was palpable over the carotid, no bruit was audible, and the temporal pulses were equal. The following note was made:—

"Motor system:—Right upper extremity: No rigidity; all movements of the shoulder and elbow are possible, but weaker than those of the other limb. Extension of the wrist can be made occasionally but the effort often fails, flexion of the wrist is not obtainable, and no movements of the fingers can be made. The lower extremities are normal. Reflexes:—Arm-jerk: R. +; L. —. Knee- and ankle-jerks normal. Abdominal: Right less than left. Plantar: Flexor. Sensation:—Touch and pin-prick unaffected. Position and form lowered as to right hand. Diagnosis:—Small cortical embolism."

CASE 12. *Non-perforating injury to right internal carotid artery.*—The patient was admitted into No. 20 General Hospital under the care of Captain Burrows in July, 1916. He was wounded on July 27th by a small piece of shell-casing from a shell that burst close beside him. He fell at once, and has no definite memory of what immediately followed, but he was probably rendered unconscious. He says he was able to get up in about ten minutes' time, but was unable to walk in consequence of weakness of his left lower extremity; the left upper he only discovered to be weak after he reached the hospital on a stretcher. The wound consisted in a gaping slit about an inch long just anterior to the right tragus. X ray examination showed the piece of shell behind the pharynx on the front of the body of the second cervical vertebra, where it was also palpable. The condition on August 4th was as follows:—

"The man is quite intelligent, but has slight difficulty in articulation due to facial paresis. The right temporal artery cannot be felt to pulsate. The pupils are equal and react normally. There is incomplete right peripheral facial palsy, and slight but definite paresis of the upper neurone type upon the left side. The tongue is protruded to the left, and the left side lies higher in the mouth. Motor system:—The right upper extremity is normal, the left flaccid and toneless. There is slight power of flexion in the fingers, but no power of extension, nor of adduction or abduction. Flexion and extension of the wrist are very feeble and limited in range. The shoulder and elbow movements are stronger. In forced inspiration the right side of the chest moves better than the left. The right lower extremity is normal; the tone of the muscles of the left is fair, and all movements are possible, especially the more distal, but they are weaker than those of the right limb. Reflexes:—Arm-jerks: R. normal; L. feeble. Knee-jerks: Equal and brisk. Ankle-jerks: Equal and brisk. Abdominal: R. brisk; L. almost absent. Plantar: R. flexor; L. extensor. Sensation:—Considerable alteration in the sense of touch, but no complete loss, is present in the left side of the head and trunk and the left upper extremity. Contact produces tingling. Localisation, sense of form, and position are all very defective in the left upper extremity."

Of the above five cases four are of the class in which no perforating injury was considered to be present, but they offered no differences in history or character of the symptoms from those in which either an arterial bruit, an aneurysmal sac, or the occurrence of secondary hæmorrhage indicated the presence of an opening in the wall of the vessel, and hence the conditions for the formation of a thrombus to be still more favourable. Among the whole 14 cases, in six a wound was concluded to be present, in the large majority the missile was a fragment of shell, and in nine instances the missile was retained. In two of the cases the paralytic signs followed the application of a ligature to the common carotid artery, appearing on the second and fourth days respectively.

DIAGNOSIS.

The striking difference noted between the history of the development of the cerebral symptoms in these cases and that noted in those dependent on sudden cerebral anæmia following the application of a ligature to the common carotid artery needs only to be noted. Examples of the latter accident have been far from uncommon during the present war, when primary or secondary hæmorrhage has necessitated occlusion of the vessel. Hemiplegia has been prompt and complete, and in more than one instance followed by a fatal issue in the course of 24 to 36 hours. In the cases of embolism, on the other hand, the more tardy development and partial nature of the paresis has been the outstanding feature. The difficulties attendant upon the presence of coexistent signs of remote nervous injuries are sufficiently set out in the above description.

PROGNOSIS AND TREATMENT.

Opportunity has only existed for clinical observation of the patients concerned for a very limited period. Only one of the patients included in the table died, and on him

unfortunately no post-mortem examination was made. Of the remainder, one was sent home practically recovered from an incomplete right brachial monoplegia and aphasia, while slight improvement had occurred in some of the others. The general impression, however, was not a favourable one. As to treatment, rest is the only resort; it may, however, be remarked as an important fact in support of the diagnosis (in favour of embolism as opposed to cerebral softening from anæmia) that in the two cases in which subsequent complications necessitated ligature of the common carotid artery neither patient suffered any increase in symptoms; in fact, in one (Case 7) the man's condition improved at once and considerably, and in the second (Case 8) steady improvement followed.

I should, in conclusion, express my thanks to Captain L. W. Shelley for his aid in the collection and arrangement of the notes of the cases dealt with.

NOTES ON THE AGGLUTINATION REACTIONS WITH OXFORD STANDARD AGGLUTINABLE CULTURES

IN A SERIES OF PATIENTS, INCLUDING THOSE EXAMINED IN A RECENT CIVILIAN OUTBREAK.

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THE following analysis is based on a series of 275 cases, of whom 184 were soldiers ill or convalescent from overseas, 53 were civilians suffering from "enteric" during the recent outbreak in Reading, and 38 were civilians ill from causes other than "enteric." The sera of these cases were examined once or oftener at the request of the medical men in charge of the cases, while for permission to examine the sera of the non-enteric civilians we are indebted to Lieutenant-Colonel Freeman, R.A.M.C. (T.), and Major Abram, R.A.M.C. (T.). The examinations were part of the routine work carried out in the laboratories of the civil and military hospitals, and were not specially selected.

Agglutination technique.—At the outset a small number of sera were examined strictly in accordance with the instructions laid down in the "Directions" issued from the Standards Laboratory, Oxford, at the instance of the Medical Research Committee. As we found a difficulty in dealing with many sera at one time with the small number of "outfits" supplied, and as some of these agglutination tubes did not hold the amount of fluid necessary for the test, we discontinued their use.

In their stead we employed accurately graduated pipettes to deliver known volumes of serum, saline, and bacillary suspension. The standard agglutinable cultures issued from the Oxford laboratory were used exclusively, the volume of standard suspension added to each tube being 1 c.c. With this were mixed serum and saline in such proportions that when the three constituents had been added the resulting serum dilutions were 1 in 27, 1 in 54, 1 in 120, and 1 in 150 in the first four tubes respectively.

The tubes were placed in a water-bath for two hours at 50° C., but instead of reading the result at the end of 15 minutes after their two-hours' heating we left them on the bench till the following morning. Where many sera had to be done we found it most convenient to use for heating purposes Hearson's water-bath, as supplied by them for Wassermann purposes. In place of their rack we had another one of the same size made, but constructed to hold 225 agglutination tubes at once.

The readings were all carried out by the same observer, and to facilitate accurate reading we always used the same source of light. This was an electric-light bulb let in through a slit in the top of an ordinary soap-box whose interior had been painted black. Against this background the readings were made, and to detect the end-point we used a pocket lens.

The sera were always put up in the first instance in the four dilutions named above against standard suspensions of

B. typhosus, B. paratyph. A, and B. paratyph. B respectively. If the end-point had not been reached in 1 in 150 a further series was put up, and so on till an end-point was obtained. The highest dilution we had occasion to use was 1 in 21,333.

As, with a few exceptions, all the sera were examined by the volumetric method and not that recommended by Professor Dreyer and his colleagues, no attempt was made to interpret the results, hence the varying sensitiveness of the bacillary suspensions was not reckoned with.

Comments.—The substitution of the graduated for the Oxford dropping pipette was made not because we regarded the latter as less accurate for ordinary practical purposes, but because of the greater convenience of the former. We mention this because Hort has stated that he has not found the method of sufficient accuracy for routine work on a large scale.

Strictly speaking, inasmuch as the Oxford pipette is used to deliver a certain number of drops of fluids whose surface-tension differs, there must be a small margin of error in the accuracy of the resulting serum dilution. In practice, however, this appears to be negligible where the macroscopic end-point to be determined is a crude one in comparison with an end-point in terms of chemistry. Inaccuracies, however, may occur in the counting of the drops delivered. This is all the more likely where the operator has many sera to put up and when there is more opportunity for temporary mental distraction from the immediate work in hand—viz., the counting of drops. This may account for Hort's statement.¹

Of course, the volumetric method is not absolutely free from the same objection, but it is considerably less liable to error in so far as there is no need for arithmetical concentration. In our series of cases wherever any large discrepancy was observed between the two readings, or wherever a doubt existed as to the dilutions made, the test was repeated. We believe that the volumetric method takes up less time than the drop method and that the operator is less liable to make mistakes.

Collection of blood.—In order to obtain a sufficiency of blood there was only one method to adopt and that was to remove a reasonable quantity by venipuncture, generally from the median basilic vein. This was done in every case. It involves considerably more trouble where several sera have to be examined in the same day, inasmuch as the syringe must be sterilised between each case. Apart from the time spent during sterilisation, the actual collection of the specimen is easier since there is no trouble encountered in securing a sufficiency, and as far as the patient is concerned no greater pain is felt than by other methods. It has a further advantage over other methods in that blood can be secured at the same time for culture purposes.

Results: Group I.

This group comprises a series of 115 men whose bloods were examined twice or oftener. These are divided into three sections.

Section 1 includes those whose bloods showed the presence of agglutinins for paratyphoid bacilli with or without agglutinins for B. typhosus. This comprises 71.6 per cent. of the cases in Group I. They are divided up as follows:—

TABLE I.

No. of cases...	...	...	...	...	81
Agglutinating B. para. A	...	...	...	...	31 cases or 38.2 per cent.
" B. para. B	...	...	...	43	" 53.0 "
" B. para. A and B	...	...	...	7	" 8.6 "

Comments.—When the cases began to come in from the Mediterranean towards the end of 1915 it speedily became evident that the clinical diagnosis was quite untrustworthy. The majority of the cases arrived as "enterics" or "dysenteries," but without any bacteriological record to support this. Out of 133 men who were questioned only 11, or 8.2 per cent., were able to state that their blood had been examined. While the actual number examined was probably larger than could be ascertained from the patients, it was obvious that if the clinical diagnosis were to be accepted at its face value the percentage of typhoids would indeed be large. That this was not so, and incidentally furnishing indirect evidence that the diagnosis made abroad rested on clinical grounds only, became evident on bacteriological investigation.

The serological methods above described were employed, as by the time the patients reached us the possibility of finding the causal organism in the blood was reduced to a minimum. This latter method was employed in many of

the cases alongside the serological with results so poor that it was discontinued except in special cases. Bacteriological examination of the excreta we found to be of only a little more value than blood culture owing to the number of negative results obtained, and to be of little value for immediate diagnosis, for instance, in those cases which were negative on two or three occasions, to be followed on a subsequent occasion by the presence of infective bacilli. As a matter of interest B. para. A was isolated from the faeces of 5 men only in this section of 81 cases, and B. para. B from 8 men, the blood of one of the latter, however, being devoid of agglutinins for B. para. B.

As most of the men had been inoculated against typhoid, but not against paratyphoid, an endeavour was made to obtain the bloods on at least two occasions. It was pointed out that while one examination was probably sufficient to enable one to state that the patient had been infected with paratyphoid it was not sufficient for a diagnosis of typhoid, inasmuch as the inoculated persons would show the presence of typhoid agglutinins in the blood. It ought not to be necessary at this stage to refer specially to the need for two or more examinations, but the fact does not yet appear to be generally recognised by clinicians. Moreover, as the men are now being immunised with a triple vaccine the need for repeated serological examination will be greater, since hitherto a single examination has sufficed to determine whether a paratyphoid infection was present or absent.

Of the 115 men referred to in Group I., 81, or 71.6 per cent., showed the presence of para. agglutinins in the blood, indicating a more or less recent paratyphoid infection. The relative proportions of those with para. A and para. B agglutinins respectively are given in Table I.

Omitting for the moment those which agglutinated both paratyphoid A and B, it will be seen that para. B infections were more frequent, so far as our results go, than para. A infections. This is further supported by the figures which we give above relative to the proportion of cases from which paratyphoid organisms were recovered. The relative proportions in our series differ from those recorded by some other observers, notably those who were working in the East, while they agree more or less closely with the figures given by Professor E. Glynn and Dr. E. C. Lowe,² who suggest that the difference may be explained by the fact that after the month of October paratyphoid A was more common than para. B. In 68 of our series the date of onset of illness was more or less accurately ascertained, with the result that up to the end of October the proportion of para. A to para. B was 1:1.79, while after that date the proportion was 1:1.07. These figures, while not so striking as those of Glynn and Lowe, show a slight increase in para. B over para. A up to the end of October, while after that month they are practically equal.

While the seasonal incidence may be the explanation, we cannot help thinking that the reason for the difference is to be sought for in the character of the stimulus which calls forth the production of agglutinins. In other words, it would be explained by the assumption that B. para. A is a weaker stimulus than B. para. B, or that the agglutinins resulting from the A stimulus are more transient than those produced by B. para. B.

If this assumption be correct, one would expect to have a larger number of positive A agglutinins, the nearer to the infecting point the blood is examined, and conversely, the longer the interval between onset and agglutination the fewer will be the number of those previously infected with B. para. A which will show the presence of A agglutinins. The same thing, of course, will hold good as regards B typhosus and B. para. B, but the point we wish to make is this, that the interval during which para. A agglutinins can be found in the blood is probably shorter than in the case of the other two infections.

Further, when a paratyphoid infection occurs in a person already inoculated against typhoid only, a new stimulus to specific agglutinin formation is introduced. That this new stimulus is not without an influence on the agglutinin-producing mechanism already called into being as a result of the typhoid inoculation appears to be the opinion of several observers. This being so, we should expect the typhoid agglutinin output to vary directly as the ability of the new stimulus to produce agglutinins. Accordingly, if the explanation offered for the discrepancy between various observers in the relative numbers of para. A and para. B

infections be correct, we should expect to be able to trace the same influence at work on the typhoid agglutinin content.

TABLE II.—Showing Percentages of Cases with Agglutinins for *B. Typhosus* in a Dilution of 1 in 150 or more.

Of those agglutinating Typhoid only	62.5 per cent.
" " " Typhoid and Para. A	65.5 " "
" " " Typhoid and Para. B	90.2 " "

From Table II. we see that those who have been infected with paratyphoid bacilli after a previous inoculation with typhoid bacilli yield a greater percentage having a high typhoid agglutinin content than those who have not been so infected. In other words, those with agglutinins for T. only do not agglutinate T. so strongly as those with a mixed infection. It is interesting in this connexion to note that

TABLE III.—Showing Nature of Curve of Paratyphoid Agglutinins Compared with that of Typhoid Agglutinins in Same Series.

	Paratyphoid.				Typhoid.			
	No. of cases.	Station-ary.	Rise.	Fall.	No. of cases.	Station-ary.	Rise.	Fall.
Para. A ...	31	3.2 %	22.5 %	74.1 %	29	89.6 %	0	10.3 %
Para. B ...	43	25.5 %	13.9 %	60.4 %	41	60.97 %	7.3 %	31.7 %
Total ...	74	16.2 %	17.5 %	66.2 %	70	72.8 %	4.2 %	22.8 %

TABLE IV.—Showing Average Time Elapsing from Onset till First Agglutination and Average Interval between Readings.

	Stationary.		Rise.		Fall.	
	Months from onset.	Interval.	Months from onset.	Interval.	Months from onset.	Interval.
B. Para. A	2.5	11 days.	1.9	16.6 days	1.65	23.3 days.
B. Para. B	2.2	19 "	1.8	21 "	2	22.3 "
Typhoid in Para. A Series	1.78	21.8 "	0	0 "	1.5	20 "
Typhoid in Para. B Series	1.97	20.8 "	2.75	18 "	2.2	23.1 "

TABLE V.—Showing Percentage Rise or Fall by more than One Tube in the Series of 113 Cases.

	T. agglutinins in typhoid only series.	T. agglutinins in paratyphoid series.	Paratyphoid agglutinins in paratyphoid series.
Rise	6.25 %	1.23 %	9.8 %
Fall... ..	18.75 %	8.6 %	39.5 %
No. of cases ...	32	81	81

the para. A series is not much higher than that with T. agglutinins only, whereas in the para. B series the increase in the T. titre is very marked. This, it seems to us, is further evidence that a paratyphoid infection raises the T. titre, but that the rise is greater or takes place in a greater number of cases where the infecting agent is B. para. B than where it is B. para. A. Another possibility is that while both may be stimulated to produce an equal rise in the T. agglutinin content, the effect of the stimulus may be more evanescent where the infecting agent is B. para. A. The same stimulus effect has been noticed in non-inoculated civilians who were suffering from a mixed typhoid and paratyphoid infection. (Table VI.)

TABLE VI.—Showing Comparative Percentages of Cases with T. Agglutinins with a Titre of 1 in 150 or more.

	Of those agglutinating T. only.	Of those agglutinating T. and Para. T.
Cases examined twice	62.5 %	79.2 %
Cases examined once	57.8 %	68.9 %
Uninoculated civilian cases	64.5 %	80.0 %

In view of this we should expect a considerable difference in results according to the interval which has elapsed

between onset and agglutination. It does not follow, because there is a great subsequent fall in the para. titres, that we should expect a corresponding great fall in the T. titres of the same cases. We must take into account another factor—viz., the interval between the point of infection and the point at which agglutination is carried out. It is interesting to see how far this is borne out by a comparison of Glynn and Lowe's figures, bearing in mind that their cases were examined well on in convalescence, whereas ours were examined on an average 1.9 months from the outset of the illness. In their analysis we found that 14 per cent. of the T.A series and 51 per cent. of the T.B series showed an agglutination titre for T. in 1 in 250 or more as compared with 48.2 per cent. and 73.1 per cent. respectively, which in our series agglutinated in a dilution of 1 in 320 or more. Moreover, 10.3 per cent. of our T.A series fell as regards their T. agglutinin content, while 31.7 per cent. of the T.B series fell as regards their T. agglutinin content.

These figures suggest that the influence of the A stimulus on the T. agglutinin production had probably largely disappeared, while the influence of the B stimulus was still present. That a fall took place in the short period of 23 days between the two agglutinations rather points to the conclusion that the induced T. agglutinin curve was falling rather more rapidly than a simple T. inoculation curve would do. In other words, that after the induced rise the fall was not a repetition of the slow fall seen after inoculation.

We are inclined, therefore, from a consideration of the above to think that paratyphoid infection raises temporarily the existing inoculation titre for typhoid. In para. A infections the influence of the A stimulus on the T. agglutinin production is less in amount or more transitory in its effect than in the case of B infections, and in both cases the fall from the top of the induced T. curve is probably more rapid up to a certain level than would be the case if the decline of the induced curve imitated that which follows a simple T. inoculation. After that certain point (probably near the level which the T. curve would naturally have arrived at) the curve goes on to decline at the same rate as it would have done if no undulation of the curve had occurred.

With one doubtful exception, of the 74 cases agglutinating para. A or para. B none had been inoculated with a triple vaccine. The doubtful case is said to have had a triple vaccine four months before agglutination. He agglutinated B. para. A, the curve showing a marked decline between the two readings, while the T. titre remained stationary, and agglutinins for B. para. B were absent on both occasions. None of the other para. agglutinins in this series of 74 cases can be ascribed to a previous immunising inoculation. While a few were cases of relapse, 50 per cent. of the number were still in a condition of pyrexia, and, as a matter of fact, only 16.2 per cent. gave stationary para. readings, as compared with 72.85 per cent. of a total of 70 of the same cases which showed a stationary T. curve, and with 16.6 per cent. in a series of 12 civilian typhoids. The movement in the T. curve in the paratyphoid series we have tried to show above is probably largely explained by the effect of an A or B stimulus to a person already provided with a mechanism for the ready production of T. agglutinins. We have no evidence in the above section or in any of the others to follow that the para. agglutinins were to be regarded as co-agglutinins occurring in the course of a true typhoid infection. In 4 of the 74 cases paratyphoid agglutinins only were present, while paratyphoid bacilli were isolated from 17.5 per cent. of the series.

In addition to the fact that a high para. titre was present in a great number of the paratyphoid cases, we found that there was a corresponding difference in the extent of excursion of the curve between the two readings. Taking together all cases agglutinating T. A, T. B, or T. A B, we find that 49.3 per cent. of them showed a rise or fall in the paratyphoid curve of more than one tube as compared with 9.8 per cent. of the same series showing a rise or fall of the T. titre to the same extent. The total number of cases from which this is estimated was 81. Further, out of 81 cases 43.2 per cent. possessed a paratyphoid titre greater than their T. titre, which latter we believe to have been due to T. inoculation, while 7.4 per cent. yielded a para. titre of equal value with the T. titre.

These figures are further evidence that the paratyphoid curve was due to infection, while the typhoid curve was the result of inoculation, but disturbed by the para. stimulus.

In only 3 cases out of 70, or 4·2 per cent., was there a rise in the typhoid curve. In all 3 the paratyphoid titre was higher than the T. titre. On the first agglutination, and in only one, did the T. titre reach the same level as the para. titre. These we regard as examples of active para. infections where the para. titre is increasing and, owing to this stimulus, the T. titre is also on the rise.

Seven cases were found to agglutinate both A and B, the average time between onset and agglutination being 2·5 months, while the interval between the two readings was 17·5 days. None of these, so far as we could learn, had been inoculated with a triple vaccine except one, and he was done for the first time on the morning of the very day on which he reported sick. From his urine we have isolated B. para. A, and on inquiry we learnt that some months previously he had an attack of diarrhoea for two weeks, which, however, did not lay him up. His agglutination result was 1 in 320 for para. A and 1 in 54 for para. B on both examinations. In this case it is just possible that the para. B agglutinins were due to his inoculation. In 3 of the others the para. A titre was considerably greater than the para. B titre, and in 2 other cases the reverse held good. In the seventh case the titre of A and B was equal, but the A fell between the two readings, while the B remained at the same level.

In 4 of the 6 cases just mentioned the T. titre remained stationary, in 1 it fell below the higher of the two para. readings, and in 1 it rose while the para. A element rose in equal amount. In the same series of 6 cases the para. element which gave the higher titre fell in 3 cases between the readings, in 2 it remained stationary, and in 1 it rose. Of the lower of the two paratyphoid elements in the series of 7 cases none gave a higher reading than 1 in 54 except one which agglutinated both A and B in 1 in 533. Of these 2 had fallen to zero in an interval of 20 days. Finally, of the 6 cases mentioned 3 gave a lower T. titre than para. titre, 1 agglutinated T. and A in equal amount, and 2 were slightly higher as regards their T. titre, but one of these fell on a subsequent reading below the para. level.

Having regard to these figures and to the fact that none had received a paratyphoid vaccine (except one from whom paratyphoid bacilli were recovered), we are of the opinion that all of these are examples of paratyphoid infection, and that there is no evidence of infection by B. typhosus. The only point of difficulty is to explain the co-existence of A and B agglutinins. That the higher of the two titres in each case represents the response to the immediately active infection seems indicated from the first of the series, from which we recovered the organism. The question as to whether the weaker of the two paratyphoid sets of agglutinins in each case is the result simply of co-agglutination, is a co-infection, or represents the end result of a previous para. infection must be left for the present.

Section 2—Section 2 includes all those cases, 32 in number, which were found to agglutinate T. only, after two examinations. These form 27·8 per cent. of the total bloods examined twice. An analysis of these is set out in comparison (Table VII.) on the lines of that given by Glynn and Lowe.

TABLE VII.

—	Station- ary.	Months from onset.	Interval.	Rise.	Months from onset.	Interval.	Fall.	Months from onset.	Interval.
	%		days.	%		days.	%		days.
32 cases with T. agglutinins only	50	1·9	16·2	6·2	3·5	18	43·7	1·5	17·7
Typhoid in para. A and para. B series, 70 cases	72·85	1·8	21·3	4·2	2·75	18	22·8	2	22·5
Para. agglu- tinins, 74 cases	16·2	2·25	18·4	17·5	1·85	18·8	66·2	1·84	22·3

In inoculated persons a serological diagnosis can only be made after two or more readings separated by an interval. Since, however, there is a steady fall in titre after the inoculation maximum has been reached, it follows that a fall in titre between two readings may indicate either a typhoid infection or simply the natural diminution in the agglutinin content after inoculation. In order to discriminate between the two, one must take into consideration the time factor, more especially the interval between the readings. In the

case of the normal fall in a person not suffering from typhoid, the fall is a comparatively slow one, and in order to detect an appreciable difference the interval between the readings must be considerable. Some indication of this is given in the comments on the paratyphoid series where, we believe, no typhoid infection occurred.

In our series of 70 cases we find that 72·85 per cent. were stationary as regards their T. curve between the two readings, although we must bear in mind that these cases were also under the influence of a paratyphoid infection. Where the case is genuinely one of typhoid infection occurring in an inoculated or uninoculated person, we shall expect to find a steeper decline in the curve of the T. agglutinins, or, in other words, the interval allowing of a fall in the curve will be considerably shorter than in the case of non-typhoid persons. Another way to put it is that the downward excursion of the curve in a given period will probably be greater.

In this series we intend to claim all those as typhoid infections whose curve had altered during the two readings for the following reasons. In our series of 32 cases 50 per cent. only were stationary as compared with the 72·85 per cent. whose T. curve did not alter in the case of those infected with paratyphoid.

Of the 50 per cent. which showed a variation in titre 6·2 per cent. were of the nature of a rise. As these patients were not infected with paratyphoid bacilli so far as we know, we claim them as genuine cases of typhoid fever. One had been inoculated eight months before, and showed a rise of three tubes in 16 days, followed by a drop of two tubes after a 77-day interval. The extent of rise in both cases suggests that it was not due to any variation of sensitiveness on the part of the standard cultures used, supposing for argument's sake that the same batch of culture was not used on both occasions.

There remain 43·7 per cent. which showed a decline in the agglutination titre. As this decline occurred in the short average interval of 17·7 days, the first reading being taken on average 1·5 months from the onset, we believe we are justified in calling them cases of typhoid infection. Had the interval between the readings been longer, as, for instance, 4·8 weeks mentioned in Glynn and Lowe's paper, the fall in titre could not have been ascribed to a typhoid infection with the same degree of certainty. Further, those which fell one tube only were 8 in number, or 25 per cent. of the 32.

It is interesting to note that in those which fell one tube only the first reading was made 2·1 months from the onset, whereas those which fell two or more tubes were examined first 1·3 months from the onset, suggesting that the nearer we get to the infecting point the more rapid or the steeper is the rise or fall.

Of those which fell two or more tubes one had never been inoculated, while the others had been inoculated 21, 12, 11½, 11, and 9 months before respectively, the average interval between their two readings being 21·1 days. It is further interesting to note that in 62·5 per cent. of the 32 cases the titre was 1 in 150 or more. Of the 16 cases whose curve altered 14, or 87·5 per cent., had a titre of 1 in 150 or more, while of the 16 whose curve remained stationary only 5, or 31·2 per cent., had a titre of 1 in 150 or more.

For these reasons we are inclined to claim all 16 cases (50 per cent.) whose curve altered as cases of typhoid infection. Out of 115 men whose bloods were examined twice this forms a total of only 13·8 per cent. This is certainly a slightly larger percentage than that found by Glynn and Lowe (under 10 per cent. in a series of 300 cases). But it must be borne in mind that their cases were examined when well advanced in convalescence, whilst our agglutinations were carried out nearer the infecting point. Even our figure is a remarkable and irrefutable argument in favour of preventive inoculation, especially as only one of the 115 men died, whereas in the epidemic amongst uninoculated civilians out of 53 cases 15 per cent. died.

The single case which died agglutinated B. typhosus on both occasions in 1 in 50, but had no agglutinations for para. A or B. From him, however, we isolated B. para. B, which we take to be the causal organism in this case. We can therefore say that none of those which agglutinated T. only, died of a T. infection.

Section 3.—Out of this series of 115 cases only 2 were found which did not agglutinate T., A or B, at either agglutination. One had been inoculated once and against typhoid

only, five months before, and the interval between the two examinations was 22 days. The other had never been inoculated; the interval between the two readings was 19 days. As both were examined for the first time two and a half months from the onset of the illness we think it rather difficult to state definitely whether they had typhoid or not. The strong probability is that neither had had typhoid, though it is possible, especially in the second case, that the infection agglutinins had disappeared owing to the slightly longer interval which elapsed between the onset and the first agglutination.

Group II.

In this group are included 69 soldiers whose bloods were examined once only. The majority of these were convalescent, the first agglutination being made 2.25 months after onset in the case of those with agglutinins for T. only, and 2.69 months in the case of those with para. agglutinins as well. Several were examined, not because there was any suspicion of "enteric," but because the blood was being taken for some other purpose. In these cases the patients were suffering from some other disease—e.g., tuberculous meningitis, pneumococcal meningitis, and wound infections.

In view of a recent statement made by Captain H. L. Tidy, R.A.M.C.,³ which has already been completely refuted, we desire to state that in none of those cases suffering from diseases other than typhoid did we find any evidence of a disappearance of agglutinins, although in every case the patient was in a condition of pyrexia. Only one, suffering from wound infection, had no agglutinins for T., A or B, and he had never been inoculated! Since then we have had occasion to make many blood cultures in cases of pyrexia due to wound infection, and in these cases also we have found no evidence to support the contention that inoculation agglutinins disappear in cases of pyrexia not due to typhoid.

With 12 exceptions who came from France, all the others, so far as could be learnt, came from the East. An analysis of these cases appears in Table VIII. 43.4 per cent. of

TABLE VIII.

—	T. only.	T., A.	T., B.	T., A, B.	A, B.	No agglutinins.
Cases ...	38	6	15	8	1	1
Per cent. ...	55	8.6	21.7	11.5	1.4	1.4

these 69 cases showed the presence of para. agglutinins. Out of 41 of the 69 cases whose inoculation data were available only 1 had been inoculated against paratyphoid, and his blood showed complete absence of paratyphoid agglutinins, even in a 1 in 27 dilution. We think it highly probable, therefore, that all the 43.4 per cent. had had a paratyphoid infection. It is of interest in passing to note the smaller percentage—viz., 8.6 per cent.—which show the presence of para. A agglutinins, as compared with 26.9 per cent. recorded in the series whose bloods were examined twice and who were nearer the infecting point.

Of those which agglutinated T. only, 2 had been bacteriologically examined elsewhere and had been diagnosed as typhoid on an increasing Widal. Other 2 stated that they had been diagnosed as "enteric" abroad, but there was no documentary evidence of this. There are thus 2 more cases of definite typhoid, and probably 4, to be added to the figures already given in another section. It may even be slightly larger still, inasmuch as 6 out of 17, or 25 per cent., whose data are known agglutinated in a titre of 1 in 150 or more, especially in view of the fact that the average time elapsing since inoculation in these 17 cases was 9.8 months. Another possible explanation is that some of these had had a previous para. infection, the agglutinins for which were no longer present, but the effect of their stimulus was still felt in a heightened T. curve. At the same time, from a consideration of the typhoid agglutinins occurring in uninoculated civilians, we think it right to state that inferences based on mere height of titre may be fallacious, owing to the great variation in individual response to a T. infection or to a T. inoculation.

Of those agglutinating para. B that organism had been recovered from the blood in France in 2 cases, while a third had been diagnosed para. B on an increasing Widal. From one agglutinating T. + A and B we recovered B. para. B from the blood, stools, and peritoneal cavity, though no perforation was found at the operation. From two others we

isolated B. para. B from the faeces. Of this series, in all except one, the titre for one of the paratyphoid groups of organism was greater than for the other. In only 3 was the lowest of the 2 para. titres 1 in 27. The typhoid titre was 1 in 150 or more in 57.8 per cent. of those agglutinating T. only, while in those agglutinating paratyphoid as well it was 68.9 per cent., again showing, though not so markedly, the stimulus effect of a para. infection.

Of the 41 whose data are known all had been inoculated against typhoid except one, and his blood contained no agglutinins for T., A or B. He was suffering from wounds. One who had been inoculated against typhoid had only agglutinins for paratyphoid bacilli. Out of the remaining 28 whose data are unknown one had no agglutinins for T., A or B.

It is interesting to note that a titre of 1 in 150 or more for para. A was present in only 33.3 per cent. of the cases agglutinating B. para. A, while it was present for B. para. B in 53.3 per cent. of the cases agglutinating B. para. B, showing that the further we go from the infecting point the less does the agglutinin content for the infecting organism become, while what we regard as the T. inoculation titre, after being reinforced by the para. infection, remains higher for a longer period of time. It is of interest to compare these figures with the much higher ones, viz., 70.9 per cent. of those agglutinating B. para. A and 62.79 per cent. of those agglutinating B. para. B in the series of 74 examined twice, where the examination was made near the infecting point.

Group III.

In this group we record the results of 53 civilians whose agglutinations were carried out as described above, using the Oxford Standard cultures. The cases formed the bulk of a small epidemic which broke out in Reading at the end of December, 1915. As none of these had been inoculated nor had typhoid fever before, we thought it might be of interest to record the results. Unfortunately it was only possible to make two examinations in 13 out of the 53, but as they were all uninoculated one examination was sufficient as a rule to establish a diagnosis.

It was evident at the outset that we were either dealing with cases some of which contained co-agglutinins or we were dealing with a mixed infection. The latter we believe to have been the case, and in all probability the infection came from a military source, either through some flaw in the system of water-carriage or through direct contact.

Of the 53 persons suffering from an "enteric"-like disease 50 cases, or 94.3 per cent., had agglutinins for B. typhosus and a few for paratyphoid bacilli as well. Of the remaining 3 one was able to agglutinate B. para. B only, while the other two did not agglutinate T., A or B even in a 1 in 27 dilution. One of these was examined first on the eighth day of illness and again on the twelfth, with negative results each time. Post-mortem, however, revealed extensive ulceration and B. typhosus was recovered. The other case was examined once only—viz., on the thirty-first day—and although negative this patient had a hæmorrhage, which, taken in conjunction with the other clinical features of the case, justify us in calling it typhoid or paratyphoid. An analysis is shown in Tables IX. and X.

TABLE IX.

No. of cases.	T. agglutinins only.	T., A.	T., B.	T., A, B.	B only.	No agglutinins.
53	58.4%	5.6%	26.4%	3.7%	1.8%	3.7%

TABLE X.

No. of cases.	Stationary.	Days from onset.	Interval.	Rise.	Days from onset.	Interval.	Fall.	Days from onset.	Interval.
12	% 16.6	9.6	days. 7.6	% 58.3	11	days. 16.8	% 25	14.7	days. 19.2

Those in Table X. were agglutinated on two occasions. The ages ranged from 6 to 54 years, and the two sexes were almost equally divided. 37.7 per cent. showed the presence of paratyphoid agglutinins as well as or without T. agglutinins. The percentage agglutinating B. para. A is much smaller than that agglutinating B. para. B. As regards titre, 76.9

per cent. of these with T. agglutinins, whether or not accompanied by para. agglutinins, were able to agglutinate B. typhosus in 1 in 120 or more, the highest point reached in any being 1 in 5333. Of those agglutinating T. only 64.5 per cent. had a titre of 1 in 150 or more. Of those agglutinating paratyphoid as well as typhoid, the titre was 1 in 150 or more in 80 per cent. This suggests that the T. titre is higher in cases infected with typhoid and paratyphoid organisms than it is in pure T. infections. 60 per cent., or 3 cases out of 5 yielding A agglutinins were able to agglutinate B. para. A in 1 in 120 or more, the highest being 1 in 150. In 1 of these cases the A titre was higher than the corresponding T. titre. 52.9 per cent., or 9 cases out of the 17 which agglutinated B. para. B were able to agglutinate in a dilution of 1 in 120 or more, the highest being 1 in 5333. In one of these cases also the B titre was higher than the T. titre in the same serum. In another case the T. titre fell four tubes as against one tube in the B titre in the same time. The percentage of high para. titres would probably have been greater if all cases had been examined at least on two occasions. Of those whose data are available, whether examined once or twice, the average time elapsing between onset and first agglutination was 15.8 days.

That there was a definite co-existing paratyphoid infection in a certain number of the cases we believe is supported by the following facts. B. paratyphosus B was isolated from the stools of one patient (age 1 year) whose blood was not sent for agglutination, and who is therefore not included in the above series of 53. This case was clinically one of typhoid or paratyphoid infection. It is interesting to note that in the above series are included 3 other members of the same family from which this child came. One of them had agglutinins for T. and A. another for T. and B. and the third for T. only.

From another case with agglutinins for T. and B. B. para. B was recovered from the urine. In still another case already mentioned in the table there were agglutinins for B. para. B only. Finally, in one or two cases absorption tests were carried out which showed that the para. agglutinins in the cases examined were not of the nature of co-agglutinins.

Only 4 of these with agglutinins for paratyphoid bacilli agglutinated in 1 in 27 only on the single occasion of examination. One of these examined on the eighth day of illness also agglutinated B. typhosus in 1 in 120 only. Two were examined on the sixteenth day and had also T. titres of 1 in 150 and 1 in 1600 respectively, and the fourth with T. A agglutinins, examined on the twenty-first day, had a T. titre of 1 in 150. From the stools of a brother of this patient B. paratyphosus B was recovered. The remaining 31 cases, or 58.4 per cent., showed no trace of paratyphoid agglutinins even in 1 in 27, although 64.5 per cent. of them agglutinated B. typhosus in 1 in 150 or more, the titre in 22.5 per cent. running into four figures, of which the highest was 1 in 5333. Of these which agglutinated both para. A and para. B, one had a titre of 1 in 150 for A and 1 in 120 for B, while the other was 1 in 150 for A and 1 in 320 for B.

In view of the above facts we conclude that those cases with paratyphoid agglutinins from whom no paratyphoid bacilli were isolated were in all probability cases of mixed infection, and that none of them is to be regarded as examples of co-agglutination. Probably paratyphoid and typhoid bacilli would have been isolated in a greater number of cases had the excreta been systematically examined. Only a certain number were examined, and that on one occasion only when the patient was convalescent and ready to be discharged.

Group IV.

The last group embraces a series of 38 civilians whose bloods were examined once or oftener, but who were suffering or convalescent from other diseases. Of these, 15 were at first clinically suspected of typhoid, but none of them agglutinated T., A or B, even in 1 in 27, and as a matter of fact turned out to be ill from other causes. The remaining 23 bloods were taken at random from in-patients suffering from diseases other than typhoid. 19 of them were negative to T., A and B even in 1 in 27 dilution, while 4 gave different readings. That is to say, out of 38 civilians not suffering from typhoid or paratyphoid 89.4 per cent. contained no agglutinins for T., A or B even in a dilution of 1 in 27, while 10.5 per cent., or 4 cases, showed the following.

Two agglutinated B. typhosus up to 1 in 120. One—a woman—was suffering from colitis of two years' duration and empyema. She had not been inoculated, and could furnish no history of an attack of, or contact with, typhoid. The other was an old soldier who had served in India, and who said he had never consciously had typhoid and was never inoculated. In this case, however, we think there is a strong suspicion that he had at least been in contact with typhoid.

A third—a woman of 71—agglutinated B. typhosus up to 1 in 50. She stated that she had never had typhoid and had never been inoculated. At the age of 21, however, she had lived in the same house where one of the inmates died from typhoid. The fourth agglutinated B. para. B up to 1 in 50. He was in a hospital with Charcot joints. He had never had typhoid, had never been inoculated, and could give no history of contact. Out of the 4 cases, therefore, in one there was a certainty of contact with typhoid and in the other a strong suspicion.

Summary and Conclusions.

1. The paper is based on the serum reactions of 275 patients, of whom 184 were soldiers. 165 of these came from the Mediterranean, 18 from France, and 1 was an R.A.M.C. orderly who contracted paratyphoid in Reading. 91 were civilians, of whom 53 were cases of typhoid or paratyphoid occurring during the recent Reading outbreak, while the remaining 38 were not suffering from the typhoid-paratyphoid group of diseases, but were used as controls.

2. The tests were carried out on the principle recommended by Dreyer and his colleagues, but with a slightly different technique. We believe the drop method is more liable to personal error, takes up more time, and, owing to the difference in surface tension of the various liquids used, to a certain extent possesses an inherent inaccuracy. While the drop method is scientifically inaccurate, we should think that, owing to the gross nature of the end-point of the reaction, the margin of error arising from surface-tension may be neglected for all practical purposes. In our examinations we have used a volumetric method, delivering the fluids from accurately graduated pipettes.

3. We have used the standard agglutinable cultures throughout as supplied by the Standards Laboratory, Oxford, at the instance of the Medical Research Committee. We have found them thoroughly satisfactory for routine work, and the provision of such cultures has been a great boon at a time when the resources of the laboratory were being taxed to their utmost.

4. We believe this method to be the simplest, most accurate, and the most suitable of all agglutination methods where routine work is concerned.

5. The same observer should, if possible, carry out all the readings; these should be made against a black background, with a constant intensity of artificial illumination, and a hand lens employed to detect the end point.

6. Collection of blood is best obtained by venipuncture, by which means sufficient blood can at the same time be got for culture purposes.

7. Statistics based on clinical grounds alone can be of no value where typhoid and paratyphoid are concerned in cases which come from the East. The great majority of our cases arrived with a diagnosis of "enteric," whereas out of 115 cases only 13 per cent. at the outside could be claimed as true typhoid. The mortality among these typhoid cases was nil, and offers a marked contrast to the 15 per cent. mortality among 53 uninoculated civilians.

8. Out of the 115 cases examined twice, 71.6 per cent. contained agglutinins for paratyphoid bacilli, and out of the whole number of military cases 111, or 60.3 per cent., agglutinated paratyphoid organisms.

9. Paratyphoid infections raise the T. titre in inoculated persons. The stimulus effect, however, appears to vary according to the nature of the para. infection. The para. A stimulus is probably less in amount or more transitory in its effect than that of para. B. It is possible that as a result of a para. infection the T. inoculation curve rises from the point to which it has normally attained to a certain maximum. Having attained this, there is a somewhat sharper decline of the T. curve in T. A cases as compared with that in T. B cases. Hence in such cases an alteration of the T. curve between two readings, separated by a short interval, is more likely to be detected the nearer the examination is to the onset of the illness. If examined well on in convalescence, the T. curve, at least in T. A cases, has

probably begun to fall at its normal post-inoculation rate, and a change in T. titre will be less frequently encountered where the interval between the readings is a short one. We do not think that, having reached its maximum, the induced rise in the T. curve falls continuously from that point at the steady rate seen after simple inoculation.

10. That para. B infections are more common in these cases is probably more apparent than real, and is probably not to be explained merely by saying that one type of case was more common during certain months. Our results rather point to an explanation based on the time elapsing from the onset of illness till the first agglutination. The nearer to the onset that the first agglutination is made the greater will be the number of positive A agglutinations. The further on in convalescence one goes before taking a reading the more likely is it that the less persistent A agglutinins will have disappeared, and consequently there will be a greater apparent percentage of B infections.

11. We are unable to furnish any clear evidence of co-agglutination of A for B or *vice versa*, and there is no evidence of B. typhosus evoking co-agglutinins for paratyphoid organisms in the lowest dilution we employed—viz., 1 in 27. Paratyphoid infections in persons inoculated against typhoid have acted as a stimulus to the agglutinin-forming mechanism which had previously been called into being by a preventive T. inoculation.

12. We desire to emphasise the need for repeated agglutinations at intervals in the case at least of persons previously inoculated. We do not think that any diagnostic inference can be drawn from a consideration of the titre in inoculated persons, where only one examination has been made, as individuals vary so diversely in their agglutinin response to infection or inoculation.

13. We have found no evidence to support the view advanced by Tidy that marked pyrexia for some days is associated with diminution or disappearance of T. inoculation agglutinins. Diagnosis on Tidy's assumption would be more misleading even than that based on clinical observation alone.

14. The civilian epidemic in Reading was not due to B. typhosus alone, but was complicated by a paratyphoid infection in a certain proportion of the cases. The source of infection here was probably a military one, and the small epidemic broke out at a time when large numbers of typhoid and paratyphoid cases were in the military hospitals.

15. None of the 38 uninoculated controls showed any trace of agglutinins for T., A or B, except 4. In one of these there was a definite history of contact and in another a strong probability. Two only could not be accounted for.

References.—1. E. Hort: Brit. Med. Jour., 1915, vol. ii., p. 892.
2. E. Glynn and E. C. Lowe: THE LANCET, 1916, vol. ii., p. 222, et seq.
3. H. L. Tidy: THE LANCET, vol. i., 1916, p. 241.

AN APPLICATION OF DROP-MEASURING TO WIDAL TECHNIQUE:

A REPLY TO ADVERSE CRITICISM.

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IN a recent article by R. Donald on a Method of Drop-measuring Liquids and Suspensions¹ the writer finds occasion to make certain unfavourable criticisms of the drop-method and apparatus recommended for use with the standardised agglutinable cultures issued from the Standards Laboratory of the Department of Pathology at Oxford on behalf of the Medical Research Committee. The fact that the author of the article has for several years been engaged in a series of very detailed and extended researches on drop-measuring lends evident importance to his statements on this subject, though it does not qualify him to assume that the physical principles underlying such methods of measurement, which, as he rightly says, "have been more or less widely known for generations," are unfamiliar to other workers who have employed drop-measurement for many years past.

In associating with the publication of his article the name of Professor W. Bulloch, F.R.S., the writer adds greatly to

the apparent weight and value of his criticisms of Dreyer's method of measuring the agglutinin reaction for purposes of clinical pathology. Nevertheless, it can be demonstrated without difficulty that those of his objections whose triviality is not transparent entirely fail to establish themselves in regard to any essential detail of the method in question. Yet since the author appears to put forward all his objections with equal seriousness, it might seem discourteous not to make some attempt to meet them *seriatim* and with suitable gravity.

The misleading character of the statements which form the foundation of this very vigorous criticism has already been indicated briefly on a former occasion.² But, in the absence of my friend and colleague, Major G. Dreyer, on active service, I may now be permitted to undertake the duty of defending in more detail his agglutination technique against aspersions which I venture to think disfigure a contribution possessing otherwise no little interest. Unfortunately, it is often found to be the case that when such aspersions prove to be ill-founded and to spring from hasty judgment or inaccurate deduction, they tend to create a feeling of dubiety regarding the value of other and doubtless unexceptionable conclusions which the writer has put forward.

Donald's criticisms are directed chiefly against: (a) the pipettes provided in the agglutination sets supplied for the Medical Research Committee, because he finds considerable variation in the volume of their drop-delivery; (b) the technique, because it seems on cursory examination to disregard the fact that drops of different fluids and suspensions are of different volumes. On the former count he credits the method with an error of 40 per cent., and on the latter he awards it errors of 20 per cent. and 10 per cent. respectively. Further (c) he animadverts on the "Directions" for the "Use of Standardised Agglutinable Cultures" both for things they say and for things they omit to say, as well as for their general neglect of "the six precautions" which he mentions as being "necessary for practical accuracy in drop-measuring."

These are very serious and sweeping charges which can hardly have been read with equanimity by any who have committed themselves during the past year or more to the use of Dreyer's method. Since if they were well established it would be evident that this method entailed inaccuracies almost approaching the same order of magnitude as those inherent in the older methods of performing agglutination tests. The appearance of accuracy obtained by standardising both the opacity and the sensitiveness to agglutination of the cultures issued would then be quite illusory. For the quantitative measure which it was hoped thus to obtain would vanish in a mass of incorrigible errors from other sources. Fortunately this is not in any degree the case, as I shall try to show. But first it may be of assistance in clearing the ground to state briefly (a) what in essence Dreyer's method is, and (b) what it attempts to do.

a. The essence of Dreyer's method consists in having so arranged the details of the technique that in any given agglutination test the serum acts throughout in all its dilutions in the same volume of fluid, and on the same number of bacilli, in a suspension whose sensitiveness to agglutination has been ascertained exactly, instead of acting in unequal volumes of fluid as is frequently the case, on unequal numbers of bacilli as is usually the case, and in suspensions of unknown and highly variable sensitiveness to agglutination as is always the case in other methods which were previously in use. The method also endeavours to ensure that the actual volume of the mixture of the fluids used shall, so far as possible, always be approximately the same in every test that is made, and shall be contained in tubes of approximately the same form and diameter. But these matters are not regarded as being quite so fundamental since, so long as the *ratios* of the fluids remain unaltered, such differences in the results as may occur from failure in these details are so small that they can only be detected with difficulty, if at all.

β. What the method attempts, and not unsuccessfully, is to provide a means of performing agglutination tests of such precision as will enable a diagnosis to be made even in inoculated persons; and this not only where well-equipped laboratories are at hand, but without any laboratory at all, or any other apparatus than that provided, and under all the various difficulties of active service conditions.

¹ THE LANCET, Sept. 2nd, 1916, p. 423.

² THE LANCET, Sept. 9th, 1916, p. 493.