DETECTION OF TYPHOID CARRIERS BY AGGLUTINATION TESTS

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Use of the Vi agglutination test in the detection of chronic carriers of *Salmonella typhi* (*S. typhosa*) first was suggested by Felix *et al.* (1935). Since that time study of the test by many workers has resulted in the accumulation of a voluminous literature which has been reviewed by Klein (1943), Felix (1951), Landy and Lamb (1953), and Spaun (1957). Bhatnagar *et al.* (1938) recommended the use in Vi agglutination tests of *S. typhi* Vi 1 of Kauffmann which the latter worker had isolated and found inagglutinable in O and H sera for *S. typhi*. Since that time the Vi 1 strain has been used almost exclusively in agglutination tests for the detection of typhoid carriers. Desranleau (1943) and Saint-Martin and Desranleau (1951) recommended the use of a slide test using a glycerolated antigen prepared from *S. typhi* Vi 1.

The results obtained by different workers in agglutination tests differed widely in the percentage of carriers detected and in the number of reactions obtained with sera of apparently normal people. Since the Vi 1 strain is quite rough, agglutination tests done with the culture are subject to the difficulties inherent in the use of a rough culture for antigen in agglutination tests. Use of other cultures of *S. typhi* or of cultures of the Citrobacter (*Escherichia freundii*) group which contained Vi antigen was rejected either because they reacted with O and H agglutinins for *S. typhi* or because they were insufficiently sensitive to Vi agglutinins. This unsatisfactory condition led Spaun (1951), Bier (1951), Corvazier (1952), LeMinor *et al.* (1952), and Tomasíc *et al.* (1953) to investigate the sensitization of red cells with various preparations of Vi antigen. The detection of typhoid carriers by Vi hemagglutination tests was investigated by Staack and Spaun (1953) using heated extracts of Vi cultures of *S. typhi* for sensitization and by Landy and Lamb (1953) using purified Vi antigen for sensitization. Human type O red cells were employed in both instances. The number of carriers tested by these investigators was not large, and some control sera, particularly those of vaccinated individuals, gave positive results whereas a small number of known carriers yielded negative tests. The conflicting results obtained in bacterial agglutination tests and hemagglutination tests in the serological investigation of typhoid carriers stimulated the work here reported.

MATERIALS AMD METHODS

Through the cooperation of a number of state health departments and an army laboratory a number of sera of known typhoid carriers and of persons who were not known to be typhoid carriers were collected. The control sera were of two lots. The first consisted of 100 sera from persons subjected to premarital tests for syphilis. The second lot was collected from 100 persons being tested for syphilis before discharge from the army. This lot was included because it was known that the persons from whom the sera were derived had been vaccinated repeatedly. The carrier sera were composed of sera from 183 persons who were known to have excreted *S. typhi* for long periods. Stool or stool and urine specimens were obtained from 70 of these persons at the time the blood was drawn and *S. typhi* was isolated from 55 (78 per cent) on the single examination. Sera from 33 people, who were involved 6 months previously in a sharply circumscribed outbreak of typhoid fever, also were tested. *S. typhi* had been isolated from all these convalescents during the course of the outbreak and none was found to be a carrier on subsequent repeated examinations.

The sera were subjected first, to the Vi agglutination test of Felix (1951) using antigen prepared by the Standards Laboratory, Central Public Health Laboratory, London; second, to the Vi slide agglutination test performed by the method of Saint-Martin and Desranleau (1951) in which antigen furnished by Mr. J. M. Desrlaneau was employed; and third, to the Vi hemagglutination test. We wished to use a method in hemagglu-

tination tests which could be applied readily in public health laboratories so the method of Landy and Lamb (1953) was modified in three ways: (*a*) Sheep cells were used, instead of human type O cells, because they are more readily available in the average public health laboratory. A control with unsensitized cells was included in each test and those sera which contained sheep cell agglutinins were adsorbed with unsensitized cells and retested. It was necessary to adsorb the sera from 14 of the premarital specimens, 22 of the army dischargees, 15 of the carriers, and 6 of the convalescents. (b) Tests were incubated for 1 hr at 37 C in the water bath instead of for 2 hr as recommended by Landy and Lamb. (c) A crude Vi extract was used instead of purified Vi antigen as employed by Landy and Lamb.

In preliminary experiments, extracts of the Vi forms of *S. typhi* 2 of Felix and of the Citrobacter cultures generally referred to as Ballerup and Coli 5396–38 of Kauffmann, as well as purified antigen supplied by Dr. Landy were used. In general, it may be said that comparable results were obtained with the four preparations. The observation of Landy and Lamb that 10 μg per ml of purified Vi antigen was optimum for sensitization of human type O cells applied also to sheep cells. As a rule, titers obtained with purified Vi antigen were one dilution lower than those obtained with crude extracts of the above-mentioned organisms. After a number of carrier sera was examined it was decided that slightly more consistent results were obtained with the Ballerup extract than with those obtained from *S. typhi* 2 or Coli 5396–38. Thereafter, the Ballerup extract was used exclusively throughout the course of the work.

The preparation and standardization of the extract were as follows. The Ballerup strain was plated on infusion agar[1] and a Vi colony selected. This process was repeated until only Vi colonies appeared upon the plates. One such colony was inoculated into infusion broth, incubated for 5 hr, and used to seed thickly poured plates. After overnight incubation, 3 ml of 0.85 per cent saline were used to suspend the growth from each plate. The resulting heavy suspension was steamed in an Arnold sterilizer at 100 C for 1 hr. After cooling, the suspension was centrifuged to clarity and the supernatant preserved with merthiolate (0.1 mg per ml) and stored at 4 C. Using the method of Landy and Lamb, sheep cells were sensitized with dilutions of the extract extending from 1:10 to 1:1000 and these were titrated with Ballerup Vi serum. In repeated tests it was found that a 1:400 dilution of the extract gave maximal titers. Cells so treated were agglutinated to a titer of 1:10,240 in a serum which had a Vi agglutinin titer of 1:1000 for *S. typhi* Vi 1. Tested in a Ballerup O antiserum (titer 1:640), the cells were agglutinated only in a dilution of 1:2, confirming the observations of Spaun (1951) and Landy and Ceppelini (1955) on the O-inagglutinability of erythrocytes treated with Vi antigen. Numerous lots of cells treated in this manner throughout the course of the work yielded identical results. In interpreting the results of bacterial Vi agglutination tests, the recommendations of their originators were followed. A titer of 1:1 was considered positive in slide tests. A titer of 1:5 in tube tests was recorded as positive. Hemagglutination tests which had a titer of 1:10 or more were considered positive. This interpretation is discussed subsequently.

Since O and H agglutination tests occasionally have been recommended in the examination of carriers of *S. typhi*, such tests were included in the work. The O antigen employed was a heated, phenolized suspension of *S. typhi* O901. The H antigen was prepared by passing *S. typhi* H901 through semisolid agar until maximal motility was attained, inoculating the organisms into infusion broth, incubating overnight at 33 C, and adding to the culture an equal amount of saline which contained 0.6 per cent formalin.

RESULTS

Unfortunately, the amounts of sera received did not permit the performance of all tests with each specimen. For this reason the total number of sera of each group tested by various methods differs slightly from one test to another but these differences are not so great as to interfere with interpretation of results. The results obtained in the tests for O and H agglutinins are included in tables 1 and 2. No marked differences were observed in the O titers of the sera of the various groups and it should be noted that the highest proportion of sera which had an O titer of less than 1:40 was found among the sera of carriers. On the contrary, this group contained the lowest percentage of sera which had H titers of less

[1] The blood agar base medium without addition of blood supplied by Baltimore Biological Laboratories was used thoroughout the work.

TABLE 1

Titers of sera against Salmonella typhi O901

Group	Dilutions of Sera						
	<40	40	80	160	320	640	Total
Premarital	43 (46.7)	17 (18.5)	23 (25.0)	7 (7.6)	2 (2.2)		93
Army dischargees	38 (38.0)	35 (35.0)	18 (18.0)	6 (6.0)	2 (2.0)	1 (1.0)	100
Carriers	88 (53.0)	38 (22.9)	21 (12.7)	13 (7.8)	3 (1.8)	3 (1.8)	166
Convalescents	9 (27.3)	10 (30.3)	9 (27.3)	2 (6.0)	1 (3.0)	1 (3.0)	32

TABLE 2

Titers of sera against Salmonella typhi H901

Group	Dilutions of Sera								
	<40	40	80	160	320	640	1280	2560	Total
Premarital	39 (41.9)	25 (26.9)	22 (23.7)	7 (7.5)					93
Army dischargees	27 (27.0)	18 (18.0)	27 (27.0)	20 (20.0)	5 (5.0)	2 (2.0)	1 (1.0)		100
Carriers	32 (19.3)	23 (13.8)	32 (19.3)	42 (25.3)	17 (10.2)	12 (7.2)	4 (2.4)	4 (2.4)	166
Convalescents	29 (87.9)	1 (3.0)	1 (3.0)	1 (3.0)	1 (3.0)				33

TABLE 3

Titers of sera (tube agglutination) against Salmonella typhi Vi 1

Group	Dilutions of Sera							
	<5	5	10	20	40	80	160	Total
Premarital	87 (87.0)	8 (8.0)	4 (4.0)	1 (1.0)				100
Army dischargees	80 (80.0)	11 (11.0)	4 (4.0)	5 (5.0)				100
Carriers	57 (33.1)	41 (23.8)	28 (16.3)	21 (12.2)	18 (10.5)	5 (2.9)	2 (1.2)	172
Convalescents	25 (80.6)	5 (16.1)	1 (3.2)					31

TABLE 4

Titers of sera (slide agglutination) against Salmonella typhi Vi 1

Group	Dilutions of Sera								
	<1	1	2.5	5	10	20	40	80	Total
Premarital	94 (94.0)	1 (1.0)	1 (1.0)	2 (2.0)	1 (1.0)	1 (1.0)			100
Army dischargees	89 (91.8)	7 (7.2)		1 (1.0)					97
Carriers	28 (15.6)	41 (22.9)	4 (2.2)	23 (12.9)	48 (26.8)	29 (16.2)	5 (2.8)	1 (0.6)	179
Convalescents	10 (31.3)	4 (12.5)	9 (28.1)	8 (25.0)	1 (3.1)				32

TABLE 5

Titers of sera (Vi hemagglutination test)

Group	Dilutions of Sera										
	<10	10	20	40	80	160	320	640	1280	2560	Total
Premarital	100 (100.0)										100
Army dischargees	93 (100.0)										93
Carriers	15 (8.4)	14 (7.8)	11 (6.2)	28 (15.6)	29 (16.2)	29 (16.2)	23 (12.8)	12 (6.7)	11 (6.2)	7 (3.9)	179
Convalescents	31 (93.9)		2 (6.1)								33

than 1:40. The sera of army dischargees had a somewhat higher level of H agglutinins than did the group of premarital specimens, probably a reflection of repeated vaccination. However, the level of O and H agglutinins in the premarital sera indicated that many people in that group also had been vaccinated. In general, it may be said that the level of O and H agglutinins in the control sera was such that tests for these substances were of no aid in the detection of carriers. It may be mentioned that the convalescents were infected with a culture of *S. typhi* which was almost nonmotile and that fact probably accounts for the very low level of H agglutinins in that group as compared to the other groups. Finally, it should be emphasized that proved carriers repeatedly were found who had both O and H titers of less than 1:40.

The results of the three tests for the detection of Vi agglutinins are given in tables 3, 4, and 5, and are summarized in table 6. Tube tests with *S. typhi* Vi 1 antigen not only were positive with the sera of the smallest number of carriers of any of the tests, but also were most often positive with control sera. The slide test of Saint-Martin and Desranleau detected more carriers than did the tube test and reacted with less than half the number of control sera found positive in the latter. Some difficulty was experienced in reading the slide tests and in determining the exact end point of agglutination.

The Vi hemagglutination test yielded results

TABLE 6

Results of Vi agglutination tests

Group	Tube		Slide		Hemagglutination	
	+	−	+	−	+	−
Premarital	13 (13.0)	87 (87.0)	6 (6.0)	94 (94.0)	0 (0.0)	100 (100.0)
Army dischargees	20 (20.0)	80 (80.0)	8 (8.2)	89 (91.8)	0 (0.0)	93 (100.0)
Carriers	115 (66.9)	57 (33.1)	151 (84.4)	28 (15.6)	164 (91.6)	15 (8.4)
Convalescents	8 (19.4)	25 (80.6)	22 (68.8)	10 (31.2)	2 (6.1)	31 (93.9)

TABLE 7

Vi agglutination tests with sera of typhoid carriers

Tube	Slide	Hemagglutination	No.
+	+	+	93
−	+	+	40
−	−	+	10
+	−	+	11
+	+	−	7
−	+	−	6
−	−	−	1
Total.....			168

Includes only those carrier sera on which all three Vi agglutination tests were performed.

decidedly superior to those obtained with the other tests, both in the fact that it detected the highest percentage of carriers and in that no positive tests were obtained with control sera. The results obtained with convalescent sera with the three tests differed markedly. Whereas only 6.1 per cent of these persons, who upon repeated stool examinations were found not to be carriers, were positive in hemagglutination tests, 19.36 per cent were positive in tube agglutination tests, and no less than 68.75 per cent were positive in slide agglutination tests in which *S. typhi* Vi 1 was used as antigen. Results obtained in the testing of carrier sera confirmed the observation of Landy and Lamb that there was no correlation in the Vi titers of sera determined by bacterial agglutination and by hemagglutination.

The comparative results obtained with the three tests for Vi agglutinins in the sera of carriers are recorded in table 7. In spite of the greater efficiency of the hemagglutination test in the detection of carriers, sera of 13 of these people reacted positively in one or both of the bacterial agglutination tests but failed to react in the hemagglutination test. It should be remembered, however, that the hemagglutination test detected a greater number of carriers than either of the bacterial agglutination tests alone. It is of interest that the serum of only 1 carrier of 168 tested by all three methods was negative in the three tests. It is beyond the province of the average diagnostic laboratory to use all three methods.

DISCUSSION

From the data presented, there is no question of the superiority in this series of tests of the hemagglutination test over the other methods used in the serological detection of typhoid carriers. In this respect, the results confirm those of Landy and Lamb (1953). The total lack of positive reactions in control sera contrasts with the small number of positives found by Landy and Lamb in unvaccinated persons and the larger number found by them among vaccinated individuals. In contrast to those workers, Staack and Spaun (1953) found that sera of fewer vaccinated people reacted in Vi hemagglutination tests than sera of individuals not recently vaccinated. These conflicting results discount the importance of vaccination in hemagglutination tests and in this respect confirm the results obtained by the writers.

In any consideration of the value and usefulness of various methods of determining Vi agglutinin levels, it is necessary to take into account the ease with which the necessary reagents may be prepared. The Vi extracts used here to sensitize sheep cells were easily prepared and readily reproducible. Further, they were stable when stored for 10 months at 4 C or at −20 C. Spaun (1958, *personal communication*) found no deterioration in Vi extracts of *S. typhi* stored in the frozen state for 3 years. The Vi 1 antigen for tube tests was prepared repeatedly by the method of Felix (1951) and several lots were of acceptable quality. However, it must be said that antigens prepared by the writers were not considered by them to be quite as satisfactory as that obtained from the Standards Laboratory. Vi antigen prepared by this method deteriorates on storage and usually is used for only 2 to 3 months after preparation. Likewise, numerous lots of slide antigen were prepared carefully following directions supplied by Desranleau (1943). None of these antigens was of acceptable quality and of necessity antigens provided by Mr. Desranleau were used exclusively.

The total lack of positive hemagglutination tests among the control sera in this series contrasts with the positives noted both by Landy and Lamb (1953) and Staack and Spaun (1953). No obvious explanation of this difference can be offered. It can be said only that slightly different methods were used in the present work, that controls of unsensitized sheep cells were included in all tests, and that those sera which agglutinated such cells were fully absorbed and retested.

From the present series of tests as well as from the results of others, it is obvious that hemagglutination tests, as they have been done to this time, will not detect all carriers of *S. typhi*. The writers obtained 91.6 per cent positive results and this figure is in good agreement with the 90 per cent found by Landy and Lamb and the 87.9 per cent reported by Staack and Spaun in examinations of smaller series of carriers. In this series of tests, the lowest dilution considered positive was 1:10. In retrospect, it might have been more advantageous to use a dilution of 1:5. The work cannot be reevaluated fully on that basis since the premarital sera were not tested at 1:5. The sera of carriers which were negative at 1:10 were retested at 1:5 and the sera of army dischargees and convalescents were tested at 1:5. The sera of three carriers, which were negative at 1:10, were positive at 1:5; whereas 12 failed to react at that dilution. The serum of one dischargee was positive at 1:5. This indicates that the test could be poised at a lower dilution than was done here and that a few more carriers would be detected without a great increase of reactions among noncarriers. However, in this series there were proved carriers the sera of which failed to react at 1:5. For this reason, reliance cannot be placed on the hemagglutination test alone in the detection of typhoid carriers. It would provide a most useful adjunct to the bacteriological search for carriers, indicating when repeated bacteriological examinations should be done. Employed in this way, it should be much more useful than the usual bacterial Vi agglutination test which Lie *et al.* (1957) recently found very inefficient as a screening test for the detection of typhoid carriers in Indonesia.

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SUMMARY

The sera of approximately 200 apparently normal individuals and 180 chronic typhoid carriers were tested for O and H agglutinins for *Salmonella typhi* (*S. typhosa*) and also were tested for Vi agglutinins using bacterial agglutination tests done on slides and in tubes using *S. typhi* Vi 1 as antigen. Hemagglutination tests using sheep cells sensitized with heated extracts of the Ballerup strain also were done. Tests for O and H agglutinins were of no value in the detection of carriers. Of the Vi agglutination tests, hemagglutination was found to be most useful since it gave no reaction with sera of control groups and was positive with 91.6 per cent of sera from typhoid carriers. It is emphasized that no serological test nor combination of tests yet devised can be relied upon to detect all carriers of *S. typhi*.

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