AGGLUTINATION TESTS IN THE DIAGNOSIS OF ENTERIC FEVER IN THE INOCULATED

By J. F. WILSON

Although a clinical diagnosis can be made in most cases of enteric fever, the disease in inoculated individuals may be so mild and its course so short that it becomes difficult to distinguish clinically. Therefore in suspected cases in inoculated people laboratory tests may require to be started earlier and repeated more often than in uninoculated individuals.

The isolation of the causal organism remains the best method for laboratory diagnosis. Agglutination tests can be no more than highly suggestive of a fever of the enteric group. Although they are of great assistance in the uninoculated case, with recently inoculated patients their value is more doubtful (Topley & Wilson, 1936; Bhatnagar, Speechly & Singh, 1938; Bhatnagar, 1938; Boyd, 1939).

The interpretation of the original Widal test was greatly complicated by the introduction of inoculation. It became usual to repeat the test every 4 days, since enteric fever was believed to produce a steadily rising titre which was more significant than the actual level of the agglutinins (Dreyer, Walker & Gibson, 1915; Dreyer & Walker, 1916; Dreyer & Inman, 1917). Then Felix (1924) distinguished between the flagellar or H agglutinins and the somatic or O agglutinins. He emphasized the diagnostic value of the level of the O agglutinins, especially in the inoculated. While the H agglutinins give satisfactory results in the uninoculated, they do not have the same significance in the inoculated. H agglutinins are usually present in the sera of inoculated patients, and any fever may produce in them an anamnestic rise which cannot be distinguished from that in enteric fever (Dulaney & Wikle, 1933; Giglioli, 1933; Damon, 1937; Boyd, 1939; Hac, Flynn & Perry, 1939). Therefore in a population with a large preponderance of inoculated individuals the estimation of H agglutinins is of little help in the diagnosis of enteric from other fevers.

As noted above, Felix emphasized the value of the O agglutinins. These are not specific and can only indicate whether an infection is of the enteric group or not. In enteric fever the O agglutinin response appears to be erratic: in some cases these agglutinins have not been found, and in others they have not developed until convalescence. When they are present, a fluctuation rather than a steady rise appears to occur (Manson-Bahr, 1940). The actual titre reached depends (*a*) upon the technique used for the estimation, and (*b*) upon the effect of any previous inoculation, that is, upon the type and dose of vaccine, the individual variation in the production of antibodies, and the lapse of time since the vaccine was given (Perry, 1918; Felix, 1941). Inoculation may not produce agglutinins. If they are produced, titres of 1 : 160 for the O agglutinins of *Bact. typhosum* appear to be rare more than 6 months after inoculation (Hac *et al.* 1939); within 6 months the upper limit is about 1 : 320; and within 1 month 1 : 640 (Beattie & Elliot, 1937). Non-specific increases to 1 : 640 have been reported in various diseases (Bensted, 1940). The O agglutinins reacting with *Bact. paratyphosum* A are found less frequently and in smaller amounts.

The Vi agglutinins remain to be considered. In typhoid fever these appear about the same time as the H and O agglutinins. Bhatnagar (1938) considered that by estimating the Vi agglutinins in an inoculated subject suspected of having enteric fever, a definite conclusion as to the diagnosis could be reached by the end of the first week. But the production of this antibody in amounts that can be titrated seems to be irregular and transitory. In some cases examined regularly throughout the disease Vi agglutinins were not demonstrated (Felix, Krikorian & Reitler, 1935; Almon, Read & Stoval, 1937; Pijper & Crocker, 1939; Almon & Stoval, 1940; Bensted, 1940; Eliot, 1940). Further, although the Vi agglutinins were at first considered specific for typhoid fever (Bhatnagar, 1938), they have been found in fevers that were not of the enteric group (Seshadrinathan & Pai, 1940; Bensted, 1940), and appear in normal subjects after inoculation with the newer vaccines (Felix, Rainsford & Stokes, 1941). After inoculation with the army phenolized vaccine a titre of 1 : 25 is rarely reached (Hamilton, 1939), though titres of 1 : 160 have been described (Bensted, 1940). The level of the Vi agglutinins has also been used in the detection of chronic carriers; but doubt was cast on their value when people who were not carriers were found to have Vi agglutinins, and carriers were described in whose sera Vi agglutinins could not be demonstrated (Bensted, 1937; Almon *et al.* 1937; Felix, 1938; Almon &

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Stoval, 1940; Eliot, 1940; Horgan & Drysdale, 1940; Scott, 1943).

From the foregoing it appears that O and Vi agglutinins may not be produced in proven cases of enteric fever; that both agglutinins may occur in comparatively high titres in cases that are not enteric fever; and that no definite level for either has been established as of diagnostic significance in suspected cases of enteric. When a diagnosis has to be made in a recently inoculated individual, their significance is even more doubtful.

The work of which the present paper is a report represents an attempt to establish their value more exactly.

MATERIAL AND METHODS

The work was done in the closed community of an Indian frontier post where movement beyond the perimeter wall was confined to organized columns. Over 4000 sera from 2707 individuals were examined. The material falls into the following groups:

A. 2170 sera from (1) 1973 normal inoculated soldiers of many races, (2) 127 normal uninoculated Indian males.

B. 1689 sera from 557 inoculated and eighteen uninoculated patients suffering from febrile diseases other than enteric fever.

C. 140 sera from thirty-two cases of enteric fever in recently inoculated individuals.

The agglutination tests were carried out in Felix's tubes. Doubling dilutions of serum were made in a total volume of 0·75 c.c. Standard concentrated suspensions of *Bact. typhosum* 901 O and Vi 1 (Bhatnagar), and a standard concentrated suspension of *Bact. paratyphosum* AO were obtained from the Enteric Laboratory, Kasauli, which supplied laboratories throughout India. One drop of the concentrated suspensions from a Dreyer's pipette was added to a tube. The tubes were incubated for 2 hr. at 37° C. and read after a further 20–22 hr. at room temperature (Bensted, 1940). The end-point for both O and Vi agglutination was taken as the tube next above that in which there was complete dispersal of the deposit and complete clearing of the supernatant fluid.

The Army phenolized vaccine containing 1000×10^6 *Bact. typhosum* and 750×10^6 *Bact. paratyphosum* A and B was used for all inoculations. 0·5 c.c. was given yearly following an initial inoculation of 0·5 and 1 c.c. at 10 days' interval.

A. *Agglutinins in normal inoculated soldiers*

The O and Vi agglutinins found in the sera of normal inoculated soldiers are shown in Table 1. The percentages of sera with *Bact. typhosum* O agglutinins at titres above 1 : 40 decreased as the time from the last inoculation increased. Thus five of the ten sera with titres of over 1 : 160 were from the 335 sera taken from men tested within 1 month of inoculation, and the other five were from among the 1638 men inoculated for a longer period. The sera with Vi agglutinin titres above 1 : 10 showed a similar decrease in numbers as the time from the last inoculation increased. The χ^2-test indicated that these decreases were statistically significant. Histories taken from the men with high TO or Vi titres produced no evidence of previous attacks of enteric fever. No pathogenic organisms were isolated from 265 stool and urine cultures made from nineteen of these men (Table 7). No high titres for the *Bact. paratyphosum* AO agglutinins were found, and the time from the last inoculation had no significant effect on these agglutinins.

Previous inoculations caused no increase in the numbers of sera with AO or Vi agglutinins, nor in the numbers with TO agglutinins when inoculation had been carried out more than 6 months previously. Within 6 months of inoculation TO titres over 1 : 40 were found in 4·7 % of 149 sera after a first inoculation and in 9·7 % of 869 sera after a second or subsequent inoculation. The difference was 5 ± 2 %.

B. *Agglutinins in diseases other than enteric fever*

1689 sera from 575 patients of many races suffering from diseases other than enteric fever were examined usually on the third, seventh and eleventh days of disease. The results from the different diseases, which included dysentery, malaria, mumps, respiratory tract infections, sandfly fever and tuberculosis, were similar and were considered together (Table 2). At the lower titres the percentages of sera with TO or AO agglutinins showed no significant change as the time from the last inoculation increased. The sixteen sera with TO titres over 1 : 160 were from nine cases only four of which had more than one serum with such a high titre. All these cases had been inoculated within 9 months and had received more than one inoculation. Six sera from three cases had AO titres of 1 : 160. All three had been inoculated within 6 months and had received more than one inoculation. The history, clinical examination and blood, stool and urine cultures gave no evidence of enteric fever or of the carrier state in any of these twelve cases (Table 7).

Within 6 months of inoculation fewer sera with TO titres over 1 : 80 or AO titres over 1 : 20 were found after a first inoculation than after subsequent inoculations. More than 6 months after inoculation there was no difference between the two groups.

There was no significant change in the number of sera with Vi agglutinins as the time from the

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Table 1. *Agglutinins in normal inoculated soldiers*

Time from last inoculation	No. of sera	Percentage of sera with titres of 1 : 10	1 : 20	1 : 40	1 : 80	1 : 160	1 : 320	1 : 640	1 : 1280
		Bact. typhosum O agglutinins							
1–6 weeks	386	—	—	10·1	10·1	2·1	1·3	0	0
2–6 months	749	—	—	13·8	5·5	1·6	0·2	0	0
7–12 months	684	—	—	9·8	5·6	0·7	0·3	0	0
Over 12 months	154	—	—	11·7	3·9	0·6	0	0	0·6
Total	1973	—	—	11·4	6·2	1·3	0·5	0	0·05
Uninoculated	127	—	—	10·2	7·9	0	0·9	0	0
		Bact. paratyphosum AO agglutinins							
1–6 weeks	386	—	—	1·3	0·5	0	0	0	0
2–6 months	749	—	—	1·7	0·1	0	0	0	0
7–12 months	684	—	—	1·5	0·3	0	0	0	0
Over 12 months	154	—	—	1·3	1·3	0	0	0	0
Total	1973	—	—	1·4	0·4	0	0	0	0
Uninoculated	127	—	—	3·9	0	0	0	0	0
		Bact. typhosum Vi agglutinins							
1–6 weeks	386	3·1	1·3	1·0	0·3	0·5	0	0	0
2–6 months	749	2·9	0·5	0·1	0	0	0	0	0
7–12 months	684	2·4	0·1	0	0	0	0	0	0
Over 12 months	154	2·6	0	0	0	0	0·6	0	0
Total	1973	2·8	0·5	0·3	0·05	0·1	0·05	0	0
Uninoculated	127	0	0	0	0	0	0	0	0

Table 2. *Agglutinins in diseases other than enteric fever*

Time from last inoculation	No. of sera	Percentage of sera with titres of 1 : 10	1 : 20	1 : 40	1 : 80	1 : 160	1 : 320	1 : 640	1 : 1280	1 : 2560
		Bact. typhosum O agglutinins								
1–6 weeks	206	—	—	9·7	6·3	4·4	1·0	0·5	1·0	0
2–6 months	706	—	—	9·5	8·5	3·1	0·4	0·1	0·1	0·1
7–12 months	539	—	—	11·2	6·4	3·9	0·6	0·4	0	0
Over 12 months	176	—	—	7·9	3·9	4·0	0	0	0	0
Total	1627	—	—	10·5	7·0	3·6	0·5	0·2	0·2	0·1
Uninoculated	53	—	—	0	1·9	0	0	0	0	0
		Bact. paratyphosum AO agglutinins								
1–6 weeks	206	—	—	4·8	0·5	1·5	0	0	0	0
2–6 months	706	—	—	3·7	1·4	0·4	0	0	0	0
7–12 months	539	—	—	3·5	0·6	0	0	0	0	0
Over 12 months	176	—	—	3·9	1·2	0	0	0	0	0
Total	1627	—	—	3·9	0·9	0·4	0	0	0	0
Uninoculated	53	—	—	0	0	0	0	0	0	0
		Bact. typhosum Vi agglutinins								
1–6 weeks	203	3·0	1·0	1·5	0·5	0	0	0	0	0
2–6 months	703	3·3	1·3	0·4	0	0·3	0·3	0	0	0
7–12 months	534	2·8	1·1	0·2	0·2	0·2	0	0	0	0
Over 12 months	172	5·2	0·6	0	0	0	0	0	0	0
Total	1612	3·3	1·1	0·4	0·1	0·2	0·1	0	0	0
Uninoculated	56	8·9	1·8	0	0	0	0	0	0	0

Note. Cases for which the exact date of inoculation was not known were omitted.

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last inoculation increased. Thirty-two sera from twenty-five inoculated cases had agglutinins at titres of 1 : 20 or over. These were found from 3 to 12 months after inoculation and after first and subsequent inoculations. When thirty-one blood, 151 faeces and 139 urine cultures were made from these twenty-five cases, no evidence of enteric fever or of the carrier state was obtained (Table 7) and the history gave no evidence of previous attacks of enteric fever.

The percentage of sera with Vi titres of 1 : 10 and over found after first inoculation showed no significant difference from that after subsequent inoculations.

C. *Inoculated cases of enteric fever*

Eighteen cases of typhoid fever, nine of paratyphoid fever and five fevers of the enteric group occurred within 18 months of inoculation. Fourteen were typical cases and could be diagnosed clinically. The help of the laboratory was required to distinguish the remaining cases from more common fevers early in the disease. The mildest cases suffered only from headache and fever for a few days, and six were discharged from hospital incorrectly diagnosed before the results of the laboratory tests were known.

Twenty-three cases had positive blood cultures, twelve positive stool or urine cultures and from five no organism was isolated. One of these five was diagnosed from the agglutination tests alone, one from the clinical condition and three from these two factors considered together. Laboratory tests were started on twenty-five cases during the first week of the disease, and nineteen gave positive blood cultures on the average about the fifth day. Eight of these cultures, in broth containing 1 : 160,000 gentian violet, were incubated for more than 5 days before a growth appeared. Cultures became positive most slowly in patients inoculated within 6 weeks of the onset of the disease.

High titres were present during the disease irrespective of the time since the last inoculation (Table 4) and after both first and subsequent inoculations. Seven of these high TO titres, five high AO titres and six high Vi titres were present by the tenth day of disease. One case of paratyphoid (B) fever had a titre of 1 : 640 of BO agglutinins on the eighteenth day of disease. The AO titres were relatively higher than the TO titres in three cases of typhoid fever, and the TO titres were relatively higher than the AO titres in one case of paratyphoid (A) fever.

Although a comparison had to be made with caution due to the small number of enteric fever cases, high O and Vi titres were found in a greater proportion of cases with enteric fever than with other diseases (Table 5).

Fluctuations in the TO, AO *and* Vi *agglutinins in inoculated patients with enteric fever and other diseases*

Cases with agglutinin titres showing a steady progressive change were placed in the 'Rising' or 'Falling' group. When the titres did not vary or varied by one dilution only, the cases were placed in the 'Stationary' group. Cases showing an irregular change in titre greater than one dilution were included in the 'Fluctuating' group.

Table 3. *Agglutinins in inoculated cases of enteric fever*

Agglutinins	No. of sera	Percentage of sera with titres of 1 : 10	1 : 20	1 : 40	1 : 80	1 : 160	1 : 320	1 : 640	1 : 1280	1 : 2560	1 : 5120
TO	140	—	—	—	11·4	17·8	9·3	6·4	2·9	0·7	0·7
AO	140	—	—	—	9·3	7·9	4·3	1·5	1·5	0·7	0
Vi	135	1·5	10·4	3·7	3·7	3·0	0	0	0	0	0

Only five of the twenty-seven patients with enteric fever, on whom three or more agglutination tests were carried out, had steadily rising titres for the TO agglutinins, an indefinite fluctuation was found in thirteen and there was no change in five. No TO agglutinins above a titre of 1 : 20 were found in the remaining four cases. The AO and Vi agglu-

Table 4. *Enteric fever cases with high agglutinin titres*

Time from last inoculation	No. of cases	Cases with titres above TO 1 : 160	AO 1 : 80	Vi 1 : 10
1–6 weeks	8	5	2	2
2–6 months	8	2	0	2
7–12 months	10	2	2	6
Over 12 months	5	2	3	1

Table 5. *Inoculated cases of enteric fever and other diseases with high agglutinin titres during the first 2 weeks of disease*

Group	No. of cases	Cases with titres above TO 1 : 160	AO 1 : 80	Vi 1 : 10
Enteric	29	8	6	6
Others	468	8	3	25

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tinins behaved in a similar manner, except that cases with rising titres were fewer, two with AO agglutinins and one with Vi agglutinins. When only those cases from which three sera were examined during the first 2 weeks of disease were included, the results were very similar. Thus, enteric fever in previously inoculated patients produced a fluctuating titre more often than a rising titre.

The changes in the titres during the first 2 weeks of enteric fever were compared with those found during the first 2 weeks of other diseases (Table 6). Both rising and fluctuating titres were found in diseases that were not enteric fever. The TO agglutinins had a rising titre in single cases of bacillary dysentery, tuberculosis, pharyngitis and sandfly fever all of which had been inoculated within 6 months (three within 2 months) of examination and had received more than one inoculation. One case of sandfly fever, inoculated 8 months previously, had a rising AO titre. The Vi agglutinins had a rising titre in two cases of sandfly fever and one of mumps, all of which had been inoculated within 2 months of examination and had received more than one inoculation. Therefore rising titres were usually found in diseases other than enteric fever when inoculation had been recent. It appeared also that rising and fluctuating titres were more often present in enteric fever than in other diseases, but the number of cases of enteric fever was too small for a definite conclusion to be drawn.

Table 6. *Fluctuations in the titres of the agglutinins during the first 2 weeks of disease in enteric fever and the group of other diseases*

	No. of cases											
	Total		Rising		Falling		Stationary		Fluctuating		No agglutinins	
Agglutinins	Ent.	Other	Ent.	Other	Ent.	Other	Ent.	Other	Ent.	Other	Ent.	Other
TO	13	435	3	4	0	18	2	33	6	118	2	262
AO	13	435	1	1	0	6	2	8	4	37	6	383
Vi	12	435	1	3	0	3	0	1	5	52	6	376

Table 7. *Faeces and urine cultures made to exclude carriers in the normal inoculated group and group of diseases other than enteric fever*

	Cases with TO titres above	Cultures		Cases with AO titres above	Cultures		Cases with Vi titres above	Cultures	
Group	1 : 160	Faeces	Urine	1 : 80	Faeces	Urine	1 : 10	Faeces	Urine
Normal	8	22	18	0	0	0	12	132	129
Other disease	7	63	58	3	21	21	28	204	179
Total	15	85	76	3	21	21	40	336	308

Carriers and the O *and* Vi *agglutinins*

One chronic carrier of *Bact. paratyphosum* A was found whose sera reacted at a titre of 1 : 10 with the Vi antigen but did not react at a 1 : 20 dilution with the TO and AO antigens.

847 stool and urine cultures were made from fifty-eight individuals who had high titres for the O or Vi agglutinins without any carriers being found (Table 7).

DISCUSSION

The number of normal soldiers with O and Vi agglutinins after inoculation decreased as the time from the last inoculation increased (Table 1). This was not found at the lower ranges of titres in recently inoculated patients with diseases other than enteric fever (Table 2). Therefore febrile diseases other than enteric fever increased the time for which inoculated people would retain O and Vi agglutinins. But this referred only to the lower range of titres which would be little used in diagnosis. At the higher titres the difference was too small to be statistically significant for the size of the material examined (Dalberg, 1940).

With inoculated patients a significantly greater number of TO titres above 1 : 160 were found in cases of enteric fever than in other diseases. At a titre of 1 : 160 the difference between the two groups was not so great, but this level was reached by only 1·8 % of the normal sera and 3·9 % of sera from patients with diseases that were not enteric fever. This appeared to be the limit below which *Bact. typhosum* O agglutinins would have little significance in diagnosis. For the AO agglutinins the level appeared to be slightly lower at a titre of 1 : 80.

In the enteric, other disease and normal groups there was no significant difference in the percentages of inoculated cases with Vi titres over 1 : 10. But the figures for the enteric group included the cases of paratyphoid fever, only one of which

had Vi agglutinins at this level. Five of the eighteen cases of typhoid fever had Vi agglutinins at titres over 1 : 10 during the first 2 weeks of the disease. Only about 2% of sera in the normal and disease groups had agglutinins at this level, but when sera with a titre of 1 : 10 were included the percentage was between 5 and 6%. Therefore a titre of 1 : 10 appeared to be the level above which these agglutinins might be used in diagnosis.

These results confirmed that no titre for the TO, AO or Vi agglutinins nor any particular variation in the titres during the disease could be taken as absolutely diagnostic for enteric fever. But in previously inoculated patients titres at the levels suggested and rising or fluctuating titres appeared to be found more often in enteric fever than in other diseases. The tests were least specific when they were made within 6 months of the last inoculation, especially if previous inoculations had been given. When these findings were applied to the present series of thirty-two cases, the agglutination tests suggested a diagnosis of enteric fever in sixteen cases, while the blood cultures gave a definite diagnosis in twenty-two cases.

SUMMARY

1. Early blood culture is the best method of laboratory diagnosis in the inoculated case of enteric fever, but agglutination tests may give useful information.

2. In inoculated cases of enteric fever O and Vi agglutinins may be absent or present only at low titres. Steadily rising titres are not the rule and the titres more often fluctuate.

3. Complete agglutination at titres over 1 : 80 for TO, over 1 : 40 for AO, or over 1 : 10 for Vi is suggestive but not diagnostic of an active infection.

4. Non-specific stimulation of the O and Vi agglutinins in the inoculated affects mainly the lower range of titres which are probably of little diagnostic significance.

5. Previous inoculations increase the proportion of people with residual O agglutinins within 6 months of the last inoculation but do not affect the Vi agglutinins.

6. Vi agglutinins may be present in normal inoculated persons who are not carriers.

REFERENCES

ALMON, L., READ, J. & STOVAL, W. D. (1937). *Amer. J. Publ. Hlth*, **27**, 357.
ALMON, L. & STOVAL, W. D. (1940). *J. Lab. Clin. Med.* **25**, 844.
BEATTIE, C. P. & ELLIOT, J. S. (1937). *J. Hyg., Camb.*, **37**, 36.
BENSTED, H. J. (1937). *J. R. Army Med. Cps*, **68**, 1.
BENSTED, H. J. (1940). *J. R. Army Med. Cps*, **74**, 19.
BHATNAGAR, S. S. (1938). *Brit. Med. J.* **2**, 1195.
BHATNAGAR, S. S., SPEECHLY, C. G. J. & SINGH, M. (1938). *J. Hyg., Camb.*, **38**, 663.
BOYD, J. S. K. (1939). *Brit. Med. J.* **2**, 902.
DAHLBERG, G. (1940). *Statistical Methods for Medical and Biological Students*. London.
DAMON, S. R. (1937). *Amer. J. Hyg.* **26**, 40.
DREYER, G. & INMAN, A. C. (1917). *Lancet*, **1**, 365.
DREYER, G. & WALKER, E. W. A. (1916). *Lancet*, **2**, 419.
DREYER, G., WALKER, E. W. A. & GIBSON, A. G. (1915). *Lancet*, **1**, 324.
DULANEY, A. D. & WIKLE, W. T. (1933). *J. Immunol.* **24**, 235.
ELIOT, C. P. (1940). *Amer. J. Hyg.* B, **31**, 8.
FELIX, A. (1924). *J. Immunol.* **9**, 115.
FELIX, A. (1938). *Lancet*, **2**, 738.
FELIX, A. (1941). *Brit. Med. J.* **1**, 391.
FELIX, A., KRIKORIAN, K. S. & REITLER, R. (1935). *J. Hyg., Camb.*, **35**, 421.
FELIX, A., RAINSFORD, S. G. & STOKES, E. J. (1941). *Brit. Med. J.* **1**, 434.
GIGLIOLI, G. (1933). *J. Hyg., Camb.*, **33**, 387.
HAC, L. R., FLYNN, C. S. & PERRY, C. A. (1939). *J. Lab. Clin. Med.* **24**, 567.
HAMILTON, W. H. (1939). Circular issued by Headquarters, Northern Command, India, August 1939.
HORGAN, E. S. & DRYSDALE, E. (1940). *Lancet*, **1**, 1084.
MANSON-BAHR, P. H. (1940). Manson's *Tropical Diseases*, 11th ed. London.
PERRY, H. M. (1918). *Lancet*, **1**, 593.
PIJPER, A. & CROCKER, C. G. (1939). *S. Afr. Med. J.* **13**, 255.
SESHADRINATHAN, N. & PAI, M. N. (1940). *Ind. Med. Gaz.* **85**, 735.
SCOTT, W. D. (1943). *J. R. Nav. Med. Serv.* **29**, 198.
TOPLEY, W. W. C. & WILSON, G. S. (1936). *The Principles of Bacteriology and Immunity*, 2nd ed. London.

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