AN INVESTIGATION INTO THE AGGLUTINATING PROPERTIES OF CERTAIN SERA AGAINST *B. TYPHOSUS* AND *B. ENTERITIDIS* GAERTNER (DELÉPINE, 7160).

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THE present investigation was undertaken at the suggestion and under the supervision of Professor E. J. McWeeney, as part of the work done at the Bacteriological Department, University College, Dublin, National University of Ireland, in connexion with the scheme of the Medical Research Committee.

A comparatively large number of sera was sent in to this laboratory by the Irish military authorities for the Wassermann test, and as these sera are for the most part taken from men who had received antityphoid inoculation it was thought a good opportunity of estimating their agglutinating power for *B. typhosus* by Dreyer's method, and to find out whether any of them agglutinated a certain strain of Gaertner (Delépine, 7160). As a result of investigations made by Delépine and Dawson¹ with a culture of *B. enteritidis* Gaertner (7160), isolated by the former, the claim is made that this bacillus 7160 is agglutinated by the serum of patients suffering from enteric fever and is unaffected by the sera of antityphoid-inoculated individuals. According to these authors, therefore, 7160 can be used as a valuable means of making a definite diagnosis of enteric fever in a febrile antityphoid-inoculated person when the mere agglutination of typhoid bacilli alone cannot be relied upon. To estimate fully the value of this observation it would be necessary to test the following sera for the presence or absence of agglutinins for 7160: Class A, the sera of normal non-inoculated persons; Class B, the sera of inoculated, though otherwise normal persons; Class C, the sera of non-inoculated enteric fever patients; and Class D, the sera of enteric fever patients who had been previously inoculated.

The sera which were submitted to me for examination fell under Class B—namely, the sera of persons who had received antityphoid inoculation and who were at the time not suffering from enteric fever or food poisoning. The only way in which the persons differed from normal healthy inoculated individuals was that they either had or were suspected of having syphilis. The question as to how far the presence or absence of syphilis may be considered to affect the agglutinin content of a serum will be dealt with later on.

At the beginning of the investigation it was thought sufficient to obtain information as to the date of the last inoculation, together with such clinical data as appeared important during the course of the research. It was soon realised, however, that much would be gained by obtaining more complete details, such as the number of times the men had been inoculated and the existence of any previous history of enteric fever. Also, in view of Tidy's contention³ "that pyrexia or pyrexial conditions lead to the removal from the serum of agglutinins due to inoculation," the importance as to whether the men were in the secondary (frequently febrile stage) of syphilis became apparent.

I have to express my thanks to the officers of the Royal Army Medical Corps at Portobello Hospital for their kindness in furnishing me with the details of the cases.

Technique.—The method adopted for all cases was the quantitative macroscopic agglutination test of Dreyer, which is advocated by Ainley Walker² and others, by means of which the agglutinin content of each serum is stated as the number of standard agglutinin units per 1 c.c. of serum. In the case of *B. typhosus* a standard agglutinable culture was prepared, stored in bulk, and standardised, 250 c.c. being taken as required, each 250 c.c. being again standardised against standard culture and antiserum kindly provided by Dr. Gardner, of the Standards Laboratory, Oxford. The positive results obtained with the sera against the standard typhoid culture are stated as standard agglutinin units per 1 c.c. of serum examined.

In the case of *B. Gaertner* (7160) a culture was used which had been kindly forwarded by Professor Delépine to Professor McWeeney. From this a uniformly agglutinable formalinised culture was made. This could not, however, be standardised in the same manner as in the case of *B. typhosus* owing to the lack of a standard specific antiserum, so that the results in the case of this micro-organism are expressed as the highest dilution of serum with which agglutination was obtained. This culture was found to give uniform results with positive and negative cases, the positive agglutinations exhibiting the same naked-eye clumping as the Oxford standard agglutinable typhoid cultures did with antityphoid serum. This formalinised 7160 culture was not affected by the Oxford standard antisera for *B. typhosus*, *B. paratyphosus* A and B, when put up in the dilutions recommended for standardisation of cultures.

In dealing with a comparatively large number of sera at a time it was found advisable to modify the details of the original Oxford method to some extent, though its general scheme was followed throughout.

The primary 1 in 10 dilution of serum was made with calibrated pipettes, the drop-method being used for the subsequent dilutions. The saline solution for making the further dilutions of serum was kept in a double-controlled burette. This was made by attaching the dropping pipette to a separating funnel (with tap) by means of a short length of rubber tubing, a screw clip being fixed in this connexion. The whole apparatus was fixed in an ordinary burette stand with one holder for the funnel and another to steady the dropping pipette. A double control was thus maintained—viz., (1) the glass funnel tap for the main flow of saline; and (2) the screw clip for regulating the drops.

In practice the advantages of this reservoir dropper can be set down as follows:—(a) The dropping pipette is kept vertical and delivers uniform drops at a uniform rate. (b) It is easy to manipulate, as many racks full of agglutination tubes can be filled as required by moving each tube in turn beneath the dropper. (c) If at any time it is found necessary to stop the flow of saline the funnel tap can be turned off without disturbing the screw clip, which regulates the size of the drop. (d) The dropping pipette can be readily detached and used for adding the serum, so that the same dropping pipette is used throughout the experiment.

It was also found that the original zinc racks provided with the Oxford outfit (though extremely useful) take up a large amount of space in a water-bath, and if there be much variation in the size of the agglutinating tubes used the mixtures of saline, serum, and culture, though of the same volume, will stand higher in some tubes than in others, and thus may be partly above the level of the water in the bath. To obviate these two drawbacks a sheet of strong cork half an inch thick was perforated with holes half an inch apart, to hold 15 to 20 rows of five tubes. The tubes are pushed through the cork as far as their rims, and when the whole rack is placed in water it floats, with the important part of the tubes submerged, the cork rack taking up much less space than would be required by the requisite number of zinc racks. Index numbers can be readily marked with a grease pencil on the cork, if it be first rubbed over with a little xylol.

The water-bath was kept at 52° C. for two hours, and the results read off by artificial light.

It may be here noted that by Dreyer's method the variations in the intensity of the agglutination are very striking at different stages of dilution between the strongest positive reaction and a negative. On several occasions zone phenomena were observed—viz., with some sera no agglutination occurred in the 1 in 25 dilution, when marked clumping was present in the 1 in 50 or over.

Material for examination.—The sera were not particularly selected, each batch being dealt with in a routine fashion as they were received. Consequently, in drawing up the results the sera have to be grouped under various heads. The number of specimens of serum examined was 495. With regard to antityphoid inoculation, they may be divided into the following groups:—

Group I.—Sera of men about whom definite information as to number of inoculations and date of last inoculation was obtained	143
Group II.—Sera of men about whom information as to date of last inoculation only was obtainable	127
Group III.—Sera of men known to be non-inoculated	32
Group IV.—Sera of men about whom no information as to inoculation was obtainable	64
Group V.—Repeated examination of sera which had already been examined previously	129

Results.—It is proposed to deal with the results obtained in the following order.

I. Agglutination results with *B. typhosus*.—A. General results (inoculated cases, Groups I. and II.). B. Factors which influence the standard agglutinin content of the sera of inoculated individuals (Groups I. and II.). C. Influence of syphilitic lesions upon the typhoid-agglutinating power of the sera of antityphoid-inoculated men (Groups I. and II.). D. Remarks on the sera of inoculated cases, examined more than once for agglutinins against *B. typhosus* (Groups I. and II.). E. Results with cases known to have not been inoculated (Group III.).

II. Agglutination results obtained with *B. enteritidis* of Gaertner (Delépine, 7160).—A. Sera of antityphoid-inoculated individuals (Groups I. and II.). B. Influence of antityphoid inoculation on the agglutinating power of sera for bacillus 7160, Delépine (Groups I. and II.). C. Influence of syphilis on the agglutinating power of sera of antityphoid-inoculated individuals for Delépine, 7160 (Groups I. and II.). D. Remarks on sera (of inoculated cases) examined more than once for agglutinins against Delépine, 7160 (Groups I. and II.). E. Results with cases definitely known to have been uninoculated (Group III.). F. Results with sera of cases about whom information as to inoculation was not obtainable (Group IV.) and total results with 7160 (Groups I., II., III., and IV.).

III. General results with cases which had a previous history of typhoid fever (which occurred among Groups I., II., and III.).

IV. Summary.

I. AGGLUTINATION RESULTS WITH *B. TYPHOSUS*.

A. General Results (Inoculated Cases, Groups I. and II.).

Total number of sera of inoculated cases examined, 270. These may be divided into two groups—viz.:—

Group I. The sera of men about whom definite information as to number of inoculations and date of last inoculation was obtainable... 143

Group II. The sera of men about whom information as to date of last inoculation only was obtainable... 127

Note.—In the above cases the length of time which had elapsed between the date of inoculation and the date of examination was from one month to eight years.

General results: Group I.—Number of sera 143, of which 125 (87.5 per cent.) gave agglutination for *B. typhosus*, and 18 (12.5 per cent.) gave no agglutination.

Group II.—Number of sera 127, of which 112 (88.2 per cent.) gave agglutination for *B. typhosus* and 15 (11.8 per cent.) gave no agglutination.

When the general results of these two groups are combined:—Total number of sera of inoculated cases, 270: of these 237 (87.1 per cent.) gave agglutination with *B. typhosus*, and 33 (12.9 per cent.) gave no agglutination.

Results in Positive Cases: Group I.

No. of cases.	Contained up to—	Per cent. of total.	No. of cases.	Contained up to—	Per cent. of total.
77	25 a.u.	53.9	1	225 a.u.	0.7
25	50 "	17.5	1	450 "	0.7
10	75 "	7.0	1	500 "	0.7
5	100 "	3.5	1	550 "	0.7
2	175 "	1.4	1	900 "	0.7
1	200 "	0.7			

Group II.

33	25 a.u.	26.0	2	250 a.u.	1.6
34	50 "	26.7	2	275 "	1.6
16	75 "	12.6	3	300 "	2.3
3	100 "	2.3	1	550 "	0.8
4	125 "	3.2	1	575 "	0.8
5	150 "	4.0	1	725 "	0.8
3	200 "	2.3	1	900 "	0.8
1	225 "	0.8	2	1000 "	1.6

Groups I. and II.

110	25 a.u.	40.7	2	275 a.u.	0.7
59	50 "	21.8	3	300 "	1.1
26	75 "	9.7	1	450 "	0.3
8	100 "	3.0	1	500 "	0.3
4	125 "	1.4	2	550 "	0.7
5	150 "	1.8	1	575 "	0.3
2	175 "	0.7	1	725 "	0.3
4	200 "	1.5	2	900 "	0.7
2	225 "	0.7	2	1000 "	0.7
2	250 "	0.7			

a.u. = Agglutination units per 1 c.c.

B. Factors which Influence the Standard Agglutinin Content of the Sera of Inoculated Individuals.

It has been abundantly proved by numerous workers, one of the most recent being Dakeyne,⁵ that two factors influence the presence of agglutinins in the blood of inoculated individuals—namely, (1) the time elapsed between date of inoculation and date of examination of blood; and (2) the number of inoculations the person has received. Generally speaking, the more recently men have been inoculated the stronger will be the agglutinating power of their serum for *B. typhosus*; also fewer sera will be met with which may fail to agglutinate this bacillus. In addition, men who have been inoculated twice have a much greater chance of developing and retaining a reaction than those who have been inoculated once.

The majority of these results have been obtained by the microscopical method of performing Widal's reaction, and, as is pointed out by Dreyer and Ainley Walker,² the results obtained by different workers are not strictly comparable, as cultures of different strains of the same organisms are well known to vary in their agglutinability. Furthermore, a more or less variable standard of the actual strength of a given serum has to be adopted, such as strong reaction, moderate reaction, &c., with certain dilutions of serum. All workers will agree as to whether a serum gives a positive or negative reaction, and the majority will agree as to the difference between a strong and a weak reaction, but in gauging the finer differences between these two the personal element, together with the varying conditions under which different experiments may be carried out, render the results obtained by different workers not strictly comparable. On this account more stress has necessarily been laid upon the percentage of negatives obtained in one series of cases as compared with another, rather than a comparison of the degree of agglutinating power obtained with various positive sera.

By Dreyer's macroscopic standard method results can be uniformly recorded, and it has the distinct advantage that, in addition to being able to state that a case is positive or negative, it enables one to give an exact estimate as to the strength of a given serum in standard units. In addition, it is possible to estimate accurately the effect of time and the effect of the number of inoculations, on the serum of inoculated individuals which give a positive Widal's reaction.

Influence of number of doses of antityphoid vaccine on agglutinin content of sera of inoculated men.—Firstly it is necessary to determine what is the average agglutinin content of the sera of inoculated persons. This can be studied from the totals obtained in Groups I. and II.: total number of inoculated cases, 270. On reference to Chart 1 it will be seen that the average percentage of negatives is 12.9 per cent. of the total.

The remaining 87.1 per cent. sera which gave positive agglutination for *B. typhosus* may be divided up as follows:—40.7 per cent. gave up to 25 agglutinin units per 1 c.c.; 21.8 per cent. gave up to 50 units; 9.7 per cent. gave up to 75 units; 3.0 per cent. gave up to 100 units; and 1.4 per cent. gave up to 125 units. After this, comparatively few sera gave higher agglutinin units.

From this it will be seen that the greater number of sera giving positive agglutination gave only up to 25 agglutinin units; after this the percentage of those giving more units drops rapidly until it is found that sera containing more than 125 agglutinin units form but a small percentage of cases.

CHART 1.

Having determined the average agglutinin content of the sera of 270 inoculated persons, we can analyse the average agglutinin content of the sera of persons inoculated

CHART 2.

The continuous line indicates those inoculated once; the interrupted line those inoculated twice.

Influence of number of inoculations and lapse of time on agglutinin content of sera.—The influence of lapse of time can be ascertained by comparing the sera showing the highest titre, stated in terms of agglutinin units, obtained in the various months which have elapsed since the administration of antityphoid inoculation. In the first place it is necessary to determine what are the highest titres obtained in the sera of inoculated individuals taken as a whole. This may be studied from the total figures of Groups I. and II. Total number of inoculated cases, 270. On reference to Chart 3 it will be seen that at 1 to 2 months the highest number of agglutinin units obtained was 1000. From this maximum they drop suddenly at the third month to 575, and then with another sudden drop at the fourth month to between 300 and 100, where they remain up to the fourteenth month. After they are generally found below 100 till they disappear from the serum altogether. As to when agglutinin units do disappear it would be difficult to say from the cases in this investigation, but in certain cases up to 50 agglutinin units were found after 3 years, 25 after 4 and 5 years, though none were present in one case after 8 years since antityphoid inoculation had been received.

Note.—One case in Chart 3 has been omitted. This man was stated to have been inoculated 11 months prior to the time the serum was examined. His serum contained a very unusual number of agglutinin units—viz., 714. It is probable that some error arose in noting the date of inoculation.

How is the maximum number of agglutinin units per month, since inoculation was received, affected by the number of inoculations the person has received? An analysis of the sera examined under Group I. has been made and set down on Chart 4. Group I., inoculated cases, 143. Sera of those who have been inoculated once, 43; inoculated twice, 70. This chart seems to bear the following interpretation. In the second, third, fourth, and fifth months after inoculation the serum of those inoculated twice is richer in agglutinins than the serum of those who have been only once inoculated. This would probably be also the case during the first month, but unfortunately the number of sera received from men so recently inoculated was very small. After the fifth month there is but little difference noticeable between the cases which had received one or two inoculations.

CHART 4.

Continuous line, those inoculated once; interrupted line, those inoculated twice.

CHART 3.

Generally speaking, the sera of these twice inoculated are stronger during the second, third, fourth, and fifth months, and the results are more lasting in those twice

than those only once inoculated. Therefore, so far as the presence of agglutinins can be regarded as an index of a person's resistance against typhoid fever, a man twice inoculated shows strong immunisation up to the fifth month

TABLE I.—Group I.: Cases Inoculated Three, Four, Five, and Six Times.

Months between date of inoculation and date of examination.	Number of sera examined.	Agglutinin units.				Months between date of inoculation and date of examination.	Number of sera examined.	Agglutinin units.				
		-0	-25	-50	-75			-0	-25	-50	-75	-100
Inoculated 3 times.						Inoculated 4 times.						
2	1	...	1	...	3	1	1	...	...	...	...	
4	2	...	1	1	5	1	...	1	...	...	...	
5	1	...	1	...	7	2	...	2	...	...	...	
6	1	...	1	...	8	1	1	...	...	...	...	
7	1	...	...	...	1	Inoculated 5 times.						
9	3	...	3	...	8	1	...	...	...	...	1	
10	2	1	1	...	13	2	...	2	...	...	...	
11	3	1	1	1	18	1	...	1	...	...	...	
13	1	...	1	...	Inoculated 6 times.							
20	1	...	1	...	Totals...	1	...	...	1	...	...	
24	2	...	...	1	10	2	6	1	...	1		
3 years.	1	1	...	...	Grand total for cases inoculated 3, 4, 5, and 6 times.							
5 years.	1	1	...	...	30	6	16	5	2	1		
Totals...	20	4	10	4	Per cent.	...	20.0	53.3	16.6	6.7	3.4	

after the last inoculation, and is in a better position to withstand infection up to 14 months than a person only once inoculated.

C. Influence of Syphilitic Lesions upon the Typhoid-agglutinating Power of the Sera of Antityphoid-inoculated Men.

In view of Tidy's contention³ "that pyrexia or pyrexial conditions lead to the removal from the serum of agglutinins due to inoculation," the following arrangements of the results obtained with the sera of antityphoid-inoculated men in relation to the clinical condition, and the presence or ab-ence of a positive Wassermann test, may prove of interest. Professor McWeeney has kindly provided me with the clinical data and the results of the Wassermann test carried out by him. To make a fair analysis it is proposed to eliminate 42 sera from the total 270 (Groups I. and II.), so as to deal only with 228 which gave either a distinct + 3 or + 4 Wassermann, or a distinct negative, and to include only those cases about which full information as to the stage of the syphilitic lesion (primary or secondary) was available.

Total number of sera of antityphoid-inoculated men who either had or were suspected of having syphilis, 228. These may be divided into two sections (Table II.):—Those which gave a positive Wassermann, 105 (Section I.), and those which gave a negative reaction, 123 (Section II.).

TABLE II.

Section I.: Positive Wassermann. Section II.: Negative Wassermann.

Lesions.	No. of cases.	Positive for <i>B. typhosus</i> .	Negative for <i>B. typhosus</i> .	Lesions.	No. of cases.	Positive for <i>B. typhosus</i> .	Negative for <i>B. typhosus</i> .
Primary ...	59	50(84.7%)	9(15.3%)	Suspected primary	100	90(90.0%)	10(10.0%)
Secondary	46	44(95.6%)	2(4.4%)	Suspected secondary	23	19(82.6%)	4(17.4%)
Totals ...	105	94(89.4%)	11(10.6%)	Totals ...	123	109(88.6%)	14(11.4%)

General results.—From the foregoing it will be seen that the presence of a positive Wassermann does not influence the agglutinating power of the serum of inoculated individuals; 89.4 per cent. of those giving a positive Wassermann reaction, and 88.6 per cent. of those giving a negative Wassermann reaction, agglutinating *B. typhosus*. Further, although the number of sera is small for statistical purposes,

it will be noticed that the sera of patients with secondary syphilitic lesions (which are occasionally accompanied by some rise in temperature) appear to agglutinate typhoid in a higher proportion (95.6 per cent.) than the sera of those cases suffering from primary lesions (84.7 per cent.).

D. Sera Examined More than Once for Standard Typhoid Agglutinin Units (Groups I. and II.).

To judge properly the observations made on the sera examined more than once it is necessary to refer to the paper by Dreyer, Ainley Walker, and Gibson⁴ in order to form an idea as to what variations in titre may be expected in the sera of typhoid fever patients. According to these writers, the main curve in the course of agglutination in a case of typhoid fever is indistinguishable in form from the curve obtained by experimental inoculation with antityphoid vaccine, though its *maximum* is lower and its *decline* more rapid than is the case in the inoculated subject. In a chart they plot a curve of the agglutination titre for *B. typhosus* from the time agglutinins appear in the serum in an actual case of typhoid fever. The curve is represented as the highest dilutions of serum with which agglutination was obtainable. For the sake of comparison we may provisionally take these dilutions as giving standard agglutination and take the factor 5.0 as representing the factor for the standard culture. In this manner, analysing the chart we get:—

During the first 14 days the agglutinins rapidly rise from 0 to 1 in 1250 = units 250.

During the following—

8 days the agglutinins rapidly fall to 1 in 550 = units 110.

24 days the agglutinins gradually fall to 1 in 150 = units 20.

20 days the agglutinins very gradually fall to 1 in 75 = units 15.

Therefore, in the first two weeks the agglutinins rise quickly to a maximum of 250 units, in the subsequent week they drop by 140 units, and during the three weeks following this they drop by 90 units. Thus, in a period of six weeks the agglutinin units undergo violent fluctuation.

The results obtained with the sera of inoculated individuals (not suffering from active typhoid fever) in the present investigation are set down in Tables III., IV., V., and VI. Number of sera of inoculated cases examined more than once, 75. They may be divided up as follows: Sera which gave (1) steady negatives, 9; (2) steady positive titres, 11 (Table III.); (3) a rising titre, 16 (Table IV.); (4) a falling titre, 26 (Table V.); and (5) a certain amount of fluctuation, 13 (Table VI.). To describe these in detail:—

1. Sera which gave a steady negative, 9; each of these was examined twice and one three times, a negative being obtained each time.

2. Sera which gave a steady positive titre, 11 (Table III.). These gave exactly the same reading to the decimal place (with the exception of one) at the second examination. Two of these sera were examined a third time with exactly similar results. The intervals between the examinations were from one to five weeks.

3. Sera which gave a rising titre, 16 (Table IV.). One of these was examined a third time and still showed a slight rise in titre. The figures obtained over a period of 11 weeks were 5.3, 13.4, and 25.0 agglutinin units. In this case the period which had elapsed between the date of inoculation and the date of the first examination was nine months.

In all these cases the difference between the two readings was well below 50 agglutinin units, except one which showed a difference of 71 units. This case had been inoculated three months previously, when agglutinins are rapidly on the wane, and though it is not justifiable to base a theory on a few cases, it would appear that when the agglutinins, the result of inoculation, are falling, after reaching their maximum, instead of coming down in a straight line, they fall in a somewhat irregular fashion, that is with small secondary up and down waves, these irregularities being more marked in the earlier months after inoculation than in the later months, when the secondary waves become, as it were, flattened out. There is some evidence of such occurrence in the subsequent Table VII. where the sera show slight variations at repeated examinations. The suggestion, therefore, that the main curve after inoculation is a rise and then a fall, but that this line has secondary curves on the down grade, where occasionally the serum either remains steady at its titre or perhaps rises and then falls. The titre of the serum, though falling, recovers itself, as it were, at intervals on its downward route.

TABLE III.

Months from date of inoculation and date of 1st examination.	Interval between examinations.											
	One week.			Two weeks			Three weeks			Five weeks		
	1st reading.	2nd reading.	Difference.	1st reading.	2nd reading.	Difference.	1st reading.	2nd reading.	Difference.	1st reading.	2nd reading.	Difference.
2	44·6	44·6	0	..	..	..	..	..	..	..	..	..
3	53·7	53·7	0	..	..	..	..	..	..	..	..	..
6	8·9	8·9	0	..	..	..	10·7	10·0	0·7	..	..	..
9	5·3	5·3	0	..	..	..	..	..	..	10·7	10·7	0
12	..	..	..	10·6	10·6	0	..	..	..	..	..	..
17	..	..	..	5·3	5·3	0	..	..	..	..	..	..
19	..	..	..	10·0	10·0	0	..	..	..	..	..	..
5 years	5·3	5·3	0	44·6	44·6	0	..	..	..	..	..	..

TABLE IV.

[illegible]

In any event, in the absence of a violent upward rise in the agglutinin unit content during a short time, these results would not lead one into the error of making a diagnosis of typhoid fever. It must be remembered that in a recently inoculated case the titre of the serum is rapidly rising or falling, and therefore the larger the number of agglutinin units one finds in such a serum the greater will be the difference between two readings taken at an interval of, say, three weeks. Fortunately for diagnostic purposes, this case is unlikely to be met with owing to the extreme rarity of typhoid fever occurring in such a recently inoculated individual.

One other point might also be mentioned—namely, in a case suspected of typhoid fever, information should be obtained as to how many antityphoid inoculations the patient had received, as in the earlier part of this paper it will be remembered that men who had been once inoculated are apparently less liable to maintain their agglutinins at a steady high titre than those inoculated twice. Finally, it is interesting to note that three sera of this series—namely, those giving a rising titre—showed no agglutination on the first examination, whereas on the second examination agglutinin units made their appearance—namely, 5·3, 8·9, 13·4—the second examination having been carried out one, three, and five weeks after the first examination. These three sera were taken from men who had received antityphoid inoculation three, eight, and nine months previously respectively.

4. Sera which gave a falling titre, 26 (Table V.). Three of these were examined a third time and one of them on a fourth occasion. These subsequent examinations showed the titre to be still falling.

(a) Inoculated 6 months previously, during a period of 6 weeks they gave 71.4, 53.5, 44.6 units.

(b) Inoculated 14 months previously, during a period of 15 weeks they gave 142.8, 26.8, 3.0 units.

(c) Inoculated 10 months previously, during a period of 15 weeks they gave 25.0, 17.8, 8.9, 5.0 units.

Generally speaking, the fall in standard agglutinin content was gradual, but with the sera of cases more recently inoculated the titre dropped more suddenly, particularly when the interval between the first and second examination was so long as ten weeks. This is what might be expected, because after the maximum has been reached the decline in titre is more rapid in the earlier months than in the later months following inoculation. There is one exception in this set of sera—namely, the serum of a man who was stated to have been inoculated so long as 14 months previously. This man showed a comparatively high agglutinin content: 142·8 at the first examination and only 26·8 after two weeks. Unfortunately, this man was lost sight of before the date of his inoculation could be verified, but it appears probable that he had been inoculated much more recently, judging by the comparatively high agglutinin content and the sudden drop in the titre of his serum. Here again the ascertaining of the date of inoculation and the number of inoculations received appears to assume a certain amount of importance in drawing conclusions from the variations in the standard agglutinin content of the serum in successive examinations.

5. Sera which gave a certain amount of fluctuation, 13 (Table VI.). These may be divided into: Sera which gave (a) a steady titre followed by a fall in titre, 2; (b) a rising titre followed by a steady titre, 2; (c) a rising titre followed by a falling titre, 2; (d) a falling titre followed by a rising titre, 5; and (e) a steady titre followed by a rise with a subsequent fall, 1. These numbers are too small to admit of any conclusions, but they show that slight variations are to be met with which are comparable to the sera of Tables IV. and V. This may be due to individual peculiarity, the existence of which is pointed out by Dakeyne,⁵ or it may represent the secondary curve inset in the main agglutination curve in the titre of the sera of inoculated persons as previously suggested.

TABLE V.

[illegible]

TABLE VI.

Months between date of inoculation and date of first examination.	First reading.	Serum examined after—															
		1st week.	2nd week.	3rd week.	4th week.	6th week.	8th week.	9th week.	11th week.	14th week.	15th week.	16th week.	18th week.				
Steady titre followed by a falling titre.																	
5	44.6	44.6	...	22.3	...	...	...	...	...	...	...	...	...	...	...	...	...
10	44.6	44.6	...	32.1	...	...	...	...	...	28.6	...	...	...	...	...	...	...
Rising titre followed by a steady titre.																	
12	8.0	17.8	22.3	22.3	...	...	...	...	...	...	...	...	...	...	...	...	...
16	10.6	...	13.4	...	...	...	...	...	...	...	25.0	25.0	...	...	...	...	...
Rising titre followed by a falling titre.																	
6	8.9	17.8	...	...	13.4	...	...	...	...	...	...	...	...	...	...	...	...
13	14.2	...	22.3	...	8.9	...	...	...	...	...	5.3	...	...	...	...	...	...
Falling titre followed by a rising titre.																	
2	178.5	...	...	...	...	...	...	...	...	...	53.7	...	50.0	100.0	...	...	...
2	215.0	...	53.5	...	107.0	...	...	...	...	...	...	...	...	...	...	...	...
7	28.6	...	10.7	...	...	31.2	...	...	...	...	...	...	...	...	...	...	...
8	22.3	8.9	...	...	13.4	...	...	...	...	...	...	...	...	...	...	...	...
10	142.8	17.8	...	26.8	...	...	...	...	...	...	...	...	...	...	...	...	...
2 years.	53.7	...	...	...	28.6	...	...	...	100.0	...	...	...	...	...	...	...	...
Steady titre followed by a rise and then a fall.																	
11	44.6	44.6	71.4	35.7	...	...	13.4	...	...	...	...	...	...	...	...	...	...

E. Cases definitely known to have been Uninoculated: Sera of Group III.

Number of cases, 32. Of these, 3 were positive for *B. typhosus* and 29 were negative. Of the 3 positive cases, 2 contained 13.4 agglutinin units (Ref. Nos. 33/24.3.16, 11/31.3.16); 1 contained 125 agglutinin units (Ref. No. 13/23.6.16).

With regard to the non-inoculated case, which gave 125 agglutinin units, the specimen was received on two occasions with an interval of a week and gave exactly the same reading. It did not affect 7160. Inquiries were instituted and it was positively stated that this case had not been injected and never had typhoid fever. The faeces were obtained and plated out, but no non-lactose fermenters were found. This case is remarkable in giving such a high agglutinin content and is similar to the case described by Dreyer, Ainley Walker, and Gibson.⁴ The serum in their case agglutinated typhoid up to 1 in 1250 dilution though the man had not been inoculated, denied that he ever had typhoid fever, and the writers failed to find typhoid bacilli in the faeces or urine.

With regard to the other two cases, 33/24.3.16 and 11/31.3.16, which gave 13.4 agglutinin units, no answer has yet been received to inquiries.

II. AGGLUTINATION RESULTS WITH *B. ENTERITIDIS* OF GAERTNER (DELÉPINE, 7160).

A. Sera of Antityphoid-inoculated Men (Groups I. and II.).

Number of sera, 270, of which 53, or 19.6 per cent., were positive, and 217, or 80.4 per cent., negative. Of these 53 positive sera, 30 agglutinated 7160 up to 1 in 25 dilution, 15 up to 1 in 50, 5 up to 1 in 125, and 3 up to 1 in 250. No agglutination was observed in any dilution higher than 1 in 250.

B. Influence of Antityphoid Inoculation on Agglutinating Power of Sera for *B. 7160*, Delépine (Groups I. and II.).

As none of the 53 sera which agglutinated 7160 (Groups I. and II.) suffered from active symptoms of enteric fever or from food poisoning, so far as could be ascertained, the question arises as to whether the presence of Gaertner agglutinins in these sera is to be ascribed to the antityphoid inoculation. If such influence were exerted it is highly probable that the length of time that had elapsed since inoculation would have some effect on the agglutinating power of 7160. With the object of revealing such influence (if it existed) Table VII. was drawn up. It shows the titre of the sera for 7160 and the number of months which had elapsed since antityphoid inoculation had been performed. It will be seen from this Table VII. that the length of time which had elapsed since a patient has been inoculated against *B. typhosus* exerts no influence over the clumping power of his serum for 7160, marked agglutination being

obtained with various sera whether the men had received inoculation one month or 25 months previously.

TABLE VII.

Number of months between date of inoculation and date of examination.	Number of sera agglutinating 7160.	Titre of sera against 7160.				Number of months between date of inoculation and date of examination.	Number of sera agglutinating 7160.	Titre of sera against 7160.			
		1 in 25.	1 in 50.	1 in 125.	1 in 250.			1 in 25.	1 in 50.	1 in 125.	1 in 250.
1	5	2	2	1	...	12	11	6	3	1	1
2	3	1	2	...	...	13	3	2	...	1	...
3	4	1	2	...	1	14	1	...	...	...	1
4	6	5	...	1	...	15	1	...	...	...	...
5	2	2	...	...	...	16	1	...	...	1	...
6	4	3	1	...	...	17	...	...	...	...	...
7	4	2	...	...	...	18	1	...	...	...	...
8	...	...	...	...	...	19	1	1	...	...	...
9	2	1	1	...	...	25	1	1	...	...	...
10	2	1	1	...	...						
11	1	...	1	...	...	Totals...	53	30	15	5	3

C. Influence of Syphilis on Agglutinating Power of Sera for 7160.

An analysis of the 7160 agglutination results has been made out in order to see whether active syphilis influences the behaviour of the sera in any way. Sera of Groups I. and II.: 270 sera of inoculated individuals. As before, 42 cases must be eliminated, only those cases which gave a +3 or +4 Wassermann, and about which full clinical details were available; this leaves 228 cases to be examined. (Table VIII.) These cases may again be divided into two sections: those which gave a positive Wassermann, 105 (Section I.); and those which gave a negative reaction, 123 (Section II.).

TABLE VIII.

Section I.: Positive Wassermann. Section II.: Negative Wassermann.

Lesions.	No. of cases.	Positive for 7160.	Negative for 7160.	Lesions.	No. of cases.	Positive for 7160.	Negative for 7160.
Primary ..	59	17 (28.8%)	42 (71.2%)	Suspected primary	100	16 (16.0%)	84 (84.0%)
Secondary	46	12 (26.0%)	34 (74.0%)	Suspected secondary	23	3 (13.0%)	20 (87.0%)
Totals ...	105	29 (27.6%)	76 (72.4%)	Totals ...	123	19 (15.4%)	104 (84.6%)

From Table VIII. it would appear that syphilis, as evidenced by the presence of a positive Wassermann, does exert some influence in increasing the agglutinating property of the serum of the subject for 7160. Those having a positive Wassermann gave 27.6 per cent. positives for 7160, whereas those having a negative Wassermann gave 15.4 per cent. positives for 7160, a difference of 12.2 per cent. Whether this difference is only apparent or real, it forms a contrast with the results obtained when the typhoid-agglutinating power of these sera is subjected to a similar analysis, those sera having a positive Wassermann showing practically no difference to those having a negative Wassermann in their agglutinating power to *B. typhosus*. It must be taken into consideration that a far larger number of sera of inoculated individuals (87.1 per cent.) agglutinate *B. typhosus* than agglutinate 7160 (19.6 per cent.), and therefore probably the results are not strictly comparable.

D. Sera of Inoculated Cases (Groups I. and II.) examined more than once against Gaertner, 7160.

Number of sera, 75. These may be divided up as follows. Sera which gave (1) steady negative, 62; (2) steady positive, 3; (3) a rising titre, 3; and (4) a falling titre, 7. To describe these in detail:—

1. Sera which gave steady negatives, 62. Of these 43 were examined twice; 11 on three occasions; 6 four times; and 2 five times. Two of the sera examined twice (Ref. Nos. 7/31.3.16 and 17/2.6.16) had a history of previous typhoid fever, the first 15 years ago and the second 16 years ago.

2. Sera which gave steady positives, 3. These were each examined on two occasions and gave the same reading each time—viz., positive in 1 in 25 after one week's interval, in 1 in 50 after three weeks' interval, and in 1 in 125 after one

week's interval. One of them, Ref. No. 9/21.1.16, which agglutinated 7160 in 1 in 125 dilution, had a history of typhoid 18 years ago. His serum did not affect *B. paratyphosus* A or B. He had never suffered from food poisoning. He had received antityphoid inoculation nine months before the first specimen was examined, and gave a slightly falling titre for *B. typhosus*, 26.5 and 14.2 agglutinin units per 1 c.c. of serum.

3. Sera giving a rising titre. Of these Ref. No. 12/10.3.16 was examined three times during the course of four weeks. The first two specimens failed to agglutinate 7160, but the third time it agglutinated 7160 in 1 in 25 dilution. He had received antityphoid inoculation two months previously and showed a slightly falling titre for *B. typhosus*. The second serum, Ref. No. 27/9.6.16 rose from a positive in 1 in 25 dilution at the first examination to a positive in 1 in 50 at the second after an interval of a week. This serum had no effect on *B. paratyphosus* A or B, and gave a slightly rising titre to *B. typhosus*. This man had been inoculated 12 months prior to the first examination. The third, Ref. No. 32/4.2.16, rose from a positive in 1 in 125 dilution to 1 in 250 after an interval of three weeks. There was a falling titre for *B. typhosus*. He had been inoculated nine months prior to the first examination. The full clinical details of this case have not yet come to hand.

4. Sera which gave a falling titre. Of these 4 fell from a positive in 1 in 25 dilution to 0 during a period of from two to three weeks. One of them was examined four times subsequently with a negative result. These cases had received antityphoid inoculation 2, 3, 4, and 7 months prior to the first examination. Three sera fell from a positive of 1 in 50 to 0. One of them was examined on two subsequent occasions during a course of three weeks and proved negative for 7160.

E. Results of Cases Definitely Known to have been Uninoculated (Group III.). Agglutinin Results with 7160.

Number of cases, 32. Of these, 6 agglutinated and 26 did not agglutinate 7160. Of the 6 positive cases, 4 agglutinated up to 1 in 25 dilution, 1 up to 1 in 50, and 1 up to 1 in 250.

The case which agglutinated 7160 in 1 in 250 dilution (Ref. No. 21/4.2.16) was received three times for examination with a one-week interval. It gave a positive reaction on three occasions as follows: 1 in 250, 1 in 125, and 1 in 250. The serum in this case did not agglutinate *B. typhosus*, *B. paratyphosus* A or B. He had never had typhoid fever and did not suffer from symptoms of food poisoning.

The case which agglutinated 7160 in 1 in 50 dilution (Ref. No. 25/9.6.16) was received twice subsequently at one-week and four-week intervals, but on the second and third occasions the serum failed to affect 7160 and was negative for *B. typhosus* and *B. paratyphosus* A and B. No non-lactose fermenters were discovered in the faeces. This case had typhoid in 1906—that is, ten years prior to the date of the examination of the serum.

F. Results with Sera of Cases about Whom Information as to Inoculation was Not obtainable (Group IV.).

Although it is primarily intended to deal with the 7160 agglutinations of this Group IV., it would be well to consider what was their probable relation to inoculation.

Agglutination results with B. typhosus.—Number of cases 64; 30 gave a positive and 34 gave a negative result. Of the 30 positive cases 16 contained up to 25 agglutinin units per c.c., 7 up to 50 units, 4 up to 75 units, 1 up to 100 units, 1 up to 125 units, and 1 up to 300 units. The percentage of negative cases is much higher than is found among inoculated cases, so it is probable that a large number of them had not received any inoculation.

Agglutination results with 7160.—Number of cases 64; of these 13 were positive and 51 negative for 7160. Of the 13 positive cases 4 agglutinated up to 1 in 25, 6 up to 1 in 50, 2 up to 1 in 125, and 1 up to 1 in 250.

Note.—Nine of these cases were examined more than once with the following result:—(a) Negative 7, examined twice 6, examined three times 1; (b) Positive at first examination, subsequently negative 1 in 25, 1; (c) Negative at first reading, and subsequently positive 1 in 50, 1.

Total Agglutination Results with Gaertner (Delépine, 7160).

As antityphoid inoculation bears no relation to the agglutinating power of a serum for Gaertner (Delépine, 7160), the results of the sera of each group of the present series of cases can be taken collectively as follows:—

	No. of cases.	Agglutinated 7160.
Groups I. and II., inoculated cases	270	53
Group III., cases known to have been not inoculated	32	6
Group IV., cases about whom information as to inoculation was not obtainable	64	13
	366	72 (19.6%)

The actual titre with which agglutination was obtained is given in Table IX.:

TABLE IX.

	Number of sera.	Positive sera.	Dilution of serum.			
			1 in 25.	1 in 50.	1 in 125.	1 in 250.
Groups I. and II.	270	53	30	15	5	3
Group III.	32	6	4	1	0	1
Group IV.	64	13	4	6	2	1
Totals	366	72	38	22	7	5
Per cent.		19.6	10.4	6.0	1.9	1.3

Therefore, any deductions one might seek to base upon the behaviour of the sera of persons who form the 366 units of the Groups I., II., III., and IV. are liable to an error of 19.6 per cent. if so strong a serum as 1 in 25 be taken. As the dilution is increased the error grows less—namely, 9.2 per cent. when a 1 in 50 dilution is taken, 3.2 per cent. with 1 in 125, and 1.3 per cent. with 1 in 250. Above a 1 in 250 dilution the error disappears altogether. These results are interesting in view of the work recently published by Ritchie,⁶ where an analysis of the "normal" mean agglutination of sera for bacilli of the typhoid-dysentery group is made and where deductions have been drawn as to how far dilution of serum is necessary before positive agglutination can be regarded as evidence of a particular disease. He points out that such a high dilution as 1 in 128 cannot be relied upon for diagnostic purposes in the case of *B. dysenteriae* (Flexner) infection, whereas in the case of *B. dysenteriae* (Shiga), *B. typhosus*, *B. paratyphosus* A and B agglutination with a much lower dilution of serum can be taken as diagnostic.

III. GENERAL RESULTS WITH CASES WHICH HAD A PREVIOUS HISTORY OF TYPHOID FEVER (IN GROUPS I., II., AND III.).

Total number 13. Of these 9 had been inoculated with typhoid vaccine and 4 had not been inoculated (see Table X.). Three sera agglutinated 7160. These were also tested against paratyphoid A and B, with a negative result.

TABLE X.

	Number.	Pre-vious history of typhoid fever.	Antityphoid inoculation.		Effect on <i>B. typhosus</i> .	Effect on 7160.	Effect on <i>para</i> - <i>typhosus</i> A and B.	Reference number.
			No. of	Date of last.	Units.	Dilution.		
Inoculated.	1	4 mths.	2	9 mths.	None	None	...	10/28.1.16
	2	6 years.	2	18 "	5.3	"	...	14/7.4.16
	3	8 "	2	15 "	20.0	1 in 25	None	30/ 6.6.16
	4	9 "	2	13 "	5.3	None	...	8/26.5.16
	5	15 "	2	5 "	13.4	"	...	7/31.3.16
	6	16 "	1	8 years.	None	"	...	17/2.6.16
	7	18 "	2	4 mths.	13.4	"	...	10/12.5.16
	8	18 "	2	9 "	26.5	1 in 125	None	9/21.1.16
	9	22 "	2	4 "	13.4	None	...	24/24.3.16
Not in-oculated	1	10 years	None.	None.	None	None	...	4/2.6.16
	2	10 "	"	"	"	"	...	5/19.5.16
	3	10 "	"	"	"	1 in 50	None	25/9.6.16
	4	15 "	"	"	"	None	...	10/23.6.16

Among the inoculated cases, in No. 1 (Ref. No. 10/28.1.16) the serum was submitted twice for examination. On the first occasion it failed to agglutinate *B. typhosus*, but on the second it did so (8.9 agglutinin units per 1 c.c.). On neither occasion did it affect 7160. It was subsequently

ascertained that the man had had dysentery five months and typhoid fever four months previously. Unfortunately, the patient left Dublin before a sample of his serum could be obtained to test for paratyphoid agglutinins, but the case is being followed up and inquiries have been instituted as to the serological findings obtained during his illness.

Case 8 (Ref. No. 9/21.1.16). The serum was examined twice; on the first occasion it gave 26.5 agglutinin units for *B. typhosus* and agglutinated 7160 in 125 dilution. On the second occasion, one week later, it gave 14.2 typhoid agglutinins and agglutinated 7160 in 125 dilution. On inquiry it was stated that he had never at any time suffered from symptoms of food poisoning.

Among the non-inoculated cases No. 3 (Ref. No. 25/9.6.16) was examined on two subsequent occasions, once after a week, and a second time five weeks after the first examination; on both these occasions it failed to agglutinate 7160. An examination of the faeces proved negative for non-lactose fermenting bacilli.

IV. SUMMARY.

1. Out of the 270 sera of inoculated men examined 87.1 per cent. gave positive agglutination for *B. typhosus*. Of these, the greater number gave up to 25 standard agglutinin units per 1 c.c. of serum; after this the percentage of units obtained dropped rapidly until the number of sera containing more than 125 agglutinin units formed but quite a small percentage of cases.

2. During the first month after inoculation the maximum number of agglutinin units is obtainable. This maximum falls rapidly during the third month after inoculation. Between the fifth and fourteenth month a fairly high agglutinin unit content may be met with, but after 15 months subsequent to inoculation the agglutinin units were not found above 100.

3. There is some evidence to show that after the highest point in the agglutination, following antityphoid inoculation, has been reached the subsequent fall is progressive, but is inclined to remain steady or even to rise slightly at intervals so as to form secondary waves on the downward course. This is more marked in the earlier months following inoculation than at the later months.

4. The sera of men inoculated twice give more positive reactions than men inoculated only once. Also a greater number of them give 25, 50, and 100 agglutinin units. Higher titres are obtained with the sera of men inoculated twice during the second, third, and fifth months after inoculation than among those who have been inoculated once.

5. The presence of syphilis (as indicated by a positive Wassermann) does not affect the agglutinating power of a serum for *B. typhosus*, though apparently it does appear to increase slightly its power to agglutinate 7160.

6. The agglutinating power of a serum for 7160 is not influenced by antityphoid inoculation.

7. Out of 366 sera, consisting of both inoculated and non-inoculated men who had either had or were suspected of having syphilis, 19.6 per cent. agglutinate *B. enteritidis* Gaertner (Delépine, 7160) when a 1 in 25 dilution is used. A much smaller percentage may agglutinate this bacillus up to a 1 in 250 dilution. None of these sera were found to affect 7160 in a higher dilution.

8. Out of 13 cases with a previous history of typhoid fever only 3 agglutinated 7160. These cases had had typhoid fever 8, 10, and 18 years respectively, previous to the date of examination. The 10 cases not agglutinating 7160 had histories of typhoid fever four months to 22 years previously.

9. Any of the sera clumping 7160 which were examined for co-agglutinins for *paratyphosus A* or *B* proved negative.

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CHRONIC GONORRHOEA CURED BY ELECTROLYSIS.

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CHRONIC gonorrhoea is an unfortunate state for any patient, and it also represents a clinical condition which often taxes the patience of the doctor in charge of the case as much as that of the sufferer. The condition is one which is the more exasperating, because after all there may be so little to get rid of, and yet it is (if the gonococcus be still present) material of such significant and potential virulence.

I think it is insufficiently realised that if treatment by injections, or bougies, or irrigation, or medicine, or combinations of these methods, have failed to cure a case of gonorrhoea after several months it is not only useless but actually harmful to continue them. How often does one find that irrigations continued for many months and having failed to cure have become changed from irrigations into irritations. Within the last year numerous patients have come under my care in whom these things had happened, and I think it well to indicate the lines on which I have attained success. Although the great majority have been cured, and I should prefer to know of any failures, I can only find record of one or two in which the result remains unknown because of the expiration of sick-leave and an unavoidable return to active service. The following are cases illustrating an advantageous change of treatment.

CASE 1.—The patient was sent to me with the following history. After a recent festivity he alleged a gradual onset of a discharge which was ascribed to another return of an old attack. As usual, I examined Gram-stained films of his copious discharge and found groups of gonococci in every field. He also admitted scalding and smarting, and in a consultation with his medical attendant, who was present, the history was withdrawn and a reinfection conceded six months before. Although his medical attendant had been misled the patient mentioned that he had from the outset the help of two specialists, who had irrigated and injected silver compounds into the deep urethra and inserted soluble bougies, and it was easy to believe his opinion that after six months he was little, if any, better. This patient had got it well on his nerves, and he had moderately severe rheumatism.

Treatment.—I began electrolysis of the anterior urethra every other day until 14 treatments had been given, and then proceeded to electrolyse the deep urethra and gave ten more administrations. The patient was absent on business for nine days after a month's treatment, and the case was finished after treatment spread over seven and a half weeks. Repeated microscopy showed the absence of pus, and the cure is assured by my continued touch with the patient up to one year after beginning his case.

The accessory treatment consisted in the use of santal oil, salol, and a few doses of vaccine. The last named seemed unable to suppress the rheumatism, which only disappeared with the absence of the gonococcal discharge.

I think that not the least important part of the cure in the above case was the immediate stoppage which I enforced of any injections or irrigations, even during the patient's enforced absence. The case illustrates the recuperative power of a previously irritated mucous membrane, which was gently relieved of its gonococci as described.

CASE 2.—This patient arrived from abroad on crutches with a history of acute gonorrhoea of ten weeks' standing. He had been thoroughly irrigated and developed a very swollen and inflamed knee full of fluid. This knee had been blistered freely and the patient was naturally full of anxiety. He had been given several doses of vaccine.

Treatment.—As usual I focussed my efforts on the discharge and did nothing at all to the knee. The irrigations were stopped and small doses of vaccine and a little santal oil were administered. After eight treatments the discharge was difficult to find, and I ordered the patient to begin walking, which was now not painful. Though the limb was wasted from disuse he soon walked easily, and after six weeks could do all bending exercises except supporting the whole body on the bad knee in the bent position (quite a strong test for healthy folk). In this case, however, the final clearance of pus and gonococci was unexpectedly prolonged, but after 28 treatments the patient recovered and took an army commission.

The interest, I think, of this case is the rapid resolution of the inflammation of the knee coincident with the overcoming of the discharge.