THIRD REPORT ON EXPERIMENTS IN CONNECTION WITH ANTI-TYPHOID VACCINE. 

By BREVET-LIEUTENANT-COLONEL W. B. LEISHMAN,
MAJOR W. S. HARRISON, MAJOR H. W. GRATTAN,
CAPTAIN A. L. A. WEBB, AND CAPTAIN J. C. KENNEDY.
Royal Army Medical Corps.

(1) To ascertain the influence on the phagocytosis of typhoid bacilli, in the presence of a heated normal serum, when using as a test organism, first, a non-virulent strain of *Bacillus typhosus*, second, a freshly isolated and virulent strain.

These experiments were undertaken for the purpose of investigating the discrepant results which had been obtained by ourselves and by Dr. G. Dean, of the Lister Institute. As may be seen from our former reports, we obtained, in almost all cases, higher readings, in respect of the phagocytic activity of the polynuclears, when the normal serum had been heated to 60° C. for half an hour, than when the same serum was added to the washed cells without preliminary heating. This led to the abandonment of our attempt, in the Aldershot work, to measure the fluctuations in the typhoid opsonins by the method usually adopted for the demonstration of these substances. Dr. Dean, on the other hand, had found consistently higher phagocytosis before heating than after. He was good enough to give us the benefit of his experience in the

1 Previous reports on this subject were published in the *Journal of the Royal Army Medical Corps* in the numbers for May, 1907, and June, 1908.
matter, and his suggestion that the cause of the discrepancy of our results might lie in the fact that we employed different strains of typhoid bacilli in our respective experiments proved to be correct. For the purpose of testing this point we exchanged cultures, with the result that, working with our strain, Dr. Dean obtained results identical with those which we had recorded, while we, on our part, were able to confirm Dr. Dean’s work when using the strain which he was in the habit of employing.

Our tests had always been carried out with an old laboratory strain, to which there remained little virulence; on the other hand, Dr. Dean’s strain had been recently isolated from a case of enteric fever, and was found to possess a high degree of virulence for guinea-pigs.

As an example of the results of the experiments which we conducted on this point, the following may be quoted:—

Two strains were employed, one, “G,” being an old strain, which required an intra-peritoneal dose of 5 cc. of a twenty-four hours’ broth culture to kill a guinea-pig; the other, “K,” had been recently isolated from the blood of a case which terminated fatally, and was lethal to guinea-pigs in a dose of 5 cc.

A sample of serum, taken from a normal rabbit, was divided into two parts, one of which was heated at 60° C. for half an hour, the other portion being unheated. Phagocytic estimations were then made with each strain of B. typhosus, the number of bacteria ingested by fifty polynuclear leucocytes being recorded in each instance, with the following result:—

<table>
<thead>
<tr>
<th></th>
<th>Strain “G.”</th>
<th>Strain “K.”</th>
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<tbody>
<tr>
<td></td>
<td>Unheated</td>
<td>Unheated</td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>Serum</td>
</tr>
<tr>
<td></td>
<td>after heating at 60° C.</td>
<td>after heating at 60° C.</td>
</tr>
<tr>
<td>No. of bacteria in 50 cells.</td>
<td>600</td>
<td>379</td>
</tr>
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</table>

(2) To ascertain the degree of bactericidal action of normal saline solution upon B. typhosus.

This question was raised in connection with the method recently advocated by Klien for the quantitative measurement of the bactericidal power of a serum. The dilutions of the serum in this method are made with normal saline, and, in the higher ones, it appeared to us that the well-known bactericidal action of salt solution might vitiate the results.

A series of capillary tubes were accurately graduated with mercury, and, after sterilisation, 20 c.mm of a 1-10,000 dilution of a twenty-four hours' broth culture of strain "G" were drawn up into five of these tubes. One was blown out at once on to an agar plate, to serve as a control, while the remaining four tubes were incubated at 37° C. and blown out on to agar plates after various periods, ranging from three-quarters of an hour to three hours. In each case the plates were found to be sterile, while the control tube, in which the contact with the diluting salt solution had only been a few minutes, showed about 150 colonies.

Bearing in mind the importance of the virulence and age of the strain, as shown in the former experiment, this experiment was repeated, with the difference that a virulent strain "D.1" was substituted for the non-virulent "G." In this case the dilution employed was 1-6,300 of a broth culture, and the tubes were blown on to the agar plates at intervals from half an hour to three-and-a-half hours. The results were the same as with the non-virulent culture; all of the tubes which had been incubated proved sterile, while the control tube, when plated on the agar, yielded about 400 colonies.

As it appeared possible that the bactericidal action, demonstrated by the above experiments, might, at least in part, have been due to the action of the mercury, employed in the calibration, upon the lumen of the capillary tubes, they were repeated with tubes which had been graduated with water, in place of the mercury. As shown by the following table, the results were practically the same, although a few germs in this case resisted destruction for some time:

<table>
<thead>
<tr>
<th></th>
<th>Virulent Strain Col.</th>
<th>Non-virulent Strain Col.</th>
</tr>
</thead>
<tbody>
<tr>
<td>After 1/2 hour at 37° C...</td>
<td>... 17</td>
<td>... 1</td>
</tr>
<tr>
<td>&quot; 1 &quot;</td>
<td>... 15</td>
<td>... 1</td>
</tr>
<tr>
<td>&quot; 2 hours &quot;</td>
<td>... 0</td>
<td>... 0</td>
</tr>
<tr>
<td>Control tube</td>
<td>... 250</td>
<td>... 200</td>
</tr>
</tbody>
</table>

In Klien's method a broth culture of *B. typhosus* is diluted with normal saline solution, and then mixed with varying amounts of immune serum solution and an excess of complement. The mixtures are then incubated for three hours and planted out on agar. A reduction in the number of colonies is taken as the end-point. Normal saline solution alone being able to kill 250 virulent typhoid bacteria after two hours' incubation, as is shown by the above
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experiments, it would appear, then, to be difficult to distinguish between the lethal effect of the salt solution and that of the immune serum. Klien, however, did not work with capillary tubes as we did, but with small test tubes, and on repeating his experiments with his own technique the bactericidal action of the normal salt solution was not apparent. However, it was not possible to adapt this method to our work, because the very large amount of blood serum which it requires, renders it unsuitable to the capillary tube methods necessitated where only a small amount of blood is obtainable from the patient's finger. It appears, however, to be a good method of estimating the bactericidal power of a given serum, and one more in accord with the dilution methods by which we estimate the titre of the agglutinins.

(3) In the following experiments it was desired to ascertain, as far as this could be done by laboratory experiment, what influence the age of a vaccine had upon its immunising properties. For this purpose a vaccine, prepared a year previously to the experiment, which had been out to New Zealand and had been subsequently returned to the laboratory, was used for the purpose of immunising a group of rabbits whose blood was subsequently “pooled” and submitted to quantitative tests of the protective substances. As controls, two other groups of rabbits were employed; one of these was inoculated with a freshly prepared vaccine, made from the same strain and by the same technique as the year-old vaccine, while the second was not inoculated, but furnished pooled serum for purposes of daily comparison with the blood of the two inoculated groups.

The details of the experiment may be followed by referring to the accompanying charts. (Charts I.-IV.) The rabbits received three doses of vaccine, 20, 40, and 80 millions respectively, at intervals of nine to eleven days, and tests were carried out as to the development of agglutinins and bactericidal substances, while the phagocytic ratio was, in each case, tested both by Wright's modification of the method devised by one of us (W.B.L.) and also by Klien's method of progressive dilutions of the serum.1

As regards the bactericidal substances, the method of serum dilution and plating, employed in our former work, was followed. As will be gathered by a glance at Chart I., there was no very marked evidence of superior development of these substances in the case of the rabbits immunised with either vaccine. An obser-

vation of the bactericidal power of the pooled serum of the uninoculated rabbits was made in every case, and the figure obtained daily was used as the normal standard. The system of recording the results on the chart is explained below in connection with the bactericidal observations on the blood of typhoid convalescents (p. 348).

![Chart I](image1)

**Chart I.**—Development of bactericidal substances in groups of rabbits inoculated (a) with a fresh vaccine, (b) with one a year old.

![Chart II](image2)

**Chart II.**—Development of agglutinins in groups of rabbits inoculated (a) with a fresh vaccine, (b) with one a year old.

**Note.**—No agglutination over 1-10 was noted in the rabbits inoculated with the old vaccine.

*Agglutinins.*—(Chart II.) A marked contrast was brought out by the analysis of these substances. While the rabbits inoculated with the fresh vaccine showed normal development of the agglutination curve, which, subsequent to the third dose, rose as high as 1-600, the blood of the rabbits inoculated with the year-old vaccine never showed any trace of these substances, the highest value
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recorded being a trace of agglutination in 1-10. The normal group were persistently negative.

Phagocytosis. (a) Wright's method. (Chart III) The same technique, as detailed in the former reports, was used, and a very striking contrast will be noted in the degree of response to the individual inoculations. The rabbits which received the fresh vaccine showed a very marked rise after the second and third doses, and, at the end of the experiment, the phagocytic ratio was above 3; i.e., phagocytosis was three times greater with the heated serum of the immunised rabbits than with the heated serum of the normal rabbits, used as controls at each observation. On the other hand, the indices of the group inoculated with the year-old vaccine showed far smaller fluctuations after each dose, although it is to
be noted that the last observation was the highest recorded and approximately the same as with the fresh vaccine, namely 2.6. It may be added that it does not by any means follow that a rapid appearance of the substances influencing phagocytosis, as a result of inoculation, is to be taken as evidence that these substances will remain for long at this high level. It is indeed possible, as indicated by some of our incidental experiments, that a more lasting impression is to be obtained by a process which would, in its initiation, appear to be slow and inadequate.

(b) Klein's method. (Chart IV.) By applying this method, coincidently with the older one, a useful contrast of the methods was obtained, and the value of the results reached with the one could be controlled by those of the other. This method has been the subject of much experiment, and, although it occupies considerably longer in the carrying out, the observer to whom fell this portion of the work preferred it as giving more striking and more easily interpreted results. It will be seen that, in this instance, a regular and steep curve is the result of the inoculations with the fresh vaccine, followed by a sharp fall, though not to normal, on the inoculations being discontinued; while, in the case of the rabbits inoculated with the old vaccine, Klein's method showed no elevation above the degree of phagocytosis exhibited by the non-inoculated control group.

From the general results of this experiment we gathered that the vaccine which was a year old and had voyaged round the world had lost a considerable portion of its immunising properties. As this was a point of the very first importance, the experiment was accordingly repeated, and the results of this second test are detailed below.

(4) In this experiment the immunising effect of three different vaccines was tested on three groups of rabbits, and the results were controlled by similar tests carried out on the "pooled" serum of a fourth group of normal rabbits. The three vaccines used had all been prepared from the same strain of *B. typhosus* and in the same manner, and differed only in respect of their ages; these were, respectively, on the day on which the experiment commenced, six days, eleven weeks and five days, and twenty-seven weeks. On this occasion all the vaccines had been kept under identical conditions as regards temperature, &c., and none of them had been out of the laboratory. The question of possible changes in the vaccines due to temperature did not, therefore, come into consideration. (The effects of temperature alone formed the subject of a further series of experiments, detailed below.)
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Each of the three groups of inoculated rabbits received, as in the former experiment, three consecutive and increasing doses of vaccine hypodermically, 20, 40, and 80 millions, at intervals of ten days, and their pooled serums were tested for agglutinins and for phagocytic substances, the latter by both the methods employed in the former investigation. Bactericidal substances were not measured on this occasion, as little useful information had been obtained from these tests in the earlier experiment, and as the time required was more than could be spared, since four separate quantitative estimations had to be made of each group and for each substance.

The results are shown in the accompanying Charts (V., VI., and VII.). The agglutinin curves (Chart V.) show a striking result; while there is little difference to be seen in the curves furnished by the groups inoculated with the fresh and with the three-months-old vaccines, in both of which there was a rapid and considerable development of these substances, there was, on the other hand, no trace of agglutinins to be found throughout the experiment in the case of the group of rabbits which had been inoculated with the six-months'-old vaccine.

The above result was borne out in a striking manner by the tests made in connection with the phagocytic activity of the immunised animals (Charts VI. and VII.). Here, again, there did
not appear to be any essential difference between the fresh and the three-months' vaccines, while, in the case of the six-months'-old vaccine, the inoculated animals showed no difference from the normal rabbits. The index furnished daily by the pooled serum of this normal group represents, in all cases, the base or normal line upon which the curves have been plotted.

This experiment further serves as an illustration of the results of these two very different methods of estimating the opsonic content of the serum, or, as we prefer to call it, the phagocytic ratio. By the older method (Chart VI.) will be seen the customary fluctuations of the ratio which, as immunisation progresses, reaches
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a higher and higher level. The small fluctuations which occur in the record of the six-months' vaccine are within the limits of experimental error, and it is apparent that this vaccine had lost its power of inducing increased phagocytic activity when employed for immunisation.

Klein's method (Chart VII.) brings out the same essential points with equal clearness, though in a different form. Here the curves show much greater regularity, and indicate a slow developing but, in the end, a very marked increase in the substances influencing phagocytosis, as evidenced by the high degrees to which it was necessary to dilute the serum before Klein's "end-point" was attained. The two methods are alike in demonstrating that this six-months-old vaccine had, in this respect, acted as an inert fluid.

The general result, therefore, of these two series of experiments has been to show that a vaccine of six months age may have lost, if not all, at least a very large part of its immunising properties; so much so, indeed, that it would appear to be useless for the purpose for which it was intended. As soon, therefore, as we had convinced ourselves of this possibility, we took steps to ensure that no vaccine of greater age than three months should be used for inoculation, and instructions to this effect have been printed on the labels of each phial of vaccine, and issued to all concerned in its distribution.

(5) Attempted immunisation by ingestion of dead typhoid bacilli.

Our initial experiments in this direction, recorded in earlier reports, had shown some indications suggesting the possibility of inducing an effective degree of protection by this means. As such a procedure would offer manifest advantages over hypodermic inoculation, especially in the case of the soldier, a further series of experiments was instituted. On this occasion four volunteers underwent the treatment, which consisted in the swallowing of sterilised emulsions of agar cultures of B. typhosus; these were suspended in fat and enclosed in gelatine capsules to permit of their passing undamaged through the stomach. The bacteria had been killed either by being heated in the fat to a temperature of 60° C., or by being exposed to the vapour of chloroform before they were suspended in the fat. Each capsule contained the growth from half an agar slope, in a tube of the ordinary size.

The tests which were carried out were the estimation of the phagocytic activity of the blood, by Wright's and Klein's methods, and the agglutinin value of the serum. The attached Charts (VIII., IX., and X.) will show the number of and the
intervals between the doses taken by each of the four volunteers, and the curves of these substances. The controls were made on each occasion from the blood of a normal and healthy individual. The experiment may be shortly summarised as follows, each case being dealt with separately:

**Chart VIII.—Ingestion of dead typhoid bacteria. Curves of agglutinin.**

**Chart IX.—Ingestion of dead typhoid bacteria. Phagocytic indices of four volunteers, tested by Wright's method.**

**Case 1.**—Major G. Previously inoculated by the usual method. He took, in all, eight doses within five weeks. The treatment was followed by a marked and persistent elevation of the phagocytic ratio, as tested by Klien's method (Chart X.), but Wright's method did not on this occasion correspond with Klien's, as it showed
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A drop below normal after the fifth dose had been taken, and a persistently low value until a fortnight after the last dose (Chart IX.). Agglutinins, which, at the beginning of the experiment, stood at 40, rose to 160, and, at the time the observations were discontinued, still remained as high as 120.

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<thead>
<tr>
<th>DAYS</th>
<th>BEFORE TREATMENT</th>
<th>DAYS</th>
<th>AFTER TREATMENT</th>
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<tr>
<td>9</td>
<td>7</td>
<td>5</td>
<td>3</td>
</tr>
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<td>1</td>
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<td>5</td>
<td>5</td>
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<td>7</td>
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</tr>
<tr>
<td>45</td>
<td>47</td>
<td>49</td>
<td>51</td>
</tr>
</tbody>
</table>

**RATIO TO NORMAL**

- 8.0
- 7.5
- 7.0
- 6.5
- 6.0
- 5.5
- 5.0
- 4.5
- 4.0
- 3.5
- 3.0
- 2.5
- 2.0
- 1.5
- 1.0
- 0.5

**DOSES SWALLOWED**

- Major already done.
- Case B, already done.
- Case D, already done.
- Case W, not done.

**CHART X.—Ingestion of dead typhoid bacteria. Phagocytic indices of four volunteers, tested by Klein's method.** (Chart reduced to unity.)

**Case 2.**—Captain D. Previously inoculated by the usual method. Only two capsules were taken in this case, as the subject was much upset by the treatment, considerable digestive disturbance resulting. His phagocytic ratio, which at the beginning of the experiment was well above the normal, fell, after some oscillations, to within normal limits. Agglutinins, which stood at 40 before the treatment was started, fell to vanishing point while it was in progress, but subsequently recovered on the capsules being discontinued.

**Case 3.**—Lieutenant W. Had neither been inoculated nor had enteric fever. He took ten capsules over a period of one month. They caused no unpleasant symptoms, but his blood throughout the whole experiment showed no signs of the development of either agglutinins or opsonins. (It may be noted in connection with this officer that, on being inoculated some months later with the usual vaccine, he showed an unusually severe type of local reaction; and his general reaction, although not very severe, was...
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prolonged into the second day. It is possible that the effect of the large number of capsules had been in some measure to induce a degree of hyper-sensibility to subsequent inoculation.

Case 4.—Private D. Had neither been inoculated nor had enteric fever. Four capsules were taken in ten days. A transient rise of phagocytic activity was evidenced by Klien's method during the period of treatment, but this disappeared rapidly on its termination. Wright's method, on the other hand, demonstrated a fall in phagocytosis when Klien's showed a rise. This contrast between the two methods is also in evidence in the other experiments, and is difficult to understand. A trace of agglutination appeared in his serum eight days after the last dose had been taken.

Taking these experiments as a whole, and bearing in mind our earlier work in this direction, it cannot be said that they furnish much hope of our being able to provide an efficient substitute for inoculation in the shape of pills or capsules of dead bacteria. It certainly appears to be possible to modify the contents of the blood, in substances bacteriotropic to the typhoid bacillus, by the ingestion of dead germs, but the results which we have obtained have been too irregular and too little under control to warrant a departure from the present system of inoculation. Immunisation by way of the digestive tract is, however, a subject which is occupying the attention of bacteriologists to a great extent at the present moment, and it is possible that some of this work may furnish us with indications as to a means of getting more efficient and reliable results than has been possible so far.

(6) The influence of the virulence of the strain upon the immunising properties of a vaccine.

In the preceding report experiments, conducted on the same lines, appeared to show that a vaccine prepared from a virulent strain of B. typhosus would induce the development of a higher phagocytic ratio than one which had been prepared from a non-virulent strain. The point being one of considerable importance, in view of the fact that the vaccine used for the inoculation of troops has always been prepared from a non-virulent strain, a similar experiment was made to see whether the apparent advantage of the virulent vaccine would be manifest in rabbits inoculated in the same manner and tested by both phagocytic methods.

Two vaccines were accordingly prepared, one from the non-

1 Journal of the Royal Army Medical Corps, vol. x., p. 583, 1908.
virulent strain in common use, the other from a virulent and recently isolated strain which was lethal to a guinea-pig of 250 grammes weight when 0.5 c.cm. of a twenty-four hours' broth cul-

![Chart XI](chart_xi.png)

**Chart XI.**—Development of agglutinins in groups of rabbits which had been inoculated with vaccine prepared from virulent and non-virulent strains of *B. typhosus*, respectively.

![Chart XII](chart_xii.png)

**Chart XII.**—Curves of the phagocytic ratios by Kien's method (reduced to unity), in groups of rabbits which had been inoculated with vaccine prepared from virulent and non-virulent strains of *B. typhosus*, respectively.

ture was inoculated intra-peritoneally. Three groups of rabbits were employed, the third serving, as usual, as a daily control of the quantitative estimations of the protective substances. As will be seen from the Charts (XI., XII., and XIII.), several observations were
made on each group prior to the first inoculations. The results of these preliminary estimations serve as a check to the values recorded after the immunising process had commenced, and, incidentally, bear witness to the accuracy of the methods themselves. The two groups of inoculated rabbits received in all three doses, the second fourteen days after the first, and the third twenty-six days after the second. The dosage was, respectively, 40, 80, and 160 million bacteria.

The development of the agglutinins may be followed in Chart XI., and a glance will show that there is nothing to choose between the two vaccines in respect of these substances, the close correspondence of the two curves being, indeed, a remarkable feature, and, in our experience, a rare one. The test strain of \( B. \) typhosus employed for measuring the agglutination was a non-virulent one, but, of course, different from the non-virulent strain employed for inoculation; it was selected for the purpose as one which furnished a good emulsion, and was of proved value for such tests.

The phagocytic ratio, tested both by Wright's and by Klien's methods (Charts XIII. and XII.), responded readily to the inoculation of each vaccine, and here again it is not possible to assign a greater value to one vaccine than to the other; both curves will be seen to follow similar lines, and, in spite of the wide range of the records, the values recorded on any one day for the two groups of rabbits are never very far apart. If one appeared to show to advantage at one time, the balance inclined to the other side at a later observation. Thus, as was the case with the agglutinins, no differ-
ence was elicited with regard to the development of the substances influencing phagocytosis. The test strain employed in these experiments on phagocytosis was a virulent one, and had been recently isolated from a case of enteric fever occurring in Bermuda.

From the above experiment, which covered a period of more than two months, we were unable to confirm the impression which the former series had given us, and it would appear that the inoculation of a vaccine prepared from a non-virulent strain of typhoid bacteria is capable of inducing as satisfactory development of agglutinins and substances influencing phagocytosis as one made from a virulent strain.

(7) The influence of the temperature at which a vaccine has been kept upon its immunising properties.

It was possible that the deterioration which some of the vaccines had been found to have undergone, after a certain lapse of time, might have been due, not to the factor of age alone, but to the conditions under which they had been stored. Of these conditions the most variable must be the temperature at which the vaccine has been kept. A vaccine sent out to the Tropics might change in some manner to which it would not be liable in a temperate climate. The influence of light can hardly be held as a possible cause of deterioration, as it may be assumed that the vaccine is left in the boxes in which it is packed until the moment of use, and the manner in which it is hermetically sealed in the glass phials should render other factors negligible.

The temperature, then, remained to be tested, and the following experiment was undertaken. After the preparation of a certain brew of vaccine, three samples of this brew were set aside for three months; one portion was kept at the ordinary temperature of the laboratory, the second in the ice-chest, and the third in the hot incubator at 37° C. All were kept in the dark, and they were not disturbed in any way until the three months had elapsed.

At the commencement of the fourth month from the date of the preparation of the vaccine, a series of immunising experiments was commenced on groups of rabbits. Four of these groups, comprising three animals in each, were employed; one served as the control group, while each of the three remaining groups was inoculated with one of the three samples of the vaccine, the doses being identical in each case.

Estimations were made on the agglutinins and of the phagocytic power of the blood, the latter by both Wright's and Klien's methods. No attempt was made to measure the bactericidal substances, partly on account of the great labour involved in carrying
out so many observations on four groups of animals, and partly because this method demonstrates great individual variations in the case of rabbits. The rabbits received, in all, four doses of vaccine, consisting of 20, 40, 80, and 160 millions respectively, the intervals being recorded on the accompanying charts (XIV., XV. and XVI.).

It is unnecessary to comment at length on the results of the experiment, as the details can be followed in the charts, and it will suffice to summarise the conclusions which appear to emerge from
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them. (1) The vaccine which had been kept at room temperature comes out of the test best. (2) The samples which had been kept in the hot incubator and in the ice-chest gave, approximately, the same results; neither was inactive, but both appeared to have lost a portion of their immunising properties, as contrasted with that which had been kept at room temperature, and, also, with the results of former immunising experiments on rabbits, carried out with vaccines of about the same age which had been kept under similar conditions.

It would appear, therefore, from this experiment, that it is advisable to keep typhoid vaccine at a moderate and, as far as may be, equable temperature, and that extremes, either of heat or cold, are deleterious and to be avoided. It seems clear, further, that nothing is to be gained by keeping vaccines in the ice-box, a suggestion which has been made on several occasions with a view to the possible preservation of their properties.

(8) Investigation of the blood-changes following an attack of enteric fever.

Our aim here was to determine the average course of development of the various protective substances after an attack of enteric fever, in the hope that the results, when contrasted with those which we know to follow inoculation of typhoid vaccine, might give us useful information as to the particular substance or substances which we should endeavour to call forth by our inoculations. This was thought to be worth attempting in view of the uncertainty

1 In this experiment we were assisted by Lieut. R. G. Archibald, R.A.M.C., whose careful and accurate work was of the greatest help to us.
which still prevails as to the relative importance attaching to the various substances which are demonstrable in the blood in acquired immunity, whether this be natural immunity, as after an attack of the disease, or artificial, as after a bacterial inoculation.

With this end in view, the following substances were estimated in a series of cases: agglutinins, bactericidal substances, stimulins and opsonins. The system adopted was to collect the serum of each typhoid patient or convalescent and to test this once, and once only, for the above substances, recording the results on a single chart under the particular week after infection in which the observation was made. The investigation was carried out as opportunity offered, and forty-one cases were collected within six months and dealt with in this way. The majority were convalescents furnished by the military hospitals of Netley and Millbank. Arrangements were made to secure uniformity in the work by allowing the serum, in all cases, to remain in contact with the clot for twenty-four hours, before it was drawn off and submitted to analysis. The charts record the individual observations made on these forty-one cases, each being entered under the particular week after infection, and the curves for each substance were subsequently constructed by taking the average of the observations recorded for each particular week. As was anticipated, considerable individual variations were encountered and, on this account, it was decided to mark a point in this curve only when it could represent the average of at least two observations made during the same week. In the later months of convalescence, however, when such individual fluctuations should be less in evidence, the average of two or more observations, made during the same fortnight, was recorded, if there was no more than one observation in a given week.

In the above manner it was hoped that the average blood-picture following an attack of enteric might be forthcoming, and that this might guide us in assessing the results of our experiments with typhoid vaccine. Information was elicited in all cases as to whether the original attack had been mild, ordinary, or severe, but those tested comprised too small a proportion of mild cases to yield us much information in this connection, and the curves in general may be taken to represent the course of events following on a severe attack. The preponderance of the severe cases is due to the fact that most of the men were soldiers who had been invalided from their foreign stations on account of the severity of their attack; mild cases are not now invalided from abroad, and the few whom we had the opportunity of testing were cases contracted in this country.
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and admitted either to Netley or to Millbank, or those invalided for some other cause.

In the accompanying charts (XVII., XVIII., XIX. and XX.) the actual observations made on the serum of each convalescent are

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**Chart XVII.**—Average curve of agglutinins after enteric fever.

**Chart XVIII.**—Average curve of bactericidal substances after enteric fever.

recorded as dots under the week in which they were tested, while the curves constructed from the averages, in the manner just mentioned, are, for the sake of clearness, recorded on the same charts, the points representing the averages obtained for each week being
shown as hollow dots. Where this average has been taken from
the mean of the observations in a fortnight instead of a week, it
is placed on the line between the two weeks.
The number of cases investigated during the actual course of
the attack was very small, and the observations during the weeks
immediately following defervescence are also few in numbers,

but, allowing for this, the general results bring out several points
which appear to us to be of considerable interest.

To take the agglutination curve in the first instance. No new
features are to be noted: the course of the agglutinins, during
and after an attack of enteric, has formed the subject of many
investigations, and the results brought out here are quite in accord­
ance with the usual experience. Observations were made on twenty­
eight cases, and the general course of these substances may be followed in Chart XVII.; the rapid fall in the agglutination value of the serum after the decline of the fever is brought out, and the low level at which it remains during the following months is also evident. At the same time, although the level is low, in no case was a negative result obtained, even eight months after the date of the attack. In one case, tested in the twenty-fifth week, the exceptionally high agglutination figure of 1-1,000 was obtained; this, although recorded in the chart, has not been taken into account in framing the average, as it is rare to find such a figure, even during the height of the disease; at the same time the observation is an interesting one, and suggests the possibility that the individual from whom it was obtained was a "typhoid-carrier," although we had no opportunity of putting this to the proof. Only two cases were classed as having been "mild," the remainder being either of ordinary severity or "severe." The only case which terminated fatally, subsequent to the date of our examination, showed, in the fifth week of the attack, a low figure of agglutination, 1-60.

Bactericidal Power.—In all, thirty-three cases, ranging from the second to the thirty-fifth week, were tested as to their bactericidal power; five of them were classed as "mild" (noted with an "m" on the chart), and the remainder were almost all recorded as "severe." The technique was that we have employed in the similar estimations recorded in former reports, namely, progressive dilutions of the serum were allowed to remain for a definite time, at blood heat, in contact with a measured test-dose of living typhoid bacteria, the mixture being subsequently plated out on agar. The chart (No. XVIII.), however, is framed on somewhat different lines, in order to show the results more graphically. Each observation of a convalescent's blood was controlled by a similar observation on the blood of a healthy man, and the system of recording the results of these tests was to enter the figure obtained from the typhoid patient as being so many points better or worse than the figure yielded by the normal individual; for instance, a serum which killed the test-dose of typhoid bacteria in a dilution of 1-60, while the control could only kill a similar dose in a dilution of 1-40, would be charted as being two points above the normal line.

The curve, plotted from the averages in the manner just described, shows the rapid decline of these bactericidal substances after defervescence and the gradual fall to normal, or even below normal, six months after the attack. Allowing for the inequality
of the two groups, it may be seen that the average value of the cases recorded as having been "mild" is considerably higher than that of the severe cases which constitute the majority. The fatal case, when tested, showed a comparatively good bactericidal power, a point which will be referred to again in considering the phagocytic results.

Phagocytic Ratio.—This was tested with the heated serum of the individual by Wright's method, and compared, on each occasion, with the phagocytic powers of the heated serum of a normal man. The individual observations are recorded and the curve is plotted from averages, as described above (Chart XIX.). The thirty-five observations made between the second and thirty-fifth weeks, although showing the great range of individual variations, bring out a marked contrast to the curve of the averages of the bactericidal observations. Beginning at a low point, little above the normal, the phagocytic power rises, sometimes to a great height, in the weeks following convalescence, and this elevation is well maintained up to the eighth month after the attack; it will be recalled that the bactericidal power appeared to decline steadily during the same period until it was the same as normal, or even below normal. On the whole, the power of the six cases classed as mild appeared to be a little higher than that of the severe cases. A further point of interest is in connection with the observation made in the fifth week on the serum of the case which terminated fatally; it will be seen that the figure here was only .8, and that this is the only instance in which a figure less than normal was recorded within the first twelve weeks.

Stimulins.—Observations were made on the serum of thirty-five cases to ascertain whether the stimulin effect of adding a trace of their heated serum to the heated serum of a normal individual could be demonstrated with any regularity (Chart XX.). For the technique of the method for eliciting this phenomenon the reader is referred to former reports. Although not very marked in the early days of convalescence, the serum of the later cases shows a considerable power of stimulating the phagocytic activity of the leucocytes of a normal man. The curve of stimulin averages, like that of the phagocytic index, shows a tendency to rise as the months go on, and no tendency to drop, even at the end of eight months. Once more, too, the only negative result within the first twelve weeks was in the case of the man who died.

Too much stress is not laid upon the results of this investiga-
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tion, the numbers are obviously too small and the individual variations too great, but, at the same time, certain general information, of the kind which we sought, does appear to us to be brought out by a careful consideration of the curves plotted from the averages. The steady declines both in the agglutination curve, and, more especially, in the curve of the bactericidal power, stand out in striking contrast to the curves of the substances connected with phagocytosis. The impression which this leaves on our minds is that the latter are produced in increasing amount after convalescence, and that they remain demonstrable in the blood-serum for longer periods than, at least, the bactericidal substances. It is, then, a matter of conjecture whether these phagocytic substances are not those which have most to do with the acquired immunity conferred on an individual by an attack of enteric fever. Some support for this view may be found in the results of our immunisation experiments on man and animals by the inoculation of typhoid vaccine, for here, too, phagocytosis is markedly increased, and the increased power is demonstrable for long periods after inoculation.

Owing to the small number of the cases classed as mild, little can be gathered as to the development of the various substances in relation to the severity of the attack. On the whole, it would appear that in the prolonged and severe cases there is a diminished production of protective substances, and that this comparative poverty is not compensated during convalescence. Finally, although the fatal case was an isolated one, the blood examination in this brought out an interesting condition, namely, that although the agglutinins were of moderate amount and the bactericidal power good, the tests as to phagocytic activity demonstrated that this was in default and even below that possessed by a normal individual. This, if found to be a general rule in similar cases, would furnish a point of prognostic value, and would also testify to the importance of phagocytosis in combating an attack of enteric fever.