.	.	.	.	.	.	.	.	2	.	1	.	
26	F	20	.	5	40	20	.	800	200	100	200	.	.	.	.	100	200	50	4	1	13	1	Vag./swab+
29	F	20	20	5	40	.	400+	200	25	25	.	.	.	.	.	.	200	.	.	.	2	.	
31	F	40	40	20	40	.	50	50	.	25	.	.	.	50	200	100	400	.	.	1	.	.	
33	F	20	5	10	40	20	6400	800	800	50	25	.	3200	.	.	50	200	.	1	.	3	.	
39	F	10	.	.	40	.	1600	200	.	.	.	.	.	100	.	50	200	.	1	.	.	.	
47	F	10	20	40	40	.	400+	50	.	.	.	.	.	.	.	100	100	.	.	.	.	.	Clinical
47	F	20	20	40	40	20	25	50	.	.	.	.	.	.	.	.	.	.	.	.	1	.	
49	F	10	.	10	40	10	25	.	.	.	.	.	.	.	.	.	25	.	.	.	1	.	
50	F	20	.	.	40	20	100	50	25	.	.	.	.	.	.	.	200	.	1	.	2	.	
51	F	10	10	40	40	20	400+	3200	12800	800	1600	100	.	.	.	.	.	.	.	.	2	.	
54	F	20	.	.	40	40	25	100	.	25	.	.	.	.	.	400	200	.	1	1	.	.	
55	F	80	.	40	80	20	100	200	400	.	.	.	25	.	.	.	.	.	1	.	2	1	
55	F	10	.	.	80	20	50	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	
55	F	10	.	.	40	10	200	25	25	.	.	.	.	.	.	.	.	.	2	.	.	.	
57	F	.	.	40	40	10	.	.	.	.	.	.	.	.	.	.	25	.	.	1	.	.	
58	F	40	10	.	40	.	25	25	.	25	.	25	.	.	.	25	50	.	.	.	.	.	Serological
59	F	20	20	10	40	.	25	.	.	.	.	.	.	.	.	.	50	.	.	.	1	.	
62	F	20	10	40	40	10	.	50	50	100	100	50	.	.	.	.	.	.	.	.	2	.	
63	F	160	.	80	20	.	100	800	400	50	25	.	.	.	.	50	50	.	1	1	1	1	
69	F	20	5	20	40	.	400+	1600	400	200	100	.	.	.	.	.	100	.	1	.	7	.	
70	F	80	10	80	80	40	12800	800	400	100	100	50	100	.	.	100	200	.	1	.	2	.	
70	F	20	40	20	40	20	50	200	50	.	.	.	.	.	.	.	.	.	1	.	4	1	
72	F	10	10	.	40	.	16000	200	50	.	.	.	25	50	.	.	100	.	.	.	3	.	
74	F	80	40	80	40	.	25	.	.	.	.	.	.	.	.	.	.	.	2	1	1	.	
18	M	20	.	.	40	20	50	25	.	.	.	.	.	.	.	100	200	.	1	1	.	.	
24	M	20	.	5	40	.	25	.	.	.	.	.	.	.	.	.	100	.	.	.	.	.	Clinical
26	M	20	.	5	40	.	25	.	.	.	.	.	.	100	.	100	200	.	1	1	3	.	
29	M	40	20	5	80	.	50	.	.	.	.	.	.	.	.	.	.	.	2	.	2	.	
39	M	20	10	40	40	.	50	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	
49	M	20	5	20	40	.	100	25	.	.	.	.	.	.	.	.	50	.	.	.	1	.	
62	M	20	.	10	40	.	500	400	50	.	.	.	.	.	.	.	.	.	2	.	4	.	

Table 8. *Details of immunized patients with 'Vi' agglutinin titres of 1/40 or over, 12 months after discharge from hospital*

Date of TAB	Age	Sex	'Vi' agglutinin titres at					Reciprocals of *S. typhi* 'H' titres at						*S. typhi* 'O' titres at						Specimens positive for *S. typhi*			
			D	3	6	12	24	H	D	3	6	12	24	H	D	3	6	12	24	B.C.	C.C.	F.	U.
1947–9	18	M	80	.	40	40	10	200	100	.	.	.	.	.	.	.	50	200	.	.	.	1	.
1952	32	M	.	320	640	320	80	100	100	50	25	25	.	.	.	.	.	200	.	.	1	4	.
1947–8*	34	M	20	20	10	40	.	25	25	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1942*	40	M	160	20	80	80	.	400+	400	800	400	1600	.	.	.	.	.	50	.	.	.	.	.
1941–3	43	M	10	160	160	40	20	25	600	800	400	400	100	.	.	.	50	.	.	2	1	1	.
1940–2*	46	M	40	5	20	40	10	200	100	100	200	400	100	.	.	.	50	200	.	.	.	.	.
1940–1	49	M	20	10	5	40	.	200	50	25	.	.	.	.	.	.	.	.	.	.	1	.	.
1943	57	M	160	160	40	40	.	800	400	100	50	25	.	.	50	25	.	.	.	1	1	8	2
1915–18*	65	M	80	20	40	320	20	400	1600	1600	400	1600	800	.	.	.	25	.	.	.	.	.	.
1917	67	M	20	20	5	40	20	500	400	400	100	800	400	.	.	.	50	50	.	1	.	.	.
1960	26	F	40	5	40	40	.	.	50	25	.	.	.	.	.	25	50	100	.	.	.	1	.
1954	45	F	40	20	40	40	20	100	25	.	.	.	.	.	.	.	.	.	.	.	1	.	.

* Diagnosis on clinical grounds

Table 9. *Details of patients who became carriers*

TAB	Age	Sex	'Vi' agglutinin titres at					Reciprocals of *S. typhi* 'H' titres at						*S. typhi* 'O' titres at						Specimens positive for *S. typhi*				
			D	3	6	12	24	H	D	3	6	12	24	H	D	3	6	12	24	B.C.	C.C.	F.	U.	
No	34	F	20	10	10	20	5	100	.	1600	3200	800	100	.	.	.	.	.	.	1	.	41	12	*
No	46	F	.	5	10	80	.	400	200	400	100	100	.	.	.	.	.	50	.	.	.	34	.	†
No	62	F	80	80	320	640	.	100	400	50	200	200	200	.	.	.	.	.	.	1	1	60	4	†
No	67	F	10	20	40	40	.	.	200	800	400	1600	50	.	.	.	.	100	.	2	.	85	3	†
1941–3	54	M	80	10	.	20	20	1600	6400	3200	3200	1600	400	.	.	50	.	.	.	1	.	93	1	‡
1914–16	70	M	40	10	10	5	5	50	800	200	100	100	100	.	.	.	.	25	50	.	.	95	.	§

* Cleared May 1965. † Still excreting 1973. ‡ Cleared with Septrin. § Died July 1966.

in this investigation amongst immunized nurses and general practice patients, it was decided to review the records of patients whose 'Vi' titre 12 months after discharge was 1/40 or over, and where available, the 'Vi' titre of these patients 24 months after discharge.

Table 7 presents the details of investigation on 47 non-immunized cases, of whom 42 were confirmed bacteriologically, 29 by clot, or blood culture. Three cases were diagnosed on serological, 2 on clinical grounds. At 12 months after discharge from hospital 5 patients had 'Vi' titres > 1/40, 4 of which however, had fallen to a lower level 24 months after discharge. Table 8 presents the results of investigation of 12 immunized patients, of whom 8 were confirmed bacteriologically (6 by clot or blood culture). Six cases were diagnosed on clinical grounds. Three of the 12 had titres greater than 1/40 at 12 months but by 24 months 2 had fallen below this level.

These tables also demonstrate the poor 'O' agglutinin responses in both immunized and non-immunized patients and does little to support the general opinion that rising titres of 'O' agglutinins are more helpful in diagnosis than are the 'H' agglutinins.

The findings with regard to the titres of 'Vi' agglutinins extend the observation of Anderson & Richards (1948) in that out of 303 non-immunized patients followed up after discharge, 196 (64·6%) still had demonstrable 'Vi' agglutinins present 12–24 months after discharge.

Carriers resulting from the outbreak

The poor response to the 'O' antigen of *S. typhi* already mentioned is best displayed in Table 9, which gives details of the 6 carriers, 4 females, 2 males, resulting from the outbreak. All the carriers had *S. typhi* 'H' and 'Vi' agglutinins.

No 'O' agglutinins were ever demonstrated in two females. The first of these, aged 34, had 'H' agglutinin titres in the 'Mid range rise' pattern. Her 'Vi' titres did not exceed 1/20, falling to 1/5 2 years after discharge. *S. typhi* was isolated from a blood culture, from 41 specimens of faeces and 12 of urine before she ceased to excrete spontaneously in May 1965.

The second, aged 62, had high titres of 'Vi' agglutinin which would have fallen into the 'Rising titre' pattern except for a negative result 2 years after discharge. Her 'H' agglutinin titres never exceeded 1/400. *S. typhi* was isolated from one blood culture, one clot culture, 60 specimens of faeces and 4 of urine. She continues to excrete.

Two female carriers showed the presence of 'O' agglutinins. The younger, aged 46, showed a single titre of 1/50 when examined 12 months after discharge. Her Vi agglutinins had risen to 1/80 at this time, but no specimens were available at 24 months after discharge. This patient became an intermittent excretor, having positive stools. She still remains an intermittent excretor.

The elder, aged 67, had the highest 'O' agglutinin titre of all the carriers – a single titre of 1/100 at 12 months after discharge. Her titres of 'Vi' agglutinin never exceeded 1/40. *S. typhi* was isolated from 2 blood cultures, 85 specimens of faeces, and 3 of urine. She still continues to excrete.

The elder male carrier aged 70 developed 'O' agglutinin titres of 1/25 12 months after discharge which rose to 1/50 at 24 months. His 'Vi' titre of 1/40 on discharge from hospital had fallen to 1/5 at 12 months after discharge and remained at this level 24 months after discharge. *S. typhi* was isolated from 95 specimens of faeces before his death from other causes in July 1966.

The younger male carrier, aged 54, had a single 'O' agglutinin titre of 1/50, 3 months after discharge. His titre of 'Vi' agglutinins at discharge from hospital was 1/80, but fell to 1/10 at 3 months and became negative at 6 months. 'Vi' agglutinins, however, at a titre of 1/20 were present in specimens at 12 and 24 months after discharge. *S. typhi* was isolated from a blood culture, from 93 specimens of faeces and one of urine before his carrier state ended after a course of co-trimoxazole in 1970 (Brodie, McQueen & Livingstone, 1970).

The conclusions drawn from findings in the investigation described agree with those of Anderson & Richards (1948) that a high 'Vi' titre was not invariably associated with a temporary carrier state and that high titres were occasionally found in convalescence in the absence of a demonstrable carrier state. These conclusions, however, differ from those of Bhatnagar (1944) who considered that the presence of 'Vi' agglutinins was invariably associated with the presence of *S. typhi* within the body.

Anderson & Richards (1948) were of the opinion that it seemed unwise to lay down such a hard and fast rule, as it had been established that a small percentage of persons in whom there is no evidence to suggest typhoid infection or the carrier state may have low titres of 'Vi' agglutinins in their serum possibly because of the presence in their intestine of 'Vi'-containing strains of *Esch. coli*. The findings in patients in the general community with no evidence of enteric infection have already been described (Table 5). Of 129 patients in the general community with no serological evidence of TAB inoculation, 27 (20·9%) possessed 'Vi' antibodies alone. 'Vi' antibodies were, however, present in 25 out of 37 (67·6%) patients showing serological evidence of inoculation with TAB and 47 out of 86 (54·6%) healthy immunized nurses also possessed 'Vi' antibodies.

The titre of 'Vi' antibodies in the sera of these immunized nurses and patients did not exceed 1/40. None of the chronic carriers arising from the outbreak, however, showed a 'Vi' titre exceeding 1/40 24 months after discharge. Even at 12 months after discharge only 2 showed titres greater than 1/40.

Apart from whatever value 'Vi' agglutinins may have in the diagnosis of typhoid fever, the screening of the sera of healthy individuals for the presence of 'Vi' agglutinins is still considered to be a valuable test for the detection of typhoid carriers, as it has been found that the frequency of a positive 'Vi' reaction in chronic carriers is remarkably high (Felix, Krikorian & Reitler, 1935; Pijper & Crocker, 1943; Felix, 1938; Bhatnagar, 1938; Almon, 1943). Other workers in addition have confirmed these findings as also do the findings regarding the patients who became chronic carriers in this outbreak (Table 9).

Less favourable results were reported, however, from the Sudan by Horgan & Drysdale (1940) and from Southern Rhodesia by Davis (1940). In addition to confirming the absence of 'Vi' agglutinins from chronic carriers, these workers

demonstrated raised 'Vi' titres in subjects from whom *S. typhi* could not be isolated.

This latter finding has been confirmed in this investigation by the demonstration of 'Vi' antibodies alone in the sera of 20·9% of 127 patients from the general community with no serological evidence of TAB inoculation (Table 5).

My own previous unpublished experience of the 'Vi' antibody test as a means of detecting chronic typhoid carriers was gained from attempts to detect a carrier in a large mental institution. All patients and staff were screened for 'Vi' antibody. Those with a positive reaction were investigated bacteriologically but all specimens proved negative. The investigation was extended and all 'Vi' negative patients and staff had serial faeces and urines examined. The carrier was found to be a 'Vi' negative female patient. With her removal to a special hospital, no further sporadic cases of typhoid fever occurred. This search for a carrier was made under ideal conditions in that the individuals examined were members of what was almost a closed community in which the necessary samples for examination could be obtained as required. If the test fails under such conditions one wonders just how efficient it is as a screen test when applied in the general community.

Antibiotics and antibody production

Chloramphenicol and ampicillin were the antibiotics mainly used during the outbreak (Walker, 1965; Brodie, 1977). Good & Mackenzie (1950) had found that there was little, if any, interference with antibody formation when chloramphenicol was given. Robertson & Wahab (1970) exploring the truth of the popular theory that chloramphenicol inhibits the development of antibody response in enteric fever, treated alternate cases with chloramphenicol and ampicillin. From their results, the authors concluded that there was no evidence that chloramphenicol inhibited antibody formation or if it did then ampicillin had the same effect.

If either or both of these antibiotics did exert any inhibiting influence on antibody formation in the Aberdeen outbreak, the antibody most inhibited would appear to be that reacting with *S. typhi* 'O' suspension. Against this possibility, however, may be set the findings of Davidson & Brodie (1944) that, in the absence of antibiotic therapy, during an outbreak of paratyphoid B fever in 1941 with over 300 cases the 'O' agglutinin responses were poor or even absent.

I am grateful to the Secretary of State for Scotland for the research grant which supported the investigations which followed the Aberdeen typhoid outbreak of 1964. My thanks are also 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 investigation.

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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