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BACTERIOLOGICAL DIAGNOSIS
OF
TYPHOID FEVER.

Aubrey H. Straus

May, 1923.

THESIS SUBMITTED FOR

M. A. DEGREE

'23/6/4 Approved.
Donald W. Davis

This paper is written from the standpoint of a practical diagnostic laboratory. It covers a period of fourteen years of observation and experience in the laboratory diagnosis of typhoid fever. There is, however, no attempt made to offer an exhaustive review of the literature but only a few necessary references. The principal value of this paper is that it makes practically useful in a broad way methods that were for many years regarded as only experimental or, at best, suitable for diagnosis under special conditions such as are found in a hospital.

As the result of a former publication,(1) several other public health laboratories have tried these methods with success. More than two years have now elapsed since this former paper was written. During this time, considerably more work has been carried on, certain methods improved and new observations made, particularly in regard to the bactericidal effect of blood.

The first method for the laboratory diagnosis of typhoid fever devised was the use of the agglutination test by Widal(2) in 1896. The phenomenon of agglutination had been observed a short time before by Gruber and Durham,(3) but it was Widal who devised the practical use of this reaction as a means of diagnosis. Widal, in his original publication, made note of the fact that agglutinins would remain present in a specimen of dried blood for several months.

As a result of Widal's work, the agglutination test came into widespread and general use and is now known as the Widal test or reaction. As it greatly facilitated the getting and sending of specimens to use dried blood, this method soon came into general

use in public health laboratories. However, like many things valuable in the hands of the person originating them but not working so well in the hands of others, this test came to be greatly abused. Too much confidence was frequently placed in it on the part of physicians, specimens of blood were sent in on any kind of material that happened to be available, such as odd pieces of glass, wood, paper, metal, etc., with the result that the value of the Widal test, as commonly carried out, has become greatly vitiated.

When the writer first began to do public health laboratory work in 1909, the first difficulties confronting him were complaints concerning the Widal test. These complaints were principally based upon the fact that negative reactions were so frequently obtained in cases that were clinically typical of typhoid fever. As many of the physicians would not report a case of typhoid fever without a positive Widal reaction, the laboratory results at times actually retarded the work of the health department instead of facilitating it. In an endeavor to improve this test, various experiments were carried out, using different strains of typhoid cultures, varying the lengths of time, dilutions, etc. However, in spite of these efforts, the test never proved satisfactory either to the laboratory or to the physicians.

An experience of about fourteen years in public health laboratory work has not changed my opinion in regard to this test.

During the last two years, a special strain of typhoid bacilli has been used, which is somewhat better than any preceding ones used. In spite of this, however, results continue to be unsatisfactory and the method would have been discontinued entirely were it practicable to do so. In the city of Richmond, the Widal test, as carried out with a dried drop of blood, was discontinued three years ago, but, in the work of rural Virginia, it has been impracticable to make this change, nor will it be practicable to do so until a long campaign of education has been carried out.

When the conclusion was reached that the Widal test (referring here again to the method as carried out with dried blood) was unsatisfactory, a search was begun to find some method, or methods, which would satisfactorily replace it. The blood culture, as described in a local journal(4), about this time, seemed to offer the best means for laboratory diagnosis, and, in 1910, with the help of two physicians, some experimental work along these lines was carried out. These experiments proving satisfactory, we began in 1911 to put this test into practical use.

During the year 1911, only eight blood cultures were examined. From this point on, the use of this test steadily increased, as shown below in Table I.

TABLE I

Showing Blood Cultures Examined in the Richmond City Laboratory from 1911 to 1922 (inc.) and the Number of Cases of Typhoid Fever Reported.

Year	1911	1912	1913	1914	1915	1916	1917	1918	1919	1920	1921	1922
Cases of Typhoid Reported for Year	251	208	233	148	155	224	98	105	85	79	72	63
Total Number of Blood Cultures Examined	8	40	55	121	219	316	123	65	110	182	252	165
Number of Positive Cultures	3	7	12	24	34	39	17	13	23	33	40	25
Per cent of Positive Cultures	37.5	17.5	21.8	21.1	17.8	13.6	15.9	20.0	21.5	19.3	16.7	15.2
Number of Unsat- Cultures	0	0	0	7	28	30	16	0	3	11	13	1
Per cent of Un-	0	0	0	5.8	12.8	9.5	13.0	0	2.7	6.0	5.2	0.6

*Based on examinations which were either positive or negative and including a few cultures showing organisms other than typhoid.

As shown above, the greatest number of blood cultures was examined in 1916. During the summer of 1916, there was a small epidemic of typhoid fever, and the bulk of these cultures was taken during that time.

While the largest actual number of blood cultures was examined in 1916, the largest number of cultures in proportion to the cases of typhoid fever was in 1921, when there were 3.5 cultures examined for every case of typhoid fever reported.

During the early years, most of the blood cultures submitted were collected by the City Medical Inspector, but during the last three or four years, only a small proportion of the cultures have been so collected, the great majority being obtained by the physicians themselves; that is, the test has now come into general use. In consideration of this fact, it is especially gratifying to note the very small percentage of unsatisfactory cultures. While this indicates better technique in the collecting of the blood, it is also partially due to a better technique in the laboratory in the examination of these cultures, due to certain improvements in methods to be described later.

Method of Collecting and Submitting Blood Cultures.

When the examination of blood cultures was commenced, various methods for collecting blood were experimented with. Several methods described were tried out and discarded. We were unable to employ successfully any of the methods where blood was procured by puncturing the ear or finger regardless of the method used for sterilization of the skin. We, therefore, resorted to taking blood directly from the vein with a sterile syringe and have continued the use of this method ever since. At that time, the Wassermann test had not come into general use and taking blood from a vein was not the common proceedure that it is today.

The first culture media used were lactose bile and plain bouillon. We used for each blood culture two test tubes of bile, containing 1 per cent peptone and 1 per cent lactose, and one test tube of plain bouillon. Lactose bile was used instead of plain bile as it was a stock medium and always on hand. Each of these tubes contained about 5 c.c. of media to which was added, as a rule, from 1½ c.c. to 2 c.c. of blood.

While this method gave very satisfactory results, improvements have been made from time to time until the system now used was evolved. The present system has been in use for four years without change and seems to meet every requirement. The first adaptation was omitting lactose from the bile medium. A series of parallel tests showed that the lactose exerted a slightly inhibitory effect and that the peptone bile without lactose made a better medium.

Likewise, it was found that plain bouillon was not entirely satisfactory. The blood formed a heavy clot in this, making the culture difficult to inspect and frequently giving a growth of streptococci only on the surface of the clot where it might not be observed. In order to obviate this, 1 per cent of sodium citrate was added to the bouillon to prevent the blood from clotting and 0.9 per cent of sodium chloride to prevent hemolysis. This has proved a considerable improvement as, when growth of any sort is present in the bouillon, it can be much more easily observed and studied. Beef infusion bouillon, being generally a better culture medium than plain bouillon, was substituted in its place.

Bottles have been substituted for test tubes as an increased amount of media was found advantageous and, also, bottles were found more convenient for handling. The only object of the two bile tubes was to permit the examination of a greater amount of blood, and a single bottle answers this purpose. The need for using the two varieties of media is brought out in Table II.

Experiments were carried on with several different types of bouillon in the hope that some single culture medium could be found that would take the place of the two media described above. A few cases of known typhoid fever were located in hospitals, blood was collected from these cases and inoculated into the various media. In each case the bile control was positive, but in no case was growth obtained in any of the various modifications of the bouillon. Trypsin media, which has been recommended for this work, was not tried as the cost of trypsin was too great to make it practical even if it worked.

Results Obtained from Blood Cultures.

The summary shown in Table II does not represent all the blood culture work done by the Richmond City Laboratory but only that part of it which has been under the immediate supervision of the author.

TABLE II

Summary of City Blood Cultures Under Personal Supervision to April 30, 1923.

	Number	Per cent
Positive	147	18
Negative	647	79
Unsatisfactory	28	3
Total	822	100

Positive cultures as follows:

125--Typhoid

7--Streptococci

5--Para-typhoid B

3--Pneumococci

1--B. Coli

1--Influenza bacillus

1--Meningococcus

2--Unidentified bacilli, probably para-typhoid

1--Unidentified bacillus, resembling the influenza bacillus

1--Hemolytic, Gram-negative bacillus, unidentified

As shown in Table II, positive blood cultures have been obtained for a number of different organisms. While the great majority of all positive organisms were typhoid, nevertheless, there have been found a considerable number of positive cultures of other kinds. Without the use of these two media, many of these results could never have been obtained. As will be brought out in detail later, organisms of the typhoid-colon group will not grow in bouillon unless the bulk of media used is so large as to give considerable dilution. This is due to the highly bacteriocidal action of freshly drawn blood upon these organisms. On the other hand, organisms such as the streptococci, pneumococci, etc., will not grow in the bile medium but will grow in blood bouillon. Therefore, where these two varieties of media are used, all organisms found in the blood can be grown in either one or the other.

While it is quite practical to use these two bottles in the work of a municipal laboratory, where the outfits do not have to be mailed, this is not so easily done in state work where specimens come in by mail. We were unable to devise any suitable and convenient mailing container for the two bottles; likewise, the danger of contamination in plain bouillon is much greater than in bile media, as many contaminating organisms will not grow in the bile. Also, the typhoid bacillus is the organism usually found in rural work, so it was decided to eliminate the bouillon and simply to send out the bottle of bile media in our state blood culture outfit. Table III, which follows, shows the results of the state blood culture work up to April 30, 1923.

TABLE III

Summary of State Blood Cultures from 1920 to April 30, 1923.

	Number	Percent
Positive	45*	31
Negative	91	63
Unsatisfactory	9	6
Total	145	100

*40 Typhoid, 4 B.Coli, and 1 Para-typhoid B.

Several of these cultures; including some that were positive, were not submitted in our regular containers, but were simply tubes of whole blood. The cultures in these cases were made by putting the blood clot into bile media and making sub-cultures from this as described below in our regular method. While we do not think it likely that as many positives would be obtained where this clot method is used as would be obtained when the blood was inoculated directly into the media, yet we do believe that a tube of blood submitted in this way, permitting, as it does, both the macroscopic agglutination test and the blood culture, gives on the whole very satisfactory results. For the country practitioner, with the difficulties that often confront him in sterilizing syringes, etc., whole blood collected in a Keidel tube is a very practical method. The use of the clot culture was first suggested to us by Dr. F. F. Russell, who had made use of it in the army. Recently a publication has appeared from the New York State Laboratory (5) showing a rather successful use of the method. They obtained positive results in

17 out of 84 clots examined. Fourteen of the 17 positive cultures also gave a positive agglutination test.

When B. Coli was first obtained, its pathogenic significance was doubted and contamination of the culture suspected. In the first case observed, a second culture was obtained for confirmation and a special study and report of the case made.(6) Later when other cases occurred, contamination was again suspected and a few tests were made to determine the likelihood of B. Coli being present as a result of poor technique in collecting the blood. Twelve persons were selected at random and a swab rubbed thoroughly over the skin of the arm; another swab was rolled between the fingers. Cultures from these 24 swabs were all negative for B. Coli. It was therefore concluded that B. Coli was not apt to be present unless actually in the blood, and no further hesitancy was felt in reporting these findings. This conclusion has further been verified by similar cases reported in the literature.(7) These cases clinically resembled typhoid fever.

Staphylococci, both albus and aureus, have frequently been found in blood cultures, but these organisms are also commonly found on the skin. Repeated requests have been made for second cultures in these cases without response. These findings have, therefore, not been reported or included in the tables. It is, however, very probable that staphylococci occur in the blood.

A recent publication by Hebert and Bloch(8) gives some blood culture results very different from those reported here. Their work was done over a period of four years, 1915-1919, in a large army hospital at Bar-le-Duc, France. All patients entering the hospital with fever had a blood culture made. Their series of 18,650 blood cultures is probably the largest number ever reported.

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The results were as follows:-

Typhoid bacilli	765	12.77%
Para-typhoid A bacilli	4,412	73.67%
" " B "	810	13.56%

The para-typhoid A cultures occurred principally during 1915 and 1916 when there was an epidemic of this infection. Other army outbreaks of para-typhoid A (9) have also been reported.

In view of these facts it is rather interesting to note that in the 967 blood cultures recorded in tables II and III there was not a single case of para-typhoid A.

Method of Examining Blood Cultures in the Laboratory.

When blood cultures are received in the laboratory, they are placed at once in the incubator. After 6 to 15 hours (according to the time of day that the cultures are received), an agar slant is inoculated from the bouillon and a tube of Russell's double sugar medium from the bile. If no growth is obtained on these cultures after 8 to 15 hours incubation, the cultures are reinoculated and a slide for microscopic examination is prepared from the red blood cells in the bottom of the bouillon culture. Growth in the plain bouillon is sometimes not visible to the eye and some organisms do not grow on the plain agar sub-culture. On the other hand, direct examination of the bile has never proved satisfactory.

If the second set of sub-cultures does not show growth, the culture may be considered negative for typhoid, provided the bile had incubated at least 48 hours before this culture was made, otherwise a third sub-culture is inoculated. All organisms of the typhoid-colon group will grow readily on Russell's medium, so that a failure to obtain growth on the sub-cultures may be considered conclusive.

When growth appears on the Russell's medium, a Gram stain is at once made and, if a Gram-negative bacillus is present, agglutination tests are set up. Macroscopic agglutination is always used and no organism is reported as typhoid or para-typhoid without a positive reaction. Should the agglutination be negative, various sub-cultures are inoculated, motility tested, etc. Where all of these things conform to the typhoid bacillus, agglutination is tried again and yet again if necessary. Freshly isolated typhoid bacilli are sometimes difficult to agglutinate. A preliminary report is often made to the physician as soon as a growth resembling the typhoid bacillus is found, but a positive report of typhoid is never made without confirmation by agglutination.

Pathogenic cocci, found in the bouillon bottle, can usually be identified without difficulty, but as shown in Table II, several bacilli have been obtained which could not be identified. These unknown organisms were carefully checked up and the hemolytic bacillus mentioned last in Table II was obtained in three different cultures from the patient. These organisms were clearly not contaminations. Several other unidentified organisms are not included in this list, as it was thought that they might be contaminations.

Contaminations, themselves, worry us much less now than formerly. Whenever we find contamination present now, the simple proceedure of plating from the bile on to Endo or E.M.B. media makes the isolation of the typhoid bacillus comparatively simple, even when the original culture is rather badly contaminated. Most of the contaminated organisms grow very poorly and often not at all on these media.

The Bactericidal Effect of Blood.

A feature that has been of particular interest in the blood culture work has been the almost complete failure of the typhoid bacillus to grow in the bouillon bottle while it grows readily in the bile. The blood exercises an inhibiting action which the bile counteracts. With a view to getting further data along this line, a few simple experiments were begun by inoculating media containing blood with typhoid bacilli.

In the first of these experiments, we endeavored to duplicate conditions in the actual taking of blood cultures. Bottles were prepared with measured amounts of plain bouillon, and sufficient blood added immediately after taking it from the vein to give dilutions of 40 % blood, 20%, 10% and 5%. As soon as these dilutions were completed, the bottles were all inoculated with a small loop of typhoid culture. Such tests were made on the blood of 12 different persons; 10 of the individuals had been vaccinated, 1 had a negative history and 1 had had typhoid fever the year before. The results were surprising in that growth was obtained in all of the cultures except in the case of the person who had had typhoid fever the previous year. In this case, no growth was obtained in the 40% and 20% dilutions, but growth did occur in the two higher dilutions.

These results led us, at first, to believe that the bactericidal, or inhibiting properties, of the blood were lacking in normal or vaccinated persons. Later experiments, however, showed that this was not the case. The probability is that this experiment was faulty in that the amount of bacteria added was far too large and out of all proportion to the number found in the blood of a typhoid fever case.

A second series of experiments was conducted along different lines. The blood when collected was added to bottles containing a solution of 1 per cent sodium citrate and 0.9 per cent saline, an amount of blood being added equal to the amount of solution in the bottle. Two c.c. of this mixture was then placed in each of several bottles. Each bottle was inoculated with 0.1 c.c. of a 24 hour bouillon culture and 0.1 c.c. of this mixture was immediately plated. Subsequent platings were also made at the time intervals shown in Table IV, using in each case 0.1 c.c. of the mixture. Plain agar was used in all plates.

TABLE IV

Showing the Bactericidal Effect of Citrated Blood on the Typhoid Bacillus and Other Organisms.

Case	Previous History	Organism	Time Plated: Immediately	½ hr.	1 hr.	1½ hrs.	3 hrs.
D 1	Had typhoid about 6 yrs previously	B. typhosus	Many*	100	12		0
		B. dysentery Flexner	Many*	1000	500		10
B 1	Vaccinated in '17, '19, '21	Typhoid	Many*	100	15		0
		Dysentery Flexner	Many*	Many*	Many*		Many*
V 1	Negative	Typhoid	Many*	400	300		20
		Dysentery	Many*	Many*	Many*		Many*
#15	Negative	Typhoid	Many*	1	0		0
		Dysentery Flexner	Many*	Many*	Many*		Many*
#16	Negative	Typhoid	Many*	350	200		3
		Dysentery Flexner	600	50	25		6
#17	Negative	Typhoid	Many*	400	300		100
		Dysentery Flexner	Many*	Many*	Many*		Many *
#19	Negative	Typhoid	1200	100		20	0
		B. Coli	50	10		3	0
		Dysentery Shiga	200	40		30	0
		Para-typhoid A	1400	500		200	40

* Too numerous to be counted.

The above table presents several interesting points. In the first place, there is a marked diminution in the number of typhoid bacilli in each case. This diminution progresses at a varying rate, and there seems to be no correlation between this and the previous history of the case. The individual, D 1, who was supposed to have had typhoid fever, and B 1, who gave a history of three complete previous vaccinations, neither show as strong a bactericidal effect in their blood as #15. Likewise, case #19 shows quite as strong bactericidal action as does either D1 or B 1.

In regard to the action toward the Flexner dysentery bacillus, however, there is a much greater individual variation. In some of these cases, for example, #15 and #17, there was, apparently, no bactericidal effect towards this organism, while in others, the bactericidal effect against this organism was almost as great as toward the typhoid bacillus. It is interesting to note that in case #15 there is the greatest diminution in typhoid bacilli with no action against the Flexner bacillus and yet this case gave no previous history of typhoid fever or preventive inoculation. To sum up, these tests, while presenting interesting features, threw no light on the problem at hand and they seemed to indicate that there was no relationship between a previous case of the disease, or inoculation against typhoid fever, and the bactericidal effect of the blood.

Employing the same technique, a series of tests were made on one individual in an effort to determine changes in this individual following typhoid inoculations. The results of these tests are shown in Table V.

TABLE V.

Showing Tests on the Bactericidal Effect of the Blood in Different Stages of One Individual.

Previous History	Agglutination Reaction	Organism	Time Plated: Immediately	½ hr.	1 hr.	1½ hrs.	3 hrs.
4 complete vaccinations, last lyr. before	1-400	Typhoid	Positive*	Pos.*		0	
		Dysentery Flexner	Many**	500			25
2 additional inoculations	Not made	Typhoid	Many**	50	8	0	
		Dysentery Flexner	Many**	400	100	0	
3rd additional inoculation		Para-typhoid A	110	1	0		
		B. Coli	10	0			
	1-1600	Typhoid	400	40	0		

*Count not recorded.
**Colonies too numerous to count.

While the above table apparently showed a marked increase in the bactericidal effect of the blood following typhoid inoculations, yet a single case was not sufficient, especially in view of the results obtained in Table IV, to justify any conclusions being drawn. Further experiments were, therefore, conducted and certain changes in technique made with the hope of making the test more delicate. In order to eliminate one chance of error, a test was made with the citrate and saline solution to see if this solution had any bactericidal activity. Our tests were conducted in the same manner as above described only, of course, no blood was added. As far as we could determine by this method, there was no bactericidal effect on the part of this solution.

As there were many plates in Tables IV and V containing colonies too numerous to be counted, it was decided to reduce the amount of culture used so as to give a more accurate quantitative determination.

Instead of using a 24 hour bouillon culture as heretofore, agar slants were used. The organisms were washed off of these with 0.9 per cent salt solution and diluted , by a density comparison with a known emulsion, to contain approximately 1,000 million bacilli per c.c. This suspension was then further diluted so that 1 c.c. contained approximately 100,000 bacteria. Of this dilution, 0.1 c.c. was used in a test with 0.9 c.c. of blood (previously mixed with an equal volume of citrate and salt solution as described above) and 0.1 c.c. of this mixture subsequently used in each plate. This was estimated to give about 1,000 colonies per c.c. and, as shown in Table VI, this estimate proved as reliable as one could expect.

A few similar tests were made by the same method, using blood serum instead of whole blood. These results are also shown in Table VI.

TABLE VI.

Showing the Bactericidal Effect of Blood on 10,000 Organisms per c.c.

Case		History	Agglutination	Organism	Blood or Serum	Time Plated				
						Immediately	½ hr.	1 hr.	2 hrs.	3 hrs.
W	1	Had typhoid in 1913, vac. '17, '18 and '21*	1-50	Typhoid	Blood	800	7	3	2	0
	"			Dysentery Flexner	Blood	1000	900	900	800	600
B	2	Negative	Neg.	Typhoid	Blood	700	700	800	1400	2500
	"	Vac. in '21**	1-3000	Typhoid	Blood	1500	500	280	150	30
	"			Dys. Flex.	Blood	900	420	280	200	150
D	1	Vac. '13, '16, '18, '20	1-100	Typhoid	Blood	1200	30	11	8	7
	"			Typhoid	Serum	900	3	2	0	
	"			Dys. Flex.	Blood	350	350	350	350	350
J	1	Had typhoid '10 Vac. '17, '18	1-200	Typhoid	Blood	1400	30	13	10	7
	"			Typhoid	Serum	1700	4	2	0	
	"			Dys. Flex.	Blood	600	120	35	7	2
T	1	Vac. '14 and '18	1-100	Typhoid	Blood	1500	1400	1000	75	30
	"			Typhoid	Serum	1500	25	2	9	
	"			Dys. Flex.	Blood	1000	1100	750	900	900
L	1	1 injection '18	Neg.	Typhoid	Blood	600	600	900	900	900
	"			Dys. Flex.	Blood	700	700	1000	1400	1400
Mc	1	Vac. '18	Neg.	Typhoid	Blood	2000	2500	2700	2700	2400
	"			Dys. Flex.	Blood	700	700	800	600	900
K	1	Had typhoid in '12, vac. '17 and '18	1-100	Typhoid	Blood	2000	1000	1400	1400	1500
	"			Dys. Flex.	Blood	1000	400	350	100	125

*Completed 4 months before this test was made.
**Completed just Before this test was made.

The results in Table VI simply confirmed the observations previously made. In the case of "B 2" the results of vaccination are again demonstrated. The serum in each case shows a greater bactericidal action than does the whole blood but this was to be expected as all anti-bodies are supposed to be contained in the serum.

Table VII demonstrates that the addition of 10 per cent ox-bile to the blood in a test, not only destroys the bactericidal action of this blood, but also makes it a culture medium.

TABLE VII.

Showing the Effect of Bile on Bactericidal Action of Blood Against Typhoid.

Case	History		Blood	Time Plated				
				Immed.	½ hr.	1 hr.	2 hr.	3 hr.
B 2	Vac. '12, '17, '21		Blood	1500	500	280	150	30
"			Blood and 10% bile	1400	1400	1200	2800	4000
		Agglutination						
Immunized typhoid rabbit		1-6000	Blood	900	350	320	100	75
"			Blood and 10% bile	700	800	900	500	1400

The Widal Reaction.

Next in value to the blood culture comes the macroscopic Widal test. For the past four years this test has been used in routine municipal work, the microscopic test with the dried drop of blood having been discontinued entirely. This change has proven entirely satisfactory and has met with almost no opposition on the part of the physicians. Most of them are now accustomed to drawing blood for a Wassermann test, and do not object to going into a vein as much as they did formerly. When the case has progressed too far for the blood culture to be reliable, this test is then advised. A tube and a blank for this test are also enclosed with every blood culture outfit. In a good many cases, especially in the second week of the disease, both tests are positive. Frequently, however, only one or the other is positive. In the first week of the disease, a positive agglutination test is very seldom obtained, while after the second week of the disease, it is not uncommon to get a positive agglutination test with a negative blood culture. When these two tests are used in conjunction, a false negative report is seldom made.

Comparative tests by the macroscopic and microscopic methods were made on 50 specimens of blood with results as shown in the following table:

TABLE VIII.

Showing Comparative Agglutination Results by the Macroscopic and Microscopic Methods.

Macroscopic Test		Microscopic Test		
		Positive	Negative	Atypical
Positive	23	11	4	8
Negative	27		24	3
Atypical	___	___	___	___
Total	50	11	28	11

To summarize these tests as shown above:

The macroscopic test gave 23 positives out of the 50 bloods examined while the microscopic gave only 11. Of the remaining 12 positives by the macroscopic method, 4 would have been reported negative and 8 atypical if the microscopic test alone had been relied on.

Of the 27 negatives by the macroscopic method, 24 were negatives by the microscopic method and 3 were atypical by that method.

The macroscopic method gave typical tests in 11 bloods which were atypical by the microscopic method, showing 8 of these to be positive and 3 negative. No specimen had to be regarded as atypical by the macroscopic method.

These comparative tests of the macroscopic and microscopic methods were made on 44 vaccinated persons (mostly recent, but some old and some who had as many as 4 series of vaccinations), on 3 persons who had had typhoid fever, and 3 persons giving negative histories. While these comparative tests were thus not made on bloods from cases suspected of being typhoid, there is no reason for believing that there would have been any difference in the outcome if they had come from such cases.

The microscopic tests were made from dried drops of blood, checked by two or three workers, but made in the usual routine manner. All macroscopic tests which were called positive agglutinated well in 1 to 100 and, in some cases, in dilution as high as 1 to 3200.

We are inclined to think that the danger of misleading results due to previous vaccinations has been frequently overestimated. Where a series of dilutions is set up and no reaction called positive unless the agglutination is distinct in a dilution of 1 to 100, or higher, the chance of error in any but those who have been repeatedly vaccinated, or else vaccinated within a few months, is, we believe, slight.

The following table shows that a single vaccination a year or more ago does not usually give a positive agglutination but repeated vaccinations may give a positive result even though the last one was two years ago.

TABLE IX.

Showing Agglutination Tests on 25 Persons Vaccinated 1 Year or More.

Number of times vaccinated	No. of years since last vaccination	No. of persons	Result	Titre
7	1	1	+	100
4	1	1	+	100
3	2½	1	+	800
3	2	1	+	100
3	2	1	0	...
2	2	2	0	...
1	1	1	+	200
1	1	1	0	...
1	2	11	0	...
1	3	2	0	...
1	4	2	0	...
1	5	1	0	...

Of these 25 tests, only 5, or 20%, were positive, 4 of these 5 having a history of more than two series of vaccinations. In only one of these cases was the titre over 200.

Contrasted to this, 25 known cases of typhoid, not especially selected but taken as they occur in our files, show the following agglutination titre:

TABLE X.

Titre	Number of cases
8000	1
1600	4
800	6
400*	4
400	6
200	2
100	1
50	1
Total	25

* Not run higher.

The above table shows that the titre of the agglutination is an important part of the test and that it usually runs much higher in a case of typhoid than it does as a result of vaccination.

Bacteriological Examination of Feces.

Stool and urine examinations are undoubtedly of value in certain instances and should be available when needed. One case which occurred several years ago will serve to illustrate. A positive agglutination was obtained at 1-400. The case had been running for several weeks so that a blood culture was not indicated and the patient gave a history of having been vaccinated one year before. The value of the agglutination test was, therefore, doubtful, as was also the clinical history of the case, but obtaining the typhoid bacillus from a rectal swab established the diagnosis.

Our method for collecting specimens of feces for bacteriological examination is to use a bottle, containing a solution of 30 per cent glycerine. A rectal swab, or a small amount of fecal material, is placed in this solution and the specimen sent to the laboratory.

A small amount of the diluted fecal material is streaked over the surface of plates of Endo media and eosin-methyline blue (Levine's modification). These plates are streaked by the use of a bent glass rod, several plates being made so as to give varying dilutions. We have also employed special dysentery media, recommended by Levine. We believe that it is always advantageous to use two different kinds of media for each specimen, as we have noted in several instances that one strain will grow best on one kind of media and another strain best on a different medium. Colonies picked from these plates are identified in the usual ways.

The idea of using 30 per cent glycerine in collecting the specimens was obtained from the New York State Board of Health. The advantage of this method of submitting specimens has been brought out in several articles.(10) These articles show that typhoid bacilli are not inhibited from growing by the use of the 30 per cent glycerine solution while other organisms, commonly found in feces, are inhibited. Comparative tests have shown that positive results are often obtained from specimens submitted in this solution while negative results have been obtained from duplicate specimens submitted without the addition of the glycerine solution. Our experience indicates that the same is true in regard to the Flexner dysentery bacillus. We have had no opportunity to try this out with the Shiga dysentery bacillus since we have been employing this method.

The examination of feces is, of course, much more important for locating typhoid carriers than for purposes of diagnosis. Even in diagnosing bacillary dysentery, we have found the agglutination test to be much more valuable than fecal examination.

Conclusions.

1st. The blood culture is the most valuable diagnostic test available in the early stages of typhoid fever. Not only is this valuable in typhoid fever but it frequently discloses unsuspected infections of other sorts.

2nd. A method of examining blood cultures has been devised which permits of general application, making this test available to all physicians.

3rd. The two kinds of culture media here used in municipal work will disclose any organism apt to be found in the blood stream.

4th Blood is generally bactericidal for the typhoid bacillus and, apparently, to a lesser extent for the dysentery bacillus (Flexner).

5th. This bactericidal effect appears to be increased by having typhoid fever or by receiving typhoid vaccine. More work is needed to establish the extent to which this is true. It might even be possible to determine when typhoid protection exists by some such means.

6th. The bactericidal properties of blood are destroyed by the addition of 10 per cent bile to the test. More work should here be carried out to determine the minimum amount of bile that will have this effect and if it is the same in all cases.

7th. The microscopic Widal test, as made with a dried drop of blood, is unreliable and should be discontinued.

8th. The macroscopic Widal test is simple, reliable and practical. When used in conjunction with the blood culture, we have a laboratory procedure which will seldom, if ever, mislead. This test should be a part of the work of every diagnostic laboratory.

9th. The low agglutination titre in vaccinated persons will usually avoid a falsely positive reaction. A second test later in the disease will always prevent this.

10th. Stool examinations are often valuable and should be available.

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