AGGLUTINATION EXPERIMENTS WITH TYPHOID BACILLI ISOLATED FROM THE BODY.

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It is well known that typhoid bacilli, freshly isolated from enteric fever patients, vary in their capacity to undergo agglutination. The following series of experiments was carried out with the object of discovering whether these differences in agglutinability are dependent on the stage of the disease at which the bacillus is isolated, or on the body substance from which it is obtained.

The bacilli were grown from patients suffering from enteric fever, chiefly from blood, faeces, and urine, but also from vesicular rose-spots, and (post-mortem) from the spleen, bile, and mesenteric glands. They were tested by such fermentative and other methods as were considered sufficient to establish their identity as typhoid bacilli. An agglutinating serum was obtained by the inoculation of a rabbit with the stock typhoid bacillus in use in the hospital laboratory. For purposes of comparison, the bacilli were tested also with the sera of rabbits immunized in the same way against four strains of paratyphoid organisms.

In most instances in which a bacillus was isolated, it was tested also with the patient's serum, and the result compared with the results of agglutination by the stock antisera.

Finally, the bacilli were examined according to the method described by Michaelis (1911) of agglutination by means of acid solutions of varying strengths.

The bacilli were isolated from blood by adding blood withdrawn from a vein in the arm to bouillon (250 c.c.), or ox-bile (10 c.c.), and

incubating. The best results were obtained when 5 c.c. of blood was added to 10 c.c. of bile. Cultures from faeces were made according to the method described by Kendall and Day (1911). The isolating medium was a modified Endo medium, which contained 1·5 % of agar instead of Endo's 4 %, and which was made just alkaline to litmus instead of strongly alkaline. A platinum loopful of faeces was mixed in 10 c.c. of bouillon at incubator temperature, the tube was incubated for an hour, and three loopfuls of this bouillon were then spread on a modified Endo plate by means of a right-angled glass rod. The plate was incubated overnight. The number of colonies obtained on a plate by this means varied considerably, but 30–40 was common. The cultures from urine were made in the same way as those from faeces. From vesicular rose-spots, spleen, gall-bladder, and mesenteric glands, the cultures were made directly on agar, and were always found to be pure.

The organisms were examined after isolation, and the following tests were considered sufficient to establish their identity as typhoid bacilli:

the production of acid without gas in glucose, maltose, and mannite;

the production of slight permanent acidity, without clotting, in litmus milk;

non-fermentation of lactose and saccharose;

non-production of indol in peptone water after 7 days' growth;

non-liquefaction of gelatin;

colourless growth on potato;

presence of motility.

The agglutination reactions were carried out by the microscopic method. The blood was collected in the usual way from the ear in capillary tubes, and the serum obtained was diluted with saline solution by a measuring pipette to 1–12½, 1–25, 1–50, 1–100... the series being continued until a dilution was found at which no agglutination occurred. The highest dilution in which slight but distinct agglutination occurred was reckoned as the limiting dilution. The bacilli were used in an 18–24 hours' bouillon culture. The use of this instead of an emulsion of a culture on agar slopes had always given reliable results with the laboratory organism.

Agglutinating sera were obtained by inoculating rabbits with a typhoid strain and four paratyphoid strains. Injections were given intraperitoneally at intervals of 10 days—1 c.c., 2 c.c., and 4 c.c. of a killed

24 hours' bouillon culture being introduced in successive inoculations. The animals were killed 10 days after the 3rd inoculation.

The following bacilli were used for immunization of the rabbits:

(1) *B. typhosus*, a stock strain used for about five years in the City of Glasgow Fever Hospitals for Widal reactions, and obtained originally from the spleen of a patient who died of enteric fever. This had proved a trustworthy organism.

(2) *B. paratyphosus A* (Brion-Kayser), which I received along with (3) and (5) from Dr R. M. Buchanan, Public Health Laboratory, Glasgow. These three strains had been obtained from Kral some time previously.

(3) *B. paratyphosus A* (Schottmüller).

(4) *B. paratyphosus B* (Schottmüller), obtained from Leeds, and brought originally from Vienna by Professor Grünbaum.

(5) *B. paratyphosus B* (Achard).

It was shown by experiment that growth of the laboratory bacillus in bile, and also on the modified Endo medium, did not alter its capacity to undergo agglutination.

Forty-six strains of *B. typhosus* were isolated and tested:

From	blood	17
,,	faeces	14
,,	urine	8
,,	vesicular rose-spot ...	1
,,	spleen	4
,,	gall-bladder	1
,,	mesenteric gland ...	1
	Total	46

These 46 bacilli were isolated from 42 patients. In three instances more than one bacillus was grown. In the first, a bacillus was obtained from blood and from urine, in the second from a rose-spot and from faeces, and in the third from the spleen, from the gall-bladder, and from a mesenteric gland.

In the study of the results obtained, attention was paid not only to the day of the disease on which a bacillus was isolated, and to its source, but also to the length of time which elapsed between its isolation and the day on which the tests were made. Certain observers have reported variations in agglutinability dependent on this circumstance, and bacilli found to be non-agglutinable, or only slightly agglutinable on isolation, have sometimes become completely agglutinable after standing for two or three months.

Agglutination by anti-typhoid serum.

The limit of agglutination of the stock bacillus with its own antiserum occurred at 1 : 80,000.

The 46 bacilli from patients were divided into three classes:

(1) Those agglutinated approximately as well as the stock typhoid bacillus (limiting dilution 1 : 80,000–1 : 45,000).

(2) Those in which there was moderate agglutination (limiting dilution 1 : 25,000–1 : 1600).

(3) Those in which agglutination was slight (limiting dilution 1 : 100–1 : 25), or absent at 1 : 25.

Class 1 includes 22 strains.
„ 2 „ 17 „
„ 3 „ 7 „

Of the 22 bacilli in Class 1, 5 were agglutinated quite as well as the stock typhoid bacillus. One of these was grown from a vesicular rose-spot, 1 from blood, 1 from the spleen, and 2 from faeces. Two bacilli showed no agglutination at 1 : 25—one from blood, and the other from the spleen.

Class 3 includes 1 bacillus isolated in the 1*st week*.

In the 2*nd week*, 17 bacilli were isolated:

Class 1 includes 11
„ 2 „ 4
„ 3 „ 2

In the 3*rd week*, 19 bacilli were isolated:

Class 1 includes 9
„ 2 „ 8
„ 3 „ 2

In the 4*th week*, 6 bacilli were isolated:

Class 1 includes 2
„ 2 „ 3
„ 3 „ 1

In the 5*th week and after*, 3 bacilli were isolated:

Class 1 includes 0
„ 2 „ 2
„ 3 „ 1

There was thus a distinct tendency for the earlier isolated bacilli to be agglutinated better than those obtained later. The earliest bacillus, however, grown on the 3rd day, was not agglutinable.

From the *blood*, 17 bacilli were grown:

Class	1	includes	6
,,	2	,,	7
,,	3	,,	4

From the *faeces*, 14 bacilli were grown:

Class	1	includes	11
,,	2	,,	3
,,	3	,,	0

From the *urine*, 8 bacilli were grown:

Class	1	includes	2
,,	2	,,	5
,,	3	,,	1

Class 1 includes also 2 bacilli from the spleen, and the bacillus from a rose-spot.

Class 2 includes the bacillus from the gall-bladder, and the bacillus from a mesenteric gland.

Class 3 includes 2 bacilli from the spleen.

A striking difference was thus evident between the agglutinability of the bacilli from faeces, and those from blood and urine, the bacilli from faeces being agglutinated much better than those from the two other sources.

The resemblance in respect of agglutinability between the bacilli from blood and those from urine suggests that the former come from the blood into the urine (which is the accepted view), and do not pass directly from the intestine to the bladder, as has been suggested by Blumer (1895).

It is worthy of note that in the case where three strains were obtained from one individual after death, the bacillus from a mesenteric gland was agglutinated to 1:25,000, that from the gall-bladder to 1:15,000, and that from the spleen not at all.

The length of time which elapsed between the isolation and testing of the bacilli appeared to have a slight influence on those obtained from the blood. The average number of days in this period for the 17 bacilli from blood was:

in Class 1 (6 bacilli) 139 days
„ „ 2 (7 „) 120 „
„ „ 3 (4 „) 79 „

In the case of the 14 bacilli from faeces the average number of days was:

in Class 1 (11 bacilli) 61 days
„ „ 2 (3 „) 70 „

The bacilli in the other groups were too few to give comparable results.

The average time between the isolation and the examination of the bacilli from the blood was 117 days, and of the bacilli from the faeces 63 days. If then agglutinability developed with the lapse of time, the difference in the agglutination reactions of these two groups of bacilli must originally have been even greater, for the bacilli from the faeces, which were more agglutinable, had been kept a shorter time.

The average age of all the bacilli in Class 1 was 88 days, of those in Class 2, 93 days, and of those in Class 3, 72 days.

Agglutination by anti-paratyphoid A (Brion-Kayser) serum.

This serum agglutinated its own bacillus in a limiting dilution of 1:3000.

The stock typhoid bacillus was not affected at 1:25.

The bacilli isolated from patients were divided into three classes:

(1) Those with the agglutination limit between 1:100 and 1:50 (1 bacillus).

(2) Those with the agglutination limit between 1:40 and 1:25 (14 bacilli).

(3) Those showing no agglutination at 1:25 (31 bacilli).

	Limit of agglutination			
Week of isol.	1:100–1:50	1:40–1:25	No aggl.	Total
1st	0	1	0	1
2nd	1	7	9	17
3rd	0	5	14	19
4th	0	1	5	6
5th and after	0	0	3	3
Source of bac.				
Blood	0	3	14	17
Faeces	1	8	5	14
Urine	0	1	7	8

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Class 2 includes also bacilli from spleen (1) and mesenteric gland (1).

Class 3 includes also bacilli from spleen (3), rose-spot (1), and gall-bladder (1).

There was a tendency for the earlier isolated bacilli to be better agglutinated.

As with the anti-typhoid serum, the bacilli from faeces were better agglutinated than those from blood or from urine, and the bacilli from urine again resembled those from blood rather than those from faeces.

The results were independent of the age of the bacilli. The average age of the bacilli in Class 1 was 33 days, of those in Class 2, 93 days, and of those in Class 3, 100 days.

Agglutination by anti-paratyphoid A (Schottmüller) serum.

This serum agglutinated its own bacillus in a limiting dilution of 1 : 200,000.

The stock typhoid bacillus was agglutinated to 1 : 60.

The 46 bacilli were divided into three classes:

(1) Those with the agglutination limit between 1 : 350 and 1 : 200 (6 bacilli).

(2) Those with the agglutination limit between 1 : 190 and 1 : 50 (28 bacilli).

(3) Those with the agglutination limit between 1 : 40 and 1 : 25, and those showing no agglutination at 1 : 25 (12 bacilli).

	Limit of agglutination			
Week of isol.	1 : 350—1 : 200	1 : 190—1 : 50	1 : 40 and less	Total
1st	0	1	0	1
2nd	3	10	4	17
3rd	2	13	4	19
4th	1	2	3	6
5th and after	0	2	1	3
Source of bac.				
Blood	0	9	8	17
Faeces	3	10	1	14
Urine	1	4	3	8

Class 1 includes also bacilli from gall-bladder (1), and mesenteric gland (1).

Class 2 includes also bacilli from spleen (4), and rose-spot (1).

The degree of agglutination was independent of the time of isolation of the bacillus.

With this serum also the bacilli from faeces were agglutinated considerably better than those from blood.

The time between the isolation and testing of the bacilli in Class 1 was 82 days, of those in Class 2, 97 days, and of those in Class 3, 133 days. Agglutination seemed to be diminished rather than increased with increase in the age of the organism.

Agglutination by anti-paratyphoid B (Schottmüller) serum.

This serum was the most active obtained and agglutinated its own bacillus to 1 : 800,000.

The stock typhoid bacillus was agglutinated to 1 : 150.

The bacilli were divided into three classes :

(1) Those with the agglutination limit between 1 : 400 and 1 : 200 (8 bacilli).

(2) Those with the agglutination limit between 1 : 190 and 1 : 50 (32 bacilli).

(3) Those with the agglutination limit between 1 : 40 and 1 : 25, and those not agglutinated at 1 : 25 (6 bacilli).

	Limit of agglutination			
Week of isol.	1 : 400—1 : 200	1 : 190—1 : 50	1 : 40 and less	Total
First 3 weeks	8	26	3	37
4th week and after	0	6	3	9
Source of bac.				
Blood	2	12	3	17
Faeces	3	10	1	14
Urine	1	5	2	8

Class 1 includes also bacilli from spleen (1), and mesenteric gland (1).

Class 2 includes also bacilli from spleen (3), rose-spot (1), and gall-bladder (1).

The bacilli obtained in the first three weeks were agglutinated better than those grown later.

The bacilli from faeces were agglutinated in somewhat higher dilutions than those from blood, but the difference was not so marked as with the preceding sera.

The average age of the bacilli in Class 1 was 91 days, of those in Class 2, 111 days, and of those in Class 3, 138 days. Agglutinability thus did not increase with age.

Agglutination by anti-paratyphoid B (Achard) serum.

This serum agglutinated its own bacillus in a limiting dilution of 1:70,000.

The stock typhoid bacillus was agglutinated to 1:400.

The bacilli were divided into three classes :

(1) Those with the agglutination limit between 1:400 and 1:200 (11 bacilli).

(2) Those with the agglutination limit between 1:190 and 1:50 (28 bacilli).

(3) Those with the agglutination limit between 1:40 and 1:25 (7 bacilli).

	Limit of agglutination			
Week of isol.	1:400—1:200	1:190—1:50	1:40—1:25	Total
First 3 weeks	10	23	4	37
4th week and after	1	5	3	9
Source of bac.				
Blood	4	9	4	17
Faeces	4	9	1	14
Urine	1	5	2	8

Class 1 includes also bacilli from rose-spot (1), and mesenteric gland (1).

Class 2 includes also bacilli from spleen (4), and gall-bladder (1).

The bacilli isolated in the first three weeks were agglutinated somewhat better than those obtained later.

The bacilli from faeces were agglutinated a little better than those from blood, but the difference was too slight to justify any conclusion.

The average age of the bacilli in Class 1 was 96 days, of those in Class 2, 84 days, and of those in Class 3, 122 days. Agglutinability did not increase with the age of the organisms.

General conclusions from the results of the experiments with antisera.

(1) The bacilli which were isolated earlier in the disease tended to be agglutinated better by artificial antisera than those isolated later, the difference being most marked between those obtained in the first three weeks, and those grown after the end of that time.

(2) The bacilli isolated from faeces were agglutinated much better by anti-typhoid serum, and somewhat better by anti-paratyphoid serum than those grown from the blood.

(3) The bacilli isolated from urine resembled those grown from blood rather than those from faeces.

(4) The length of time which elapsed between the isolation of the bacilli and their examination exercised no appreciable influence on their power to undergo agglutination.

Agglutination reactions of bacilli with the serum of the patient from whom each was isolated.

The results of the agglutination reactions carried out with the patients' sera and the respective bacilli come now to be considered.

Thirty-seven bacilli were investigated in this way:

From	blood	...	13
,,	faeces	...	14
,,	urine	...	8
,,	rose-spot	...	1
,,	spleen	...	1

In this series of experiments no fixed standard of comparison in respect of a limiting dilution was available, as the sera varied in activity. The results obtained were therefore compared quantitatively with the results of the agglutination tests with the same sera and the stock typhoid bacillus. In a few instances, it was found that the patient's bacillus was agglutinated rather better than the stock bacillus by his own serum, but the difference was so slight that I found it convenient to regard the result of agglutination with the stock bacillus as the maximum in each case.

In these cases the tests were carried out within a few days of the isolation of the bacilli.

The 37 bacilli were divided into three classes:

Class 1 includes those which were agglutinated approximately as well as the stock typhoid bacillus.

Class 2 those which were agglutinated distinctly less well than the stock typhoid bacillus.

Class 3 those which were agglutinated much less well than the stock typhoid bacillus, or were not agglutinated.

In Class 1 the ratio of the agglutination limit of the autogenous bacilli to that of the stock bacillus was from a little above 1 to $\frac{1}{2}$.

In Class 2 the ratio was from $\frac{1}{2}$ to $\frac{1}{6}$.
In Class 3 the ratio was below $\frac{1}{16}$.

Class	1	includes	26	bacilli
„	2	„	8	„
„	3	„	3	„

Class 1 includes 1 bacillus isolated in the 1*st week*.
Of 17 bacilli isolated in the 2*nd week*

Class	1	includes	13
„	2	„	4
„	3	„	0

Of 13 bacilli isolated in the 3*rd week*

Class	1	includes	10
„	2	„	1
„	3	„	2

Of 6 bacilli isolated in the 4*th week and after*

Class	1	includes	2
„	2	„	3
„	3	„	1

It is evident that the capacity of the bacilli to undergo agglutination was independent of the time of isolation.

Of 13 bacilli isolated from *blood*

Class	1	includes	12
„	2	„	1
„	3	„	0

Of 14 bacilli isolated from *faeces*

Class	1	includes	8
„	2	„	5
„	3	„	1

Of 8 bacilli isolated from *urine*

Class	1	includes	4
„	2	„	2
„	3	„	2

Class 1 includes also the bacilli from rose-spot and spleen.

The bacilli from blood were very much better agglutinated than those from either faeces or urine.

General conclusions from results of experiments with patients' sera.

(1) With the patient's serum no connection could be made out between the time of isolation of a bacillus and the degree of agglutination present.

(2) The bacilli from blood were agglutinated very well indeed, and very much better than those from faeces.

Results of agglutination tests with the patients' sera and the artificial serum compared.

The 37 bacilli tested with their respective patient's serum were included in the 46 tested with the artificial sera. A comparison of the results of agglutination tests on the bacilli with the two kinds of serum, the patient's and the anti-typhoid, shows striking differences.

With regard to the time of isolation, as has been seen, the anti-typhoid serum agglutinated bacilli isolated in the earlier stages of the disease much better than those obtained later; whereas with the patients' sera no such difference was found to exist. No connection could be made out in the latter instance between the time of isolation of the bacillus and the degree of agglutination present.

A consideration of the bacilli from the point of view of their origin showed the following results. (The classes were arranged as before.)

Class 1 includes bacilli agglutinated approximately as well as the stock typhoid bacillus.

Class 2 includes bacilli agglutinated distinctly less well than the stock bacillus.

Class 3 includes bacilli agglutinated much less well than the stock bacillus, or not at all.

Of 13 bacilli isolated from *blood*

	With the anti-typhoid serum	With the patient's serum
Class 1 includes	3	12
,, 2 ,,	6	1
,, 3 ,,	4	0

Of 14 bacilli isolated from *faeces*

	With the anti-typhoid serum	With the patient's serum
Class 1 includes	11	8
,, 2 ,,	3	5
,, 3 ,,	0	1

Of 8 bacilli isolated from *urine*

	With the anti-typhoid serum	With the patient's serum
Class 1 includes	2	4
,, 2 ,,	5	2
,, 3 ,,	1	2

Class 1 includes also bacilli from rose-spot and spleen.

While with the anti-typhoid serum the bacilli from blood were less well agglutinated than those from faeces, with the sera of the respective patients they were agglutinated very well indeed, and very much better than those from faeces. The latter responded less well, indeed, to the agglutinative action of the autogenous serum than to that of the anti-typhoid serum. Here again the bacilli grown from urine resembled those obtained from blood rather than those from faeces, agglutination with the patient's serum being decidedly better than with the anti-typhoid serum.

Summary of comparison of results with anti-typhoid serum and with patients' sera.

(1) With the anti-typhoid serum the bacilli isolated earlier in the disease were agglutinated much better than those obtained later; with the patients' sera, no such difference was found.

(2) With the anti-typhoid serum the bacilli from faeces were agglutinated much better than those from blood; with the patients' sera, the bacilli from blood were agglutinated very much better than those from faeces. These results were obtained with the same strains of bacilli.

(3) The bacilli from faeces were agglutinated rather less well by the patients' sera than by the anti-typhoid serum.

Discussion of variations in agglutinability.

It is known that under certain circumstances bacilli which normally are well agglutinated by an appropriate serum may become non-agglutinable (Paltauf, 1912). One of these circumstances is the passage of the bacillus through the body of man or an animal. According to Porges and Prantschoff (1906) "lessened agglutinability is chiefly observed in cultures freshly isolated from the body, or passed through animals: in bacilli from exudates: and in bacilli which have been passed through media containing agglutinins."

The cause of this phenomenon has been variously regarded. Porges (1905) showed that typhoid bacilli which had been rendered completely non-agglutinable by heating to 80° C. had their agglutinability restored by washing in normal saline solution. He supposed that the nuclein split off the nucleo-protein of the organisms was the substance which inhibited agglutination, and that when this was removed by washing, the bacilli again became agglutinable. The capsulated bacteria, such as Friedländer's bacillus, are normally non-agglutinable, and Porges and Prantschoff (1906) attributed this to increased formation of protein. In four non-agglutinable strains of typhoid bacilli, isolated from spleens, these observers thought they could detect the presence of a capsule.

Another suggestion (Paltauf) is that certain strains of typhoid bacilli are really composite strains containing both agglutinable and non-agglutinable members. Under certain circumstances, the latter may come to predominate.

Culture in agglutinin-containing media brings about non-agglutinability. Sacquépée (1901) caused strains of typhoid bacilli to become less agglutinable by growing them in collodion sacs in the peritoneal cavity of rats immunized against *B. typhosus*. The change, however, took place slowly, and it was only after treatment of a series of subcultures in the same way that the agglutinability was reduced, at the end of five months, to $\frac{1}{6}$ of its original standard. He concluded that non-agglutinable strains in man were produced by the growth of the organism in an infected or immunized body.

Numerous observers have recorded the isolation of typhoid bacilli which were agglutinated only slightly or not at all (Horton Smith (1900), Remy (1901), Sacquépée (1901), Cambier (1902), Emery (1902), Nicolle and Trenel (1902)). These bacilli have fulfilled all the tests for typhoid bacilli, and in certain cases it has been found that immunization of animals against feebly agglutinable strains resulted in the production of a serum which agglutinated laboratory strains.

According to Paltauf, not infrequently agglutination is lessened with the patient's serum as well as with an artificial anti-typhoid serum.

A slow rise on the part of these bacilli to a normal standard of agglutinability has been described by most authors as taking place, either after a certain number of subcultures, or simply by the lapse of time, without subculture. (Cambier, Emery, Porges and Prantschoff, Lipschütz (1904).) Porges and Prantschoff found that four non-agglutin-

able strains from spleens were agglutinated as well as stock bacilli after about 15 subcultures on agar, and Lipschütz noted a similar rise in the case of three typhoid strains isolated from urine. In the latter instance, these three strains were not definitely agglutinated in a dilution of 1 : 200 by an active serum (agglutinating to 1 : 20,000), whereas, three months later, without subculturing, they were agglutinated by the serum to 1 : 20,000.

A change in the characters of an organism by its presence in an animal body is referred to by Besredka (1909), in a criticism of work done by Aronson (1902, 1903) on streptococci. Aronson had endeavoured to prove the identity of streptococci from various sources by means of experiments on animals. He immunized horses with a streptococcus which he had rendered extremely virulent by passage through a series of mice, and found that streptococci from other sources, also rendered virulent by passage through mice, were acted on by the serum of these horses equally with the original strain used for immunization. From this he concluded that all the streptococci tested were essentially the same. Besredka criticised Aronson's conclusions on the ground that all his streptococci had been modified by their passage through mice, and that each strain had become what he called "un streptocoque de passage."

Conclusions from personal observations.

From what has been said it is plain that when typhoid bacilli circulate in the blood they sometimes undergo a change which manifests itself in diminished agglutinability. The results of my experiments seem to show that the bacilli in the faeces are less changed from the original agglutinable type than those in the blood, which are acted on to a much greater extent by the body fluids. But this explanation, that the bacilli become non-agglutinable by growth in the body of a person whose blood contains immune substances, does not account for the fact that the bacilli isolated from blood were practically all agglutinated by the serum in which they were circulating as well as was the stock typhoid bacillus. It may be that after the organisms have been modified by the action of the serum, some alteration in the serum itself is called forth by the change in the bacilli, and this might account for the fact that the bacilli grown from faeces were agglutinated rather worse by their respective patient's serum than by the artificial serum.

As has been shown, the bacilli isolated earlier in the disease were agglutinated better than those obtained later, and this, so far as it goes, is in favour of Sacquépée's theory that non-agglutinability is produced by growth in a body containing immune substances. It is to be noted, however, that a bacillus isolated from the blood on the 3rd day of illness was unacted on by the anti-typhoid serum at 1 : 25. On the day on which this bacillus was obtained, the patient's serum caused no clumping of the stock bacillus at 1 : 25. The non-agglutination of this bacillus, therefore, must have been due to some cause other than growth in an agglutinin-containing medium.

Agglutination of bacilli by acid solutions (Michaelis).

Another method was employed in the attempt to differentiate the bacilli which had been already tested by antisera.

Michaelis (1911) pointed out that many strains of bacteria are agglutinated by acids, and that a fixed degree of acidity corresponds to the maximum of agglutination. This maximum, he says, is characteristic for individual strains of bacteria, and can be used as a help in their identification.

The test is carried through as follows:

The bacillus to be examined is grown on agar slopes for 24 hours and is then emulsified in distilled water, the emulsion being rather denser than that used for a Widal reaction. The following six solutions are required:

	Normal sodium hydrate	Normal acetic acid	Distilled water
1	5 c.c.	7·5 c.c.	87·5 c.c.
2	5	10	85
3	5	15	80
4	5	25	70
5	5	45	50
6	5	85	10

1 c.c. of each of these solutions is put into each of a series of six test-tubes, and to each tube is added 3 c.c. of the bacterial emulsion. The tubes are then shaken up and put in the incubator at 37° C. When the first agglutination appears, the row of tubes is taken from the incubator and left at room temperature for some time. In any case the tubes are not kept at 37° C. for more than an hour.

According to Michaelis, with typhoid bacilli agglutination occurs only in tubes 3, 4, and 5, as a general rule. It is commonly most

marked in tube 3, though occasionally in 4, and in 2 and 5 is much slighter, if it occurs in these at all. Tube 3 is therefore reckoned as the optimum for *B. typhosus.*

B. paratyphosus has its optimum in tubes 5 and 6, but the A and B strains cannot be distinguished from one another.

B. coli is usually not agglutinated.

Rost (1911) applied the test to eight strains of *B. typhosus,* a paratyphoid A strain, a paratyphoid B strain, and other organisms. The results he obtained with typhoid bacilli agreed with those of Michaelis; with the paratyphoid B there was marked agglutination in tube 6, and with the paratyphoid A no agglutination. He concluded that the method is "a valuable addition to our resources for diagnosing typhoid."

A later investigator, Jaffé (1912), criticised the method. He tested 41 strains of *B. coli,* 40 of *B. typhosus,* 11 of *B. paratyphosus A*, three of *B. paratyphosus B,* and three of *B. typhi murium,* with unsatisfactory results. Eleven of the *B. coli* strains showed agglutination, and the test gave no assistance in the differentiation of the atypical members of the *B. coli* group. With *B. typhosus* the results were no more certain. In the 40 strains the optimum occurred in tubes 2 and 3. In 22 agglutination was present only in 1 or 2 tubes, in five from tube 2 to tube 6, and in one in all 6. (It was found that this last bacillus was agglutinated by distilled water.) In the case of four of the bacilli no agglutination occurred. Of the 11 paratyphoid B strains, two showed agglutination in tubes 4 and 5, eight in tubes 4, 5, and 6, and one from tube 3 to tube 6. The optimum varied. Of the three paratyphoid A strains, two were agglutinated in tubes 4, 5, and 6, and one in tubes 3 to 6. Here also the optimum varied.

This method was applied to the 46 bacilli tested by means of the antisera, with the exception of one from blood, which had died out: to two stock typhoid strains, the laboratory bacillus, and the R.A.M.C. typhoid strain (Rawlings)[1]; to the four stock paratyphoid strains; and to a strain of *B. coli.*

By accident, a bacillus was tested twice on different days, and this was discovered only when the results were tabulated. The results obtained were precisely the same on each occasion. This points to a constancy of the results obtained by the method.

With regard first to the stock typhoid bacilli, both were agglutinated in tubes 3 to 6, the laboratory strain having its maximum in tube 4, and

[1] Kindly supplied to the hospital by Sir William Leishman.

B. typhosus (Rawlings) in tubes 3–5. With the four paratyphoids, the maximum occurred in each case in tube 6, agglutination in the case of Brion-Kayser A being slight, and present only in this tube, while with the three others there was some agglutination also in tube 5. *B. coli* was unaffected.

The agglutination which took place in the case of the 45 bacilli from patients varied in extent and degree, but the maximum was found to occur as follows:

In tube 1	... ...	0 times
,, 2	... ...	0 ,,
,, 3	... ...	23 ,,
,, 4	... ...	3 ,,
,, 5	... ...	4 ,,
,, 6	... ...	5 ,,
In tubes 4, 5 and 6	... ...	1 ,,
,, 5 and 6	... ...	1 ,,
No agglutination	... ...	8 ,,
	Total	45 ,,

That is to say, in half the cases the maximum of agglutination occurred in tube 3, which Michaelis regarded as typical for *B. typhosus.*

The place of occurrence of the maximum was independent of the time of isolation, and the only difference among the bacilli from the point of view of their origin was that the bacilli from urine seemed to be less "typical" in reaction than those from blood or faeces.

With regard to the number of tubes showing a reaction in each case, agglutination was found more frequently in the combination of tubes 3 to 5 than in any other. In four instances irregular agglutination was present but in each of these the maximum occurred in tube 3. In nine cases agglutination was of Michaelis' "paratyphoid type." In four of the latter agglutination was present only in tubes 5 and 6. In one case in which it was found in tubes 4–6, and in four others with agglutination in tubes 3–6, the maximum occurred in tube 5 or tube 6.

The bacilli showing the "paratyphoid reaction" with acids were not better agglutinated than the others by the anti-paratyphoid sera.

From these results it seems that the test is of interest rather than value in the examination of typhoid bacilli.

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