NOTE ON AN INVESTIGATION INTO THE BLOOD IN CASES OF TUBERCULAR DISEASE AND MALTA FEVER.

BY CAPTAIN J. C. B. STATHAM.
Royal Army Medical Corps.

The following series of experiments on the blood of tubercular and Malta fever patients have been carried out at Netley with a view to investigate—

(1) The claim made by Wright and Douglas, that the opsonic power of the blood in tubercular disease and Malta fever is lowered with regard to their respective causative bacteria.

(2) Whether such lowering of the opsonic power of the blood was specific to these diseases.

(3) Whether there was any close relation between the opsonic, phagocytic, and agglutinative powers of the blood with reference to the Micrococcus melitensis of Bruce, in cases of Malta fever.

(4) Whether, and if so at what ratio, the opsonic power of the blood decreased twenty-four hours after being drawn from the body.

The paper is divided into two parts: The first deals with the investigation into the opsonic power of the blood, with reference to the tubercle bacillus in cases of tubercular disease, as compared with that of patients suffering from diseases other than tubercle. The second part of the paper treats of a similar investigation into the blood in Malta fever, as well as a comparison between the opsonic, phagocytic, and agglutinative powers of the blood in that disease. There is also included in the second part of the paper an investigation into the blood opsonic power of patients suffering from diseases other than Malta fever on the M. melitensis; and the question of the loss of opsonic power in blood drawn from the body and kept in vitro for twenty-four hours is also dealt with.

The papers consist of a series of answers to questions on the four points mentioned as investigated.

PART I.

THE OPSONIC POWER OF THE BLOOD ON THE TUBERCLE BACILLUS IN CASES OF TUBERCLE, AS COMPARED WITH THAT OF HEALTHY ADULTS AND OF PATIENTS SUFFERING FROM DISEASES OTHER THAN TUBERCLE.

The blood of eighteen patients suffering from tubercular disease was first examined. The methods adopted for estimating the opsonic power of the blood were similar to those first elaborated by Leishman and subsequently modified by Wright and Douglas.
The object aimed at was to estimate the power possessed by the blood serum of so preparing bacteria that they are more readily ingested by the leucocytes of the blood. The modification by Wright and Douglas of Leishman's original method of estimating phagocytosis consists in working with serum and leucocytes from separate sources and thus estimating the value of the blood fluids alone, while Leishman estimated the value of the entire blood in his phagocytic method.

**Method.**—The following fluids, in the quantities specified below, were measured off in capillary pipettes:

1. Washed blood corpuscles (as prepared by Wright and Douglas' method, slightly modified) 2 parts.
2. Tubercle bacilli, heated to 100°C, to destroy their agglutinating power, dissolved in physiological salt solution containing 1 per cent. of soda citrate, and then thoroughly centrifugalised to remove clumps 1 part.
3. The blood serum of either the patient to be examined, or of a healthy man as control (this was obtained in the usual way, i.e., by centrifugalising a capsule containing blood till the serum was separated from the clot) 2 parts.

The quantities so taken were mixed on a glass slide and the mixture redrawn up into the pipette, which was now sealed and incubated at 37°C for fifteen minutes. On completion of the incubation, smears or slides were made from the mixture and stained as follows: Half a minute in hot carbol fuchsin, one minute in a 3 per cent. solution of sulphuric acid, then washed, and the slide dropped for one minute into watery methylene blue.

The slides were then washed and examined. The results obtained by this method of staining were satisfactory and constant, the bright, red-stained tubercle bacilli being easily distinguished.

1 The modification consists in using a pipette in the preparation of the washed corpuscles instead of a capsule as advocated by Wright. Equal parts of a little finger blood and a 1 per cent. solution of sodium citrate are drawn up into a pipette with a chamber two or three inches long. The mixture of blood and citrate solution is now drawn up into the chamber of the pipette while the stem is broken off short and sealed. The chamber containing the citrate blood is centrifugalised, the supernatant citrated plasma drawn off with another pipette, and an excess of physiological salt solution added to and mixed with the layer of blood corpuscles remaining in the chamber. The pipette chamber containing the mixture of blood cells and salt solution is again centrifugalised, and when the blood cells have settled down the supernatant salt solution is pipetted off. This process is repeated, and after the second washing the blood cells are considered washed, i.e., freed from plasma; the upper layers of the blood cells remaining in the chamber after this second washing and centrifugalisation—which consist largely of white blood corpuscles—are made use of in the experiment. They are called “washed blood corpuscles” by Wright and Douglas, and are so referred to throughout this paper.
and counted in the pale, blue-stained polynuclear white blood cells. The tubercle bacilli contained in from fifty to sixty polynuclear leucocytes were then counted and the average number of tubercle bacilli per cent. estimated.

Example—

Examination of Blood of Control.
(1) J. C. B. S.'s (healthy adult's) washed corpuscles ... ... ... 2 parts.
(2) J. (healthy adult's) blood serum ... ... ... ... ... 2 parts.
(3) Tubercle bacilli heated to 100° C., dissolved in a solution of physiological salt and 1 per cent. soda citrate and then centrifugalised ... ... 1 part.

The above amounts were measured off in a capillary pipette, mixed on a slide, and redrawn into the pipette, which was sealed and incubated at 37° C. for fifteen minutes. A smear on a slide from this incubated mixture and stained in the manner above described, showed the presence of 177 tubercle bacilli in the first sixty polynuclear white blood cells seen and examined, or an average of three bacilli per cell. This number (three) was taken as the control unit.

Examination of Blood of Tubercular Patient.
(1) J. C. B. S.'s (healthy adult's) washed corpuscles ... ... ... 2 parts.
(2) Blood serum of W. (case of tubercular hip-joint disease) ... ... ... 2 parts.
(3) Emulsion of tubercle bacilli prepared as described above ... ... 1 part.

These fluids were mixed, incubated, and stained in a manner identical with that of the control. They showed the presence of 102 tubercle bacilli in fifty-four polynuclear white blood cells, or an average of 1·9 bacilli per cell. The ratio of opsonic power of the tubercular patient’s blood on the tubercle bacillus to that of the control was therefore $\frac{1.9}{3.0}$, or 0·6, and this figure represented the opsonic index of the tubercular patient’s blood.

Working by these methods, the blood in eighteen cases suffering from tubercular disease, and in eight non-tubercular patients, was examined, and an endeavour made to ascertain whether the opsonic power of the blood on the tubercle bacillus was lowered in tubercular disease; and if so, whether this lowering was specific to the disease or due to general causes, such as the debility and anaemia consequent on chronic affections.

Question I.—Is the opsonic power of the blood of tubercular patients on the tubercle bacillus lower than normal?

With a view to answering this question, the following examination of the blood of eighteen tubercular patients was undertaken. The cases examined were not all proved, bacteriologically, to be tubercular; but well-marked clinical types of the disease were selected, and this method was adopted owing to the difficulties
which were met with, and will always be met with, in proving many undoubted tubercular surgical affections to be tubercular bacteriologically.

Results. Table I.

(The opsonic index of the blood of the control is taken as 1.)

<table>
<thead>
<tr>
<th>Case</th>
<th>Disease Description</th>
<th>Opsonic Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>H.</td>
<td>Case of tubercular disease of prostate (tubercle bacilli present in urine)</td>
<td>0.86</td>
</tr>
<tr>
<td>S.</td>
<td>Case of tubercular disease of testicle</td>
<td>0.63</td>
</tr>
<tr>
<td>M.</td>
<td>Case of tubercular disease of testicle</td>
<td>0.83</td>
</tr>
<tr>
<td>L.</td>
<td>Case of tubercular glands in neck</td>
<td>0.39</td>
</tr>
<tr>
<td>R.</td>
<td>Case of tubercular disease of hip-joint</td>
<td>0.8</td>
</tr>
<tr>
<td>A.</td>
<td>Case of tubercular testicle</td>
<td>0.73</td>
</tr>
<tr>
<td>Br.</td>
<td>Case of tubercular disease of hip-joint</td>
<td>0.7</td>
</tr>
<tr>
<td>W.</td>
<td>Case of tubercular testes</td>
<td>0.77</td>
</tr>
<tr>
<td>H.</td>
<td>Case of tubercular disease of hip-joint</td>
<td>1.0</td>
</tr>
<tr>
<td>M.</td>
<td>Case of tubercular glands in neck</td>
<td>1.3</td>
</tr>
<tr>
<td>W.</td>
<td>Case of tubercular disease of hip-joint</td>
<td>1.0</td>
</tr>
<tr>
<td>L.</td>
<td>Case of phthisis (tubercle bacilli present in sputum)</td>
<td>0.73</td>
</tr>
<tr>
<td>R.</td>
<td>Case of phthisis (tubercle bacilli present in sputum)</td>
<td>0.84</td>
</tr>
<tr>
<td>W.</td>
<td>Case of phthisis (tubercle bacilli present in sputum)</td>
<td>0.85</td>
</tr>
<tr>
<td>H.</td>
<td>Case of phthisis (tubercle bacilli present in sputum)</td>
<td>0.7</td>
</tr>
</tbody>
</table>

In the above eighteen examinations it will be observed that the opsonic index of the blood was below normal in thirteen cases, normal in three, and above normal in two. Of the last two cases, one (H.), Case 2, had ceased to pass tubercle bacilli in the urine (as proved by inoculation into a guinea-pig) when this result was obtained, whereas, when a tubercle bacilluria had been present the opsonic index was below normal (see Cases 1 and 2). The second of the two tubercular cases with an opsonic index above normal was suffering from a psoas abscess which had been scraped out and flushed, and the man was convalescent when his blood was examined. This second case is interesting from the point of view of the claim made by Wright and Douglas, that the opsonins of the fluids in contact with the tubercle bacilli (pus from tubercular abscesses, peritoneal lymph from cases of tubercular peritonitis) become exhausted and cannot deal effectively with the bacteria, the improvement in the patient's condition, after the opening up of an abscess or tubercular abdomen, being partly due to the inflow of fresh opsonins in blood and lymph to replace the evacuated fluids.

Answer to Question I.—There appears to be, as is claimed by Wright and Douglas, a definite lowering of the opsonic power of the blood on the tubercle bacillus in tubercular diseases.

As the lowering of the opsonic power of the blood on the
tubercle bacillus found in the great majority of the tubercular patients might conceivably be due to the depressed body vitality, anemia, and debility, consequent to a chronic disease like tubercle, another investigation was undertaken to decide this point.

**Question II.**—Is the lowered opsonic power of the blood on the tubercle bacillus, found in cases of tubercle, due to the debility and lowered vitality occasioned by a chronic disease, or is it specific?

To answer this question, the blood of seven patients in the hospital, debilitated by chronic disease other than tubercle, was examined.

**Results. Table II.**

(The opsonic index of the control is taken as 1.)

<table>
<thead>
<tr>
<th></th>
<th>Opsonic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>F., case of debility after Malta fever.</td>
</tr>
<tr>
<td>2.</td>
<td>S., , ,</td>
</tr>
<tr>
<td>3.</td>
<td>H., , , ,</td>
</tr>
<tr>
<td>4.</td>
<td>D., case of mitral disease and dropsy.</td>
</tr>
<tr>
<td>5.</td>
<td>C., case of severe secondary syphilis.</td>
</tr>
<tr>
<td>6.</td>
<td>T., case of extreme debility caused by severe tertiary syphilis.</td>
</tr>
<tr>
<td>7.</td>
<td>H., case of liver abscess.</td>
</tr>
</tbody>
</table>

It will be seen on examining these results that six out of the seven patients examined had a blood opsonic index which was normal, or above normal, while only in one case (6), where the debility was extreme, was the opsonic index below normal. Why two of these debilitated cases should have had a higher blood opsonic power on the tubercle bacillus than that of a healthy adult control I do not know.

**Answer to Question II.**—The lowered opsonic power of the blood on the tubercle bacillus present in tubercular disease appears to be specific to that disease, and is not due to the debility and depressed vitality consequent on a chronic malady like tubercle.

**PART II.**

**AN INVESTIGATION INTO THE OPSONIC POWER OF THE BLOOD ON THE MICROCOCCUS MELITENSIS IN CASES OF MALTA FEVER, AND IN CASES OF DEBILITY DUE TO OTHER CAUSES, ALONG WITH A COMPARISON BETWEEN THE OPSONIC, PHAGOCYTIC, AND AGGLUTINATIVE POWER OF THE BLOOD IN MALTA FEVER.**

An investigation on similar lines, and by methods similar to those used in estimating the opsonic index in tubercular disease, was carried out on thirteen cases of Malta fever, and on five patients debilitated by diseases other than Malta fever. The object aimed at was also the same, viz., to ascertain whether the opsonic power of the blood on the *M. melitensis* was definitely lowered in
cases of Malta fever, and, if so, whether this reduction in power was due to some specific cause, or, only to the debility occasioned by the disease. The only difference in the technique employed in this investigation from that used for the tubercular cases was, of course, the substitution of an emulsion of the *M. melitensis* for the emulsion of tubercle bacilli. This emulsion was prepared by adding a little distilled water to a recent growth of the *M. melitensis* on an agar slope. The growth was broken up in the water till a suitable emulsion had been formed; this was then drawn off into a glass capsule and heated to 100° C. for one hour to destroy the agglutinating power of the cocci. To three parts of the heated emulsion one part of a solution containing 3 per cent. of sodium chloride and a 4 per cent. of sodium citrate was added; the result being an emulsion of bacteria which contained 0.75 per cent. of salt and 1.0 per cent. of citrate of soda. Except for the method employed in staining the smears of incubated blood, the technique employed was similar to that described at length in the first part of the paper, and need not be mentioned again. After repeated trials the most satisfactory method of staining the films found was to dip an unfixed film of blood for a few seconds into weak watery gentian-violet. This method caused dehaemoglobinisation of the red cells and left the leucocytes stained faintly violet, the contained cocci being recognised by their slightly greater depth of stain.

The serum for the control was taken from W., and the washed corpuscles from J. C. B. S., both healthy adults.

*Question III.—Is the opsonic power of the blood for the *M. melitensis* lowered in Malta fever?*

With a view to answering this question the blood of thirteen patients in the hospital suffering from Malta fever was examined.

*Results. Table III.*

**Thirteen Cases of Malta Fever.** *(The opsonic index of the control is taken as 1.)*

<table>
<thead>
<tr>
<th>No.</th>
<th>Opsonic Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.75</td>
</tr>
<tr>
<td>2</td>
<td>0.80</td>
</tr>
<tr>
<td>3</td>
<td>0.90</td>
</tr>
<tr>
<td>4</td>
<td>0.83</td>
</tr>
<tr>
<td>5</td>
<td>0.35</td>
</tr>
<tr>
<td>6</td>
<td>0.92</td>
</tr>
<tr>
<td>7</td>
<td>0.35</td>
</tr>
<tr>
<td>8</td>
<td>0.39</td>
</tr>
<tr>
<td>9</td>
<td>0.60</td>
</tr>
<tr>
<td>10</td>
<td>0.77</td>
</tr>
<tr>
<td>11</td>
<td>0.78</td>
</tr>
<tr>
<td>12</td>
<td>0.70</td>
</tr>
<tr>
<td>13</td>
<td>0.70</td>
</tr>
</tbody>
</table>
Blood of Tubercular Disease and Malta Fever

It will be noticed that in all thirteen of these cases proved (by the ability of their blood in high dilution to agglutinate the M. melitensis) to be Malta fever, the opsonic power of the blood for the M. melitensis is below normal.

Answer to Question III.—The opsonic power of the blood on the M. melitensis appears to be definitely lowered in cases of Malta fever.

The next point to be investigated was, whether this lowering of the opsonic index was due to some specific cause or to debility only. Although this point had been decided where the tubercle bacillus was in question, when the lowering of the opsonic index in tubercular disease was found to be specific, it was thought advisable to investigate this point with regard to the M. melitensis also.

Question IV.—Is the lowered opsonic power of the blood in Malta fever on the M. melitensis due to the debility and depressed body vitality following this debilitating disease, or is it specific?

To settle this point the blood of five patients debilitated from diseases other than Malta fever was examined.

Results. Table IV.

(1) Case of cirrhosis of the liver and ascites. Opsonic index = 1.0
(2) Case of locomotor ataxy. Opsonic index = 1.2
(3) Case of dysentery. Opsonic index = 1.2
(4) Case of heart disease. Opsonic index = 0.9
(5) Case of enteric fever. Opsonic index = 1.1

In these five cases it will be noted that only one has an opsonic index for the M. melitensis lower than normal.

Answer to Question IV.—The lowered opsonic power of the blood on the M. melitensis in cases of Malta fever is due to a specific cause and not to the debility, &c., consequent to that disease.

The blood from the cases marked (9) to (13), inclusive, of Malta fever shown in Table III. was then examined with a view to comparing its opsonic, phagocytic, and agglutinating powers. The method adopted for estimating the phagocytic power of these cases was that of Leishman's, as slightly modified by Wright and Douglas, and consisted in drawing up equal parts of the blood of the control or of the Malta fever patient along with the emulsion of the M. melitensis (prepared as described above) into a capillary pipette. The equal parts of blood and emulsion were then treated in an exactly similar manner to that described in the first part of the
paper in estimating the opsonic power of the blood. That is to say, these equal parts of blood and emulsion of the \textit{M. melitensis} were mixed on a slide and redrawn up into the pipette, which was sealed. The pipettes were now incubated at 37° C. for fifteen minutes. In default of a regular incubator, which could not be used, as the examination was conducted on bedridden patients in a ward, an improvised incubator was used. This incubator consisted of two glass beakers placed one within the other. The inner beaker and the space between the inner and outer beakers were filled with water at a temperature of 37° C. This temperature was readily maintained during the investigation by the occasional warming of the improvised incubator over a small lamp, or by adding a little hot water to the water in the beaker as occasion required.

The agglutinating power of the Malta fever blood was determined by the sedimentation test.

\textbf{Question V.—Is there any close relation between the opsonic, phagocytic, and agglutinating powers of the blood in Malta fever?}

\textbf{Results. Table V.}

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|}
\hline
Cases & Opsonic power & Phagocytic power & Agglutinating power, (Highest dilution with which positive result obtained) \\
\hline
Control blood & 1.0 & 1.0 & Nil. \\
(9) G. & 0.36 & 0.5 & 1 in 800 \\
(10) W. & 0.77 & 0.58 & 1 in 600 \\
(11) P. & 0.78 & 0.52 & 1 in 600 \\
(12) Wo. & 0.7 & 0.53 & 1 in 800 \\
(13) F. & 0.7 & 0.79 & 1 in 300 \\
\hline
\end{tabular}
\end{table}

The control used for testing the opsonic power of the blood in these five cases of Malta fever consisted of J. C. B. S.'s corpuscles along with W.'s serum. The opsonic indices of the patient's blood were obtained by using J. C. B. S.'s corpuscles along with the patient's blood sera, as the washed corpuscles used in both cases were from the same source, the difference between the opsonic index of any of the Malta fever patients and the control represented the difference between the opsonic power of normal serum and the serum of the Malta fever patients. In the estimation of the phagocytic power of the bloods, however, the control used was W.'s whole blood (i.e., corpuscles + plasma), and the comparison obtained is between W.'s whole blood and the whole blood of the Malta fever patients. As the difference between the opsonic indices of control and Malta fever bloods has been shown to be due to the difference...
between a normal and a Malta fever serum, and the difference between the phagocytic indices of the blood of its control and the Malta fever patients is necessarily due to a difference between both corpuscles and plasma, the variation between the phagocytic and opsonic indices is a measure of the activity and vitality of the phagocytes themselves, apart from serum influences in each particular case. Thus in Cases 9, 10, 11 and 12 the phagocytic index being lower than the opsonic index shows the phagocytes of these four patients to be less active than those of W., the control, while those of Case 13 are more active, for here the phagocytic index is higher than the opsonic index. This table further shows that while there is an approximate correspondence between the opsonic and phagocytic powers of the blood, there is no definite relation between these properties and the agglutinating power.

**Answer to Question V.**—There is a more or less definite relation between the opsonic and phagocytic powers of the blood, the phagocytic index being generally lower than the opsonic index, owing to the leucocytes of the patient being less active than those of a healthy adult control, but the converse may be found. There appears to be no definite relation between the opsonic and phagocytic powers of the blood in Malta fever and its power of agglutinating the *M. melitensis*. It appears that the simpler process of estimating the phagocytic power of the blood alone might be substituted for the longer and more difficult method of estimating the opsonic power when time or opportunity would not permit the latter process being carried out.

**Question VI.**—What is the amount of the decrease of the opsonic power of blood drawn from the body and kept in vitro for twenty-four hours?

This experiment was carried out with a view to determine whether a sample of blood could be sent, say from an out-station to a district laboratory, in order to have its opsonic value tested.

The opsonic power of the blood on the *M. melitensis* was tested (a) when freshly drawn, and (b) when kept in a capsule for twenty-four hours. Three samples of blood were examined, one from W. (the control used in the Malta fever investigations), the second from a patient suffering from cirrhosis of the liver, and the third from a case of locomotor ataxy. The opsonic powers of these bloods were estimated in a manner identical with that described in the first test of the paper. The washed corpuscles used were in each case derived from J. C. B. S.

1 Wright and Douglas have proved that blood plasma and blood serum have the same opsonic power.
Results. Table VI.

<table>
<thead>
<tr>
<th>Case</th>
<th>Fresh blood</th>
<th>Twenty-four hours' old blood</th>
<th>Percentage of decrease of opsonic power</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1) W. (healthy adult's) serum + J. C. B. S.'s corpuscles; 30 cells counted</td>
<td>Average of 4 cocci in each cell</td>
<td>Average of 3 cocci in each cell</td>
<td>25</td>
</tr>
<tr>
<td>(2) Serum of case of cirrhosis of liver + J. C. B. S.'s washed corpuscles; 20 cells counted</td>
<td>Average of 5-4 cocci in each cell</td>
<td>Average of 3-8 cocci in each cell</td>
<td>28</td>
</tr>
<tr>
<td>(3) Serum of case of locomotor ataxy + J. C. B. S.'s washed corpuscles</td>
<td>Average of 4 cocci in each cell</td>
<td>Average of 3-5 cocci in each cell</td>
<td>13</td>
</tr>
</tbody>
</table>

It will be seen from these figures that though the decrease is not a constant one, yet there is a considerable decrease in the opsonic power of the blood if drawn and kept for twenty-four hours. Wright and Douglas have already shown that there is such a decrease, and estimate it at from a third to a half in the course of three days, while finding little variation after a few hours. The deterioration after twenty-four hours was estimated, as this period would about represent the time necessary for a sample of blood to be sent from an out-station for analysis.

Answer to Question VI.—There is so considerable and definite a loss of the opsonic power of the blood twenty-four hours after being drawn, that no reliable results could be obtained from an examination of such blood.

General Conclusions and Remarks.

The conclusions arrived at, after this long and somewhat tedious examination of over sixty samples of blood, need be but briefly recapitulated, as they have already been given in the answers to the questions upon the various points of investigation.

(1) The results of the investigation tend to confirm the claim made by Wright and Douglas that the opsonic power of the blood in cases of tubercular disease and Malta fever is lower than normal.

(2) This lowering of opsonic power appears to be specific to these diseases, and not due to general causes, such as the debility and depressed vitality following chronic affections.

(3) In view of these facts and the success attained by Wright and Douglas in the treatment of diseases due to the staphylococcus and tubercle bacillus, by raising the opsonic power of the blood by injections of small quantities of the killed bacteria causative of these
diseases, a similar method of treatment might, with advantage, be employed in Malta fever.

(4) That, while there is no apparent relation between the agglutinating power of the blood and its opsonic power, the opsonic and phagocytic values of the blood do not differ seriously.

(5) That, in view of the latter fact, of the simplicity of Leishman's phagocytic method, the somewhat complicated nature of the modification introduced by Wright and Douglas of using washed corpuscles, and finally of the loss in opsonic power of drawn blood, it would be generally advisable to use the phagocytic method. A sample could not usually with advantage be sent for examination of its opsonic power from an out-station to a district laboratory. It may be remarked that the method of testing the opsonic power of the blood serum apart from the corpuscles is neither an easy one nor does it give invariably satisfactory results. One cause for this is the difficulty in getting a satisfactory layer of white blood corpuscles. In an earlier part of the paper it was shown how in this method the blood serum of the patient was tested against the blood serum of a healthy man as control, an equal quantity of washed blood corpuscles and of the bacterial emulsion to be tested being added.

These washed corpuscles are derived from an independent and healthy source and are prepared by washing them with physiological salt solution, to get rid of the blood plasma, and by centrifugalising, to get a layer of white blood cells (which are lighter than the reds) to the surface. This layer of leucocytes is very small, and when one dips a pipette into it in order to take up the required quantity of washed corpuscles, one sometimes gets a considerable number of leucocytes; or, if the pipette has been dipped in the least bit too far, very few. Thus, if a series, say two or three blood sera, are being tested with the same stock of washed corpuscles, the first sample may be tested with a large number of leucocytes and the last with very few. This, if it occurs, must not only introduce a source of inaccuracy, but also make it difficult to do a phagocytic count in the last sample owing to the paucity of leucocytes on the slides. The simple original method of Leishman's (as slightly modified by Wright and Douglas) has not this source of error and annoyance, as one whole blood is tested against another, though, of course, here also the leucocytes of one blood may be slightly greater than those of the other sample taken.

There is another source of difficulty in testing the opsonic power of the blood when emulsion of tubercle bacilli and the \textit{M. melitensis}
are used. This difficulty, which is present in both the opsonic and phagocytic methods, is due to the fact that however well one may centrifugalise an emulsion, occasional small clumps of bacteria may be retained—the ingestion of even one or two clumps into phagocytes might prejudice the resulting count somewhat.

Another point which caused some trouble when dealing with emulsions of tubercle bacilli, was that there were appreciable numbers of what appeared to be branched forms; and it was occasionally difficult to decide whether this appearance was really due to branching, or to two or three bacteria joined at an angle; in short, whether these forms should be counted as one or more.

Another difficulty experienced was in doing the phagocytic counts on the Malta fever cases. In these experiments the minute \[ M. melitensis \] was stained the same colour as its containing cell, and was very difficult to define and count accurately. Unfortunately, the difficulty will always occur, as differential staining, as in the case of the tubercle bacillus, is here impossible.

These difficulties in phagocytic counts, where the tubercle bacillus or the \[ M. melitensis \] is concerned (there are few difficulties in phagocytic counts with the staphylococcus), render them liable to an appreciable margin of error. In fact, Lieutenant Proctor, I.M.S. (who helped me in some of the counts), and I endeavoured to find how great this error might be, by independent phagocytic counts from slides derived from the same sample of blood tested. We found frequent differences of as much as 10 per cent. between our respective counts, and the difference was occasionally much greater—15 to 20 per cent. As the opsonic indices of the bloods of some of the patients given in the tables in this paper are only 10 per cent. under normal, this source of error is a somewhat serious one, though I do not think it is sufficiently serious, or occurs often enough, to greatly diminish the value of Leishman's method of phagocytic counts, or the process as modified by Wright and Douglas for opsonic tests, in these cases.

In conclusion, I have to thank Dr. Wright for a supply of killed tubercle bacilli, and Lieutenants Proctor and White, I.M.S., for help in doing some of the phagocytic counts for me.

REFERENCES.
