By CHARLES E. SIMON, M.D., in Association with R. V. LAMAR, M.D., and
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(From the Laboratory of Dr. Charles E. Simon.)
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Through the researches of Wright and Douglas ¹ it has been established that the phagocytic effect of human leucocytes upon certain bacteria is essentially dependent upon the presence in the blood plasma and serum of substances which they have termed opsonins. They could demonstrate that these substances per se are to a certain extent at least thermolabile, being partly destroyed by heating the blood serum to a temperature of 60-65° C. for ten minutes or more. The phagocytic effect of such serum is accordingly diminished and may indeed be suspended. If, however, bacteria are first digested with normal serum and subsequently exposed to a temperature of 60–65° C., phagocytosis proceeds in a normal manner. The organisms in question are the staphylococci, Bacillus pestis, Micrococcus melitensis, Diplococcus pneumoniae (Fränkel), Bacillus coli, Bacillus dysenteriae, Bacillus anthracis, Bacillus typhosus, Bacillus tuberculosis and the vibrio of Asiatic cholera, while the diphtheria bacillus and Bacillus xerosis were found to be insensible to opsonic action. With the latter phagocytosis proceeds as promptly with heated as with unheated serum.

In a series of further studies Wright and his collaborators then illustrate the variations in the opsonic content of the blood in certain infections and discuss the significance of the data obtained from the standpoint of immunity. The therapeutic inferences to which they are led and the practical results which

* Conducted under a grant from the Rockefeller Institute.
¹ Wright and Douglas, Proc. Royal Soc., 1903, lxxii, 357; ibid. 1904, lxxiii, 126; ibid., 135, and ibid., 147.
they claim to have obtained are highly suggestive and have attracted wide-spread attention.

The studies described in the present paper were undertaken with the idea of gaining a further insight into the nature of the opsonins and their significance from the standpoint of immunity.

**TECHNIQUE.**

For the purpose of estimating the phagocytic power of the leucocytes Wright makes use of a modification of the method originally devised by Leishman. To this end a sample of blood is drawn off and mixed with one tenth of its volume of a ten per cent. solution of sodium citrate. A second sample of blood is allowed to clot in the usual way. In the case of the first sample the corpuscles are isolated from the plasma by repeated washing with physiological salt solution and centrifugation; washed corpuscles are thus obtained. In the case of the second sample the serum is simply separated from the corpuscles in the ordinary way by centrifugalization. Equal parts of washed corpuscles (two or three) and serum are then taken and further mixed with one volume of a suspension of the organism under consideration, obtained by rubbing up in physiological salt solution a portion of a twenty-four hours growth on agar. The resultant mixture is incubated at blood heat for fifteen minutes, when smears are made and stained with Leishman’s stain. Finally the number of ingested bacteria is counted in a series of polynuclear leucocytes, taken in the order in which they are observed, and the average for one leucocyte calculated; the resultant figure denotes the phagocytic power. This is further compared with that of a normal control person, which latter serves as unit. The ratio between the two values is termed the opsonic index of the blood.

This method has thus far been employed almost exclusively in the study of the opsonins, and upon it the results obtained by Wright and his co-workers are based.

In some of the earlier work which was carried on by one of us (Simon) before the present studies were undertaken, the original method of Leishman was used. To this end equal
parts of blood and bacterial suspension are mixed and a drop of
the mixture placed on a slide, covered with a coverglass and
incubated at 37° C.; slide and cover are drawn apart and the
resultant smears stained as above. The average number of
organisms pro leucocyte is then determined as with Wright's
method. For purposes of class demonstration of phagocytosis
this method was found quite satisfactory, but it does not appear
to us that either in the original form or in that now employed
by Wright the mere determination of the average number of
organisms pro leucocyte gives an adequate idea of the quantity
of opsonins present. It is thus quite conceivable that in a given
case the use of concentrated serum may show what would appear
to be a perfectly normal and sufficient quantity of opsonins for a
given number of organisms, which amount, however, might be
altogether inadequate for a larger number. This inadequacy
would become manifest at once upon diluting the serum, when a
rapid exhaustion of the phagocytic power would occur. Our
own investigations have shown conclusively that such an event
is by no means uncommon. Serum of the pig in concentrated
condition thus manifests a most intense opsonic effect for staphy-
lococci, which diminishes with marked rapidity, however, on
dilution, while with human serum the dilution can in most cases
be carried much further, even though the initial phagocytic
power is less. Similarly we find that with certain organisms,
such as the anthrax bacillus, the colon bacillus, Fränkel's
pneumococcus etc. the number of organisms ingested by a
leucocyte is relatively small, but that the same serum can be
quite freely diluted before phagocytosis ceases. We have found
in human beings who were supposedly in good health that a
good phagocytic power in concentrated serum may diminish
on the one hand in proportion to the degree of dilution, while
on the other rapid exhaustion may take place. Under such
circumstances we can hardly find it warrantable to conclude that
the opsonic content in the two mixtures is identical.

But apart from these considerations there are other reasons
which render the determination of the opsonic content according
to Wright's method inadvisable for general work. With the
use of concentrated serum it may thus be absolutely impossible to count the number of staphylococci in a given cell. With normal blood or in cases where the phagocytic effect is diminished this difficulty is not so great, but in infections in which the opsonic power is increased enumeration is out of the question. The same holds good when working with the blood serum of certain animals, such as the pig. The number of organisms which are here taken up in concentrated serum is most remarkable; the cells are filled to bursting and accurate counting is impossible. With other organisms there are other difficulties. In the case of the typhoid bacillus, the colon bacillus, the bacillus of Asiatic cholera, and Metchnikoff's bacillus spherulation of the organisms and loss of staining power commonly occur and frequently preclude all possibility of obtaining a satisfactory result by counting. The agglutinating effect of certain sera upon the corresponding organisms is a further drawback to the method, which presupposes that the emulsion of organisms to be added to the blood serum should be uniform and that no agglutinative effect should precede phagocytosis. This difficulty is of special importance when dealing with the tubercle bacillus, and has not yet been satisfactorily overcome. In the case of the anthrax bacillus—and the same applies to Bacillus subtilis—enumeration is for obvious reasons inapplicable and may indeed be impossible.

Led by these considerations, among others, we abandoned the idea of determining the opsonic content of the blood according to the Leishman-Wright method, and have devised the following method, which we believe is more satisfactory and free from the objections which attach to the older procedure.

Method.—By means of a suitably bent and drawn-out glass tube about one half a cubic centimeter of blood, which is readily obtained by the usual puncture of the finger or preferably the ear, is transferred, in portions, to a small tube and mixed with a large excess of a 0.2 per cent. solution of sodium chloride and 0.1

With the method advocated by Wright we could not obtain a satisfactory emulsion of tubercle bacilli. Through the kindness of Dr. E. R. Baldwin of the Saranac Research Laboratory for the study of tuberculosis we were furnished with a supply of bacilli which had been extracted with chloroform. With these much better results were obtained, the bacilli being rubbed up in an agate mortar with 1:1000 salt solution and then centrifuged. We take pleasure in acknowledging the courtesy of both Dr. Trudeau and Dr. Baldwin.
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per cent. of ammonium oxalate. The tube is filled with the same solution and blood and diluent are well mixed and centrifugalized. When the corpuscles are well packed down the supernatant fluid is drawn off with a small pipette and replaced with 1.2 per cent. salt solution, containing no oxalate. The tube is repeatedly inverted and the corpuscles again thrown down by centrifugalization. The washing is repeated once more and the fluid withdrawn down to the corpuscles. The leucocytes which have been thus washed are entirely inactive. In our experiments they were always obtained from supposedly normal individuals and not kept longer than two or three hours, though they may be serviceable for a longer time. Wright and Douglas state that in their experience there was no indication of a variation of the phagocytic power within the space of a few hours, while after the lapse of three days it had declined to less than one half or one third of that of the freshly drawn blood.

The specimen of blood in which the opsonic content is to be determined (about one half cubic centimeter) is collected in a similar manner, but without the use of a diluent. It is placed in a small dry tube and allowed to clot, after which the clot is carefully separated from the walls and packed down by centrifugalization. A large drop of the serum which separates out is placed upon a slide and from this various dilutions are made with 1.2 per cent. salt solution. With human blood we usually prepare a 1:20, a 1:30 and a 1:40 dilution, while with the blood of animals a 1:10 and a 1:20 specimen is made. The dilutions are conveniently obtained by the aid of the Thoma-Zeiss pipette used in counting white blood corpuscles. For routine work we use a volume of diluted serum corresponding to eighteen divisions of the pipette. In the case of the 1:30 dilution we thus take twelve divisions of the 1:20 specimen and six of salt solution and for the 1:40 nine of the 1:30 and nine of the salt solution. The resultant preparations are then inoculated with a small amount of the organism under consideration; this is taken from an agar culture and gently rubbed against the tube so that a slight milky turbidity results. If an organism is used which does not readily yield an emulsion in this way, an emulsion must be separately prepared in normal salt solution, or as in the case of the tubercle bacillus in a 1:1000 solution of salt and a small volume of this emulsion is added, due allowance being made for the degree of dilution. Each tube finally receives a constant volume of corpuscles which equals one half of the diluted serum. The specimens are gently agitated and placed in the incubator at 37°–40° C. for thirty minutes, when large drops of the fluid are placed on slides, spread in the usual manner, (rather thickly) and allowed to dry in the air. It is advisable that in spreading the second slide be merely kept in contact with the blood, without really touching the lower slide; otherwise it may happen that most of the leucocytes, containing organisms, are carried to one end, while only the empty cells are found in the intervening space. When dry the smears are fixed by gently warming them over a flame, when they are stained with an aqueous solution of methylene blue. This has the advantage that in the relatively thick specimens the red cells are not colored and the leucocytes accordingly are more readily found. The use of polychrome dyes is in our experience not at all advantageous. For routine work we used equal parts of a 1:10 dilution of serum and of the tubercle emulsion, thus obtaining a 1:20 dilution. With the tubercle bacillus Gabbett’s method was used.
A Contribution to the Study of Opsonins

When phagocytosis is slight and doubt arises whether in a given cell an organism lies in or upon a cell it is advisable to go over a larger number of cells. With the higher dilution and slight phagocytosis a certain amount of practice is necessary before constant results can be obtained, and even then the personal factor will enter into consideration; this, however, is less disturbing than with the Leishman-Wright method. The results are expressed as in the following example:

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Percentage of Phagocytosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:20</td>
<td>100% +</td>
</tr>
<tr>
<td>1:30</td>
<td>92% +</td>
</tr>
<tr>
<td>1:40</td>
<td>64% +</td>
</tr>
</tbody>
</table>

Incidentally note is taken in a general way whether many organisms or only a small number are present in the cells.

A problem that gave us much consideration was the selection of the organism for inoculation. As this involves the question of the specificity of the opsonins we shall deal with it under a separate heading. At this place it will suffice to state that for routine work Staphylococcus citreus was employed.

One point upon which we wish to dwell in this connection and which does not seem to have been noticed by previous observers is the necessity of using a time limit during which the tubes are kept in the incubator. In our experiments this was uniformly thirty minutes. If a longer time is allowed the results may be totally different. Generally speaking the number of phagocytizing leucocytes increases with prolonged exposure. In one instance the values with the 1:20 dilution were thus 50 after thirty minutes and 80 after two hours.

OCCURRENCE OF OPSONINS IN VERTEBRATES.

Hektoen and Rüdiger have shown that among mammals the opsonins of one species will sensitize bacteria for phagocytosis by the leucocytes of a different species. The animals examined were the guinea pig, rabbit, dog, goat, white rat, and horse. To this list we can add the cat, the sheep, the calf and pig. The serum of all these animals was found to sensitize Staphylococcus citreus for phagocytosis by human leucocytes. We have also found that the same holds good for the serum of the chicken, the terrapin, the frog, and the fish—in other words for all the large classes of vertebrates. The amount of opsonin present in the serum of the various animals is variable, however, as determined by the degree of dilution, and seems to be lower as we descend in the scale of animal life (Table I). The degree of phagocytosis, viz. the number of organisms pro leucocyte, is variable, moreover, not only among the representatives of different species, but also

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1 Hektoen and Rüdiger, *Journ. of Infect. Dis.*, 1905, ii, 128.
among individuals (Table II). To a certain extent these differences may depend upon conditions of nutrition and the process of digestion (*vide infra*), but we feel certain that there are still other factors which are of moment in this connection.

**TABLE I.**

SHOWING THE OPSONIC VALUES OF ANIMALS OF THE DIFFERENT CLASSES OF VERTEBRATES.

<table>
<thead>
<tr>
<th></th>
<th>1:1</th>
<th>1:10</th>
<th>1:20</th>
<th>1:30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish</td>
<td>active</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Frog</td>
<td>active</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Turtle</td>
<td>1:5:52</td>
<td>40</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Chicken</td>
<td>56</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>20</td>
<td>12</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>22</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Calf</td>
<td>36</td>
<td>12</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Pig</td>
<td>96</td>
<td>14</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>32</td>
<td>20</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE II.**

SHOWING THE VARIABILITY OF THE OPSONIC VALUE IN DIFFERENT ANIMALS OF THE SAME SPECIES (ON FIRST EXAMINATION).

<table>
<thead>
<tr>
<th></th>
<th>1:1</th>
<th>1:10</th>
<th>1:20</th>
<th>1:30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbits</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(122)</td>
<td>—</td>
<td>26</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>(91)</td>
<td>—</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>(123)</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>(131)</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>(132)</td>
<td>40</td>
<td>40</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>(145)</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>(162)</td>
<td>72</td>
<td>48</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>(171)</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>(188)</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Dogs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(182)</td>
<td>40</td>
<td>28</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>(214)</td>
<td>24</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>(228)</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* With the undiluted serum of the turtle there occurs a most extensive agglutination of cocci, with hemolysis of the red cells and agglutination of the stromata. Under the circumstances it was impossible to obtain any adequate idea of the extent of phagocytosis. On diluting the serum there was still marked agglutination of the cocci, but there was no evidence of hemolysis.
A Contribution to the Study of Opsonins

<table>
<thead>
<tr>
<th>Pigs</th>
<th>1:1</th>
<th>1:10</th>
<th>1:20</th>
<th>1:30</th>
</tr>
</thead>
<tbody>
<tr>
<td>(89)</td>
<td>96</td>
<td>14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(108)</td>
<td>48</td>
<td>16</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>(149)</td>
<td>100</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(202)</td>
<td>96</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(240)</td>
<td>96</td>
<td>-</td>
<td>10</td>
<td>-</td>
</tr>
</tbody>
</table>

Rabbit 91, for example, is a large buck in excellent condition, with an initial phagocytic value of 2 with a 1:20 dilution, while Rabbit 132 is a much smaller animal and exhibits a phagocytic value of 40 with the same dilution. Dog 228 on the other hand was received in very poor condition and had a phagocytic value of 4 as compared with two other dogs who showed 40 and 50 respectively with a dilution of 1:10, and were much better nourished.

THE OPSONIC CONTENT IN HEALTHY HUMAN ADULTS.

As in the case of individual animals of a given species, so also does the opsonic content of the blood vary in man. But human blood generally speaking contains rather more opsonins than the blood of the lower animals which we have examined.

TABLE III.

| Dr. B. | 16 | 2 | 0 |
| Dr. D. | 44 | 28 | 12 |
| Dr. K. | 44 | 24 | 12 |
| Dr. S. | 20 | 16 | 4 |
| Mrs. S. | 12 | 8 | 0 |
| Dr. B. | 44 | 24 | 18 |
| Dr. L. | 56 | 32 | 20 |
| M. M. | 72 | 42 | 12 |
| Dr. E. | 44 | 20 | 8 |
| Dr. C. | 48 | 28 | 12 |
| Dr. J. | 56 | 20 | 16 |
| Dr. F. | 18 | 10 | 6 |
| Dr. M. | 42 | 28 | 10 |
| C | 12 | 6 | 3 |
| Dr. B. | 54 | 24 | 8 |
| H. B. | 12 | 4 | 0 |
| Dr. R. | 68 | 36 | 24 |
| Dr. T. | 24 | 8 | 4 |
| Dr. S. | 20 | 16 | 12 |
| Dr. M. | 38 | 12 | 2 |
The values obtained in a series of twenty normal individuals are given in Table III. From this table it will be seen that with a dilution of 1:20 there is phagocytosis in all, the average being 37.2, with 72 as maximal and 12 as minimal values. With a dilution of 1:30 there is also phagocytosis in all, but already diminished in extent, the average being 19.4. With a dilution of 1:40 (average, 9.2) there is a certain percentage of negative results, which increases with the higher dilution of 1:50. Beyond this it is exceptional to find phagocytizing leucocytes.

The figures in the table show that the opsonic content of normal blood fluctuates much more than the determination of the opsonic index according to Wright’s method would lead one to believe.

In the following apparently normal individuals high values were found.

<table>
<thead>
<tr>
<th>Dilution</th>
<th>1:20</th>
<th>1:30</th>
<th>1:40</th>
<th>1:50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. K.</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>63</td>
</tr>
<tr>
<td>Dr. J.</td>
<td>98</td>
<td>92</td>
<td>48</td>
<td>36</td>
</tr>
<tr>
<td>Dr. E.</td>
<td>88</td>
<td>40</td>
<td>40</td>
<td>—</td>
</tr>
<tr>
<td>Dr. A.</td>
<td>84</td>
<td>52</td>
<td>20</td>
<td>—</td>
</tr>
<tr>
<td>Dr. L.</td>
<td>—</td>
<td>82</td>
<td>18</td>
<td>4</td>
</tr>
</tbody>
</table>

Dr. K. was an individual who was rather a large eater; hardy and vigorous, with no sign of any infection.

Dr. J. was a person of fairly large build, and well nourished.

Dr. E. was a female physician of light build; a very active person.

Dr. A. is of small stature and does not make the impression of unusual vigor. This value was obtained an hour after breakfast.

Dr. L. is a stout man, who is exceedingly prone to pyogenic infections. At the time of examination he was healthy and well. The finding in his case is certainly opposed to Wright’s idea that infection takes place because of a low opsonic content.

Our “normal” series includes a few individuals with very low values, which were not included in the table above, as the persons did not make the impression of being in normal health. Dr. N. thus gave 4 for 1:20 and 0 for 1:30 and 1:40 and Dr. B. 6 and 0 respectively. There was no evidence of any actual illness in either, but both looked pale and were rather thin.

All the individuals named in the table were in good health at the time of examination and would hence according to Wright and Bulloch have shown practically the same index, viz. one varying between 0.8 and 1.2. Supposing this to have been the case it is difficult to understand why in one individual the

* Bulloch, W., Lancet, 1905, ii, 1605.
serum could be diluted fifty times and still retain a certain degree of activity, while in another individual the limit of opsonic power was practically at 1:30, unless we assume, as we do, that the amount of opsonins in the first case was materially greater than in the second.

In a general way the phagocytic power diminishes in proportion to the degree of dilution, but it is noteworthy that in some individuals it is more rapidly exhausted than in others. Under pathological conditions we meet with the same phenomenon, and not infrequently to an accentuated degree.

While the normal range of phagocytic power with a dilution of 1:20 lies between 12 and 72, remarkable exceptions are at times encountered, as in the case of Drs. K., A., and J. and Miss E. It will be observed that in Dr. K. phagocytosis was still active with a dilution of 1:100, being well maintained from 1:20 down. This same good maintenance is seen in Dr. J. and Miss E. We are not prepared to explain these remarkable exceptions. Such findings in our experience are obtained essentially under pathological conditions, but in the instances mentioned there was no indication of the existence of an infection.

The individual variations may in part be due to differences in the state of nutrition, but are not necessarily proportionate to the body weight (Table III). As will be shown in the following section the opsonic content is to a certain extent dependent upon the process of digestion, but in the above series the individual differences cannot be accounted for upon this basis alone, as the examinations in most cases were made in the afternoon about one or two hours after the midday meal.

The values fluctuate to a certain extent from day to day, but remain within the limits indicated.

We repeat at this place that our results have reference to a time limit of thirty minutes in the incubator and that higher values are obtained if this is materially exceeded.

**Influence of Digestion Upon the Opsonic Content.**

In some individuals a marked influence of the process of diges-
tion upon the opsonic content of the blood can be readily established.

The initial opsonic value of K. at 3 P.M. with a dilution of 1:40 was 100. The same individual after fasting through the night, at 7:30 A.M., showed only 8 per cent of phagocytosis with a dilution of 1:30. The results at 2 P.M., one hour after a hearty dinner, were the following:

\[
\begin{align*}
1:30 &= 98 \\
1:40 &= 96 \\
1:50 &= 96
\end{align*}
\]

Two hours later the corresponding figures were:

\[
\begin{align*}
1:30 &= 86 \\
1:40 &= 74 \\
1:50 &= 26
\end{align*}
\]

The rapid exhaustion as compared with the results obtained one hour after dinner, is here strikingly shown. The same is seen early in the morning. In the given experiment a 1:20 determination was not made at that hour, but on another occasion the value was 82, and fell to 2 with a dilution of 1:40.

At 6:30 P.M. supper was had and one hour later higher values are again found, which are well maintained with the higher dilutions, viz.:

\[
\begin{align*}
1:30 &= 98 \\
1:40 &= 98 \\
1:50 &= 98
\end{align*}
\]

The figures given in connection with this experiment should not be regarded as averages, however, as they were obtained in the case of Dr. K., who showed an exceptionally high phagocytic value.

The same general result was obtained in the case of a rabbit.

The initial phagocytic value was 4 per cent. for 1:30, and negative for 1:40. After fasting absolutely for 43 hours there was no phagocytosis whatever with 1:30; with 1:20 only 2 per cent. of the cells were positive, and with 1:10 only 20 per cent. The animal was then well fed and about one hour later gave the following values:

\[
\begin{align*}
1:30 &= 28 \\
1:40 &= 22 \\
1:50 &= 6
\end{align*}
\]

Dr. Amberg has made a number of opsonic determinations according to our method in breast-fed babies in the obstetrical department of the Johns Hopkins Hospital. He will report the details of his results at another place, but has kindly placed his general conclusions at our disposal. From these it appears that in nursing infants the opsonic content is distinctly higher than in the average normal adult, and that dilution is well borne up to 1:100 and in some instances even higher.

<table>
<thead>
<tr>
<th>Baby No. 8</th>
<th>1:20</th>
<th>84</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1:40</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>1:80</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>1:100</td>
<td>22</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Baby No. 9</th>
<th>1:25</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1:50</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>1:75</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>1:100</td>
<td>20</td>
</tr>
</tbody>
</table>
It seems to us that these high values are to a certain extent at least referable to the fact that the nursing infant is practically in a condition of continuous digestion.

But even though a distinct influence upon the opsonic content by the process of digestion is apparent in certain cases this is not manifest in all.

In the case of one of us (S) higher values were thus obtained early in the morning before any food had been taken than two hours after the midday meal. Miss E. similarly showed high values even after fasting absolutely for twenty-one hours.

Miss E. showed high values even after fasting absolutely for twenty-one hours, viz.:

\[ 1:20 = 92 \quad 1:30 = 48 \]

The same individual was placed upon Polin's non-proteid diet for forty-three hours and at the end of this time still showed very active phagocytosis:

\[ 1:20 = 88 \quad 1:30 = 40 \]

In rabbits also we have found that good feeding per se does not increase the opsonic content of the blood beyond the initial values which were noted shortly after the animal had been obtained from dealers. Individual peculiarities exist here, of the causes of which we know nothing as yet.

From a practical standpoint in the study of infections, however, it is well to bear the possible influence of digestion upon the opsonic content in mind. The fact that such a relation may exist throws additional light upon the rationale of "feeding a fever." Moreover, the high figures obtained by us in certain infections thus stand out in even greater contrast, if we bear in mind that in many of these cases the food consumption had been much diminished.

**Distribution of the Opsonins in the Animal Body.**

In order to ascertain what part, if any, the various organs of the body take in the production of opsonins, rabbits were bled by dividing the femoral vessels and thoroughly milking the body. The organs were then removed and ground up with well washed and dried quartz sand. In the case of the liver it was possible to obtain a sufficient quantity of fluid extract in this manner without the addition of any diluent, but in the case of the other organs it was necessary to use a small amount of normal salt solution. Whenever this was done a large amount of the triturated mush was placed in a tube, digested with the salt solution, and the sand and cellular elements then separated out.
by centrifugalization. The degree of dilution was in every case as slight as possible, merely enough to procure a few drops of liquid extract. This was then placed in a separate tube, infected as usual with Staphylococcus citreus, treated with washed human corpuscles, incubated and further examined as described. The results were absolutely negative in the case of striated muscle tissue, liver, spleen, lymph glands, kidneys, intestinal mucous membrane and muscular coats. For the examination of the adrenal glands, brain, pancreas, testicles and ovaries sheep were chosen and for the thymus gland the calf. The results were uniformly negative.

Examination of the bone marrow did not yield entirely satisfactory results. The material was obtained from the bones of a cat, stirred up in a small amount of normal salt solution and incubated at 37° C. for one hour and a half. The cellular material was then removed as far as possible by centrifugalization and the liquid portion examined. It was found that phagocytosis had occurred in 12 per cent. of the washed corpuscles. We fear, however, that the positive result in this case may have been due to the presence of blood serum which could not be removed.

Transudates like blood serum also contain opsonins, but the amount is smaller in the former. With a specimen of fresh hydrocele fluid we thus found only six per cent. of phagocytizing leucocytes for a dilution of 1:20 and with a sample of ascitic fluid 10 per cent. with the same dilution. Chyle contains rather more, but also less than the blood serum (60 per cent. in one case in concentrated form).

Our results thus tend to show that the opsonins are essentially components of the blood and the inference naturally suggests itself that they may be formed here. The fact that they are increased so frequently in infections which are associated with hyperleucocytosis would seem to point to the leucocytes as their possible source. Our investigations, however, do not support this view. They show conclusively that no constant parallelism exists between the number of leucocytes and the amount of opsonins. This is well illustrated by Fig. 1, which was obtained
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in the case of a dog, in which an obstruction of the small intestine had been artificially produced; this experiment is one of a series which one of us (Simon) undertook in association with Dr. Nelson for the purpose of studying blood changes in intestinal obstruction, a detailed report of which will appear in another place. The animal in this case survived and was subsequently
killed, when it was found that the calibre of the gut had been narrowed sufficiently to allow only the passage of a large sound.

It will be observed that notwithstanding the remarkable increase of the leucocytes, to a point above 60,000, the opsonins both for the 1:10 and the 1:20 dilution gave values which were not at all in excess of what is normal for dogs; for the most part in fact the values were rather lower than those commonly found. The same is seen in Fig. 2, which was obtained from another dog that had undergone a similar operation.

Examination in the case of patients led to the same conclusion. This is well illustrated by the two following observations.

In both cases the symptoms were suggestive of appendicitis. In the one (No. 37) the leucocytes just before operation numbered 23,000; the phagocytosis with a dilution of 1:20 was 18 and negative with 1:30. The appendix in this case was practically normal; there were two patches of induration near the lower end of the ileum and one at the base of the appendix. A definite diagnosis could not be reached; but a history of recent syphilis was considered suggestive. In the second case (No. 68) the leucocytes numbered 23,200—practically the same number as in the first, while the opsonic examination showed 100 per cent. of phagocytizing leucocytes even with dilutions of 1:30 and 1:40.

Conversely cases occur where with a normal number of leucocytes the opsonins are very high. This is well shown in the following case:

The patient (No. 137) was a young married woman who was admitted to Dr. Finney's service at the Union Protestant Infirmary with a greatly enlarged spleen and liver. The differential diagnosis rested between Banti's disease and syphilitic cirrhosis. The leucocytes numbered 7500, while phagocytosis with 1:20 was 100 and with 1:30, 96, i.e. greatly above the normal.

While the clinical evidence was thus opposed to the assumption of a relationship between leucocytes and opsonins we have made further examinations in this direction, by incubating washed leucocytes for variable lengths of time, suspended in normal salt or in Ringer's solution, and subsequently examining for the occurrence of phagocytosis. The results were invariably negative.

While the above observations render it unlikely that the neutrophilic and amphophilic granulation is concerned in the production of opsonins the rôle of the eosinophiles still remained doubtful. In the case of the incubation experiments just
referred to it was conceivable that the small number of eosinophiles in our tubes were not sufficient to bring the opsonins up to that degree of concentration at which phagocytosis would occur. To throw some light upon this question a rabbit was fed about 1750 trichine. A marked eosinophilia resulted, which at first was accompanied by a rise in the opsonins. Subsequently, however, they fell and at the last examination no phagocytosis whatever occurred with a dilution of 1:10.

The lymphocytes, according to our negative findings in the case of the lymph glands, can be excluded as opsonin producers.

In the cerebro-spinal fluid from a case of spina bifida we were much surprised to find no evidence of the presence of opsonins.

The examination of fresh seminal fluid and of milk gave negative results.

In exudates the presence of opsonins is variable; but as the element of infection enters into consideration in their production they will be dealt with under a subsequent heading.

CHEMICAL STUDY OF THE OPSONINS.

Our chemical investigations were rendered particularly difficult owing to the comparative instability of the opsonins; viz. their partial thermolability and the readiness with which various chemical reagents lead to their destruction, or at least inactivation so that they are no longer subject to detection by their specific effect upon the leucocytes. Hektoen and Rüdiger have shown that various salts even in very small amounts are capable of suspending opsonic action when added to the blood serum. They determined this in the case of calcium, barium, strontium and magnesium chloride, potassium sulphate, sodium bicarbonate, trisodium citrate, sodium oxalate, potassium ferro-cyanide and formalin. It was accordingly necessary by means of a series of preliminary experiments to determine what physical processes and what chemical reagents could be utilized without interfering with opsonic action. Then again it was necessary to ascertain, ceteris paribus, how long the opsonins will remain active in the blood serum. Wright and Douglas state that the opsonic power
of the blood fluids disappears gradually on standing, even when the serum is kept in a sealed capsule, sheltered from light. Under these conditions they found the opsonic power after five or six days to stand at a little more than half of what it was originally. This observation we are able to confirm. With the serum of the pig and of the rabbit we found a vigorous degree of opsonic activity after a week and even longer, no matter whether the serum was kept in the cold or at the temperature of the laboratory. It was interesting to note that with a fair development of bacteria in the serum, we could obtain good phagocytosis after from six to eight days; leucocytes were then found which had taken up the bacteria which had grown in the serum.

We have stated before that Wright and Douglas could demonstrate a partial thermolability for the opsonins, inasmuch as the number of ingested organisms in heated serum was usually less than in unheated serum. But apart from the diphtheria bacillus and Bacillus xerosis, which were taken up as well in the one as in the other, a complete extinction was not as a rule obtained even with those organisms which Wright designates as especially susceptible to opsonic action. In his first experiment with the typhoid bacillus he thus notes that in the heated specimen there was "everywhere considerable phagocytosis" and the phagocytic index is given as 26.2 (circ.) as compared with 24 (circ.) in the unheated serum. In Experiment II with the same organism, on the other hand, the value was 8.1 in the unheated and only 0.8 in the heated one.

Other observers seem to have taken the complete thermolability of the opsonins for granted. But Dean has recently shown that such an assumption is not warrantable and that Wright's results which suggest this possibility are referable to an inherent inaccuracy of the method, viz. the small amount of serum which is used. He could show that normal horse serum, even after being kept for four hours at a temperature of 60° C., still contains a quantity of opsonins sufficient to prepare large numbers of

cocci for phagocytosis. Similar results were obtained by Smith in the case of typhoid immune sera. In a recent communication Wright attempts to show, on the other hand, that the phagocytic effect of heated sera is merely referable to opsonins which have escaped destruction and that such sera when diluted with 1.2 per cent. salt solution and subsequently heated are inactive.

Behavior on dialysis.—To study the effect of dialysis upon the opsonins small quantities of blood serum (25–50 c.c.) were placed in sacs of animal membrane or of celloidin and dialyzed against running water for from forty-eight to sixty hours. After this time it was found that the remaining fluid showed no opsonic effect whatsoever, but the appearance of the leucocytes suggested that the negative effect might very well be due to the atonicity of the dialyzed fluid. Upon the addition of a corresponding quantity of Ringer’s solution to the dialyzed serum active phagocytosis could again be obtained, although to a less extent, so far as the number of phagocytizing leucocytes goes, than with the native serum. This diminution in the amount of opsonins upon first consideration suggests that the substances are to a certain extent dialyzable, but our subsequent experiments have placed an entirely different light upon this apparent loss, so that we believe that we are justified in concluding that the opsonins are not dialyzable. The active phagocytosis which was obtained with dialyzed serum plus Ringer’s solution shows that the soluble phosphates and carbonates of the blood are not essential to the process. The fact that the same result also was obtained with dialyzed serum plus normal salt solution further shows that the soluble calcium salts and sulphates of the serum were likewise not of moment. The suggestion that the decalcification of the blood per se, by means of the ammonium oxalate or sodium citrate used in washing the leucocytes, was essentially responsible for the non-occurrence of phagocytosis we had previously invalidated by showing that the addition of Ringer’s solution to washed corpuscles, without serum, does not restore phagocytic action.

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As the addition of normal potassium chloride solution to the dialyzed serum was found to answer the same purpose as the sodium chloride it appears likely that the function of the chloride is in a manner secondary, and merely serves the purpose of maintaining the necessary osmotic tension of the leucocytes. As we have shown, its temporary abstraction during dialysis does not interfere with the subsequent action of the opsonins when the necessary quantity is restored.

While our experiments with dialysis show that the opsonins must be sought among the non-dialyzable components of the blood serum, our experience with the soluble extractives of the various organs suggests that they are specific constituents of the serum. It would hardly seem likely that lecithins, cholesterol, soaps etc. are of moment.

Bearing in mind the close relationship which seems to exist between certain antibodies and the blood albumins experiments in this direction seemed to be indicated, and were rendered possible by our discovery that ammonium sulphate, even when added in substance to saturation, does not destroy the opsonins, although their specific effect can only become manifest again after the excess of salt has been removed by dialysis.

In a series of experiments the albumins of fifty cubic centimeters of serum were thus collectedly precipitated by salting at 40° C.: the precipitate was dissolved in as small a bulk of water as possible and dialyzed against running water for at least forty-eight hours. The contents of the dialyzer were then concentrated in the vacuum to about one half the original volume, diluted with double the amount of 1.2 per cent. salt solution and examined in the usual manner. Active phagocytosis occurred, although the number of phagocytizing cells was materially smaller than with the original serum.

The filtrate, after salting out the albumins, as just described, was likewise dialyzed against running water, concentrated in the vacuum to a small volume and treated with salt solution and examined as above. There was no phagocytosis whatever. After having thus demonstrated that the opsonins can be isolated in association with the blood albumins, the albumins were
fractioned in the usual manner. The globulin fraction was isolated by half saturation with ammonium sulphate, the precipitate dissolved in a small amount of water and dialyzed. In the water-insoluble portion which separates out (euglobulins) opsonins could then be demonstrated, if a saturated solution in 1.2 per cent. salt solution was prepared and examined in the usual manner, while the pseudoglobulin fraction after concentration in the vacuum, as also the serum albumin fraction, were entirely inactive.

The opsonic content of the euglobulin fraction was less than that of the original serum, but still quite considerable. In one experiment where the examination with the native serum had shown 100 per cent. of phagocyting leucocytes, the concentrated euglobulin solution showed 74 per cent. of the cells still quite active. On diluting, however, to the extent of only 1:10 the result was entirely negative.

These experiments were repeated a number of times with the serum of pigs, calves, sheep, and also with ascitic fluid, and in all cases with the technique described the opsonic effect was obtained with the euglobulin fraction and with this only. The conclusion hence suggested itself that the opsonins, even though themselves not necessarily of the nature of globulins, were nevertheless intimately associated with them as in the case of the various antibodies as shown by Pick. But whereas Pick could demonstrate that diphtheria antitoxin, for example, was quantitatively precipitated from goat's serum in the globulin fraction, the material loss which we met with made us hesitate to regard this conclusion as altogether valid. It seemed not at all impossible that the opsonins, like ferments, were carried down mechanically and in part lost during the physical manipulations (filtration, dialysis), viz. deposited upon the walls of the dialyzer etc. While we have not been able to prove conclusively that this actually occurs, certain experiments which were undertaken to elucidate this point certainly are very suggestive. To this end the quantity of opsonin was first estimated in the native serum. Small tubes were then charged with small quantities of serum

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together with various substances which in themselves were not
thought to exert a destructive effect upon the opsonins, such as
cogulated egg albumin which had been dried and finely powdered,
wheaten flour, diatomaceous earth, precipitated chalk, filter
paper fibre, vegetable charcoal, carmin and platinum black.
After having shaken the tubes thoroughly for one half hour the
suspended material was thrown down by centrifugalization and
the serum examined in concentrated form. The figures repre-
senting phagocytosis are given in Table IV.

TABLE IV.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Phagocytosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native serum</td>
<td>48</td>
</tr>
<tr>
<td>Carmin</td>
<td>40</td>
</tr>
<tr>
<td>Platinum black</td>
<td>40</td>
</tr>
<tr>
<td>Diatomaceous earth</td>
<td>36</td>
</tr>
<tr>
<td>Precipitated chalk</td>
<td>14</td>
</tr>
<tr>
<td>Filter paper</td>
<td>4</td>
</tr>
<tr>
<td>Albumen</td>
<td>0</td>
</tr>
<tr>
<td>Charcoal</td>
<td>0</td>
</tr>
<tr>
<td>Flour</td>
<td>0</td>
</tr>
</tbody>
</table>

These experiments show conclusively that the opsonins can
be in part or entirely removed from blood serum by various
substances in a manner quite analogous to what we see in the
case of various ferments. That they are not destroyed can be
shown in the case of carmin and charcoal, for example, by
washing the material in 1.2 per cent. salt solution and then ex-
posing it to the action of washed leucocytes, when numerous
cells are seen which have taken up the fine granules.

A perfectly analogous result was obtained with cream of milk.
Cream was thoroughly shaken with serum for half an hour,
separated by centrifugalization and washed with 1.2 per cent.
salt solution. With a particle of the resultant butter a fine
suspension was prepared in salt solution and incubated for
fifteen minutes with washed corpuscles, A drop of this mixture
was mixed with a drop of a one per cent. solution of osmic acid
and examined, when it was seen that a certain degree of phago-
cytosis had occurred.

It might of course be argued that the phagocytosis which was
thus obtained with carmin, charcoal and fat globules was not
dependent upon the absorption of opsonins, but was of the character of spontaneous phagocytosis. This, however, is negatived by the fact that in all experiments a 1.2 per cent. solution of salt was used, which according to Wright eliminates spontaneous phagocytosis. Upon the basis of these experiments it seems not inconceivable that the cause for our finding of the opsonins in the euglobulin fraction of the blood serum may be sought in the fact that these are the only albumins which in the nature of our technique are precipitated on dialysis and would hence carry the opsonins with them. This possibility must be borne in mind in future research. On the other hand our loss of opsonins during our manipulation might be referred to the time element which enters into consideration and would simply represent that portion of opsonin which would become inactive in the native serum upon standing for a corresponding length of time. To enter into this question further did not seem desirable at the present time.

The absorption experiments tend to throw some light upon the nature of the sensitizing of bacteria and the differing behavior of various organisms in reference to the extent of phagocytic action. Similar conditions obtain with various ferments, such as pepsin, which is readily absorbed by animal charcoal, diatomaceous earth, coagulated serum and egg albumin, etc., while bread, flour, lecithin and cholesterol are less active.

SPECIFICITY OF THE OPSONINS.

The question regarding the specificity of the opsonins is an exceedingly important one. From the standpoint of technique in the investigation of the opsonic content it would simplify matters very much, if it could be shown that the opsonins are not specific, while such a demonstration would also modify present conception regarding the mode of action of bacterial vaccines. As Bulloch\textsuperscript{14} states, "this question has hitherto not been touched in the various memoirs," although Wright manifestly assumes that it has been settled in the affirmative. Bulloch comes to the same conclusion, viz. that a high degree of specificity

\textsuperscript{14} Bulloch, W., \textit{Lancet}, 1905, ii, 1603.
exists. His inference is based upon two classes of experiments. "In the first the opsonic power of a serum was tested against both Staphylococcus albus and the tubercle bacillus. The serum was then mixed with one or other of these microbes and after a sojourn in the incubator the mixture was subjected to the prolonged action of the centrifuge, whereby the microbe was thrown down as a deposit; the supernatant fluid was deprived of its opsonins for the particular microbe with which it had been in contact, while it 'largely' [sic] retains its opsonins for the microbe with which it has not been digested." Unfortunately Bulloch gives no details of his experiments, from which the reader can form an estimate of what is meant by the statement that the serum "largely retains its opsonin for the microbe with which it has not been digested." We must confess that we see no cogent reason why, assuming the high specificity of the opsonins, the opsonin in the experiments mentioned should have been only "largely" retained.

Our own experiments were conducted in an analogous manner and led to results which are diametrically opposed to Bulloch's conclusion, so far as Staphylococcus citreus, on the one hand, and the typhoid bacillus and colon bacillus on the other are concerned. In the end, as usual, we determined the number of phagocyting leucocytes. In one of these experiments the opsonic power of the native serum (of the pig) was 100 both for the colon bacillus and Staphylococcus citreus. A considerable portion of an agar culture of Staphylococcus citreus was then emulsified in about three cubic centimeters of serum, the mixture stirred for at least fifteen minutes and the organisms thrown down by prolonged centrifugalization. Specimens of the supernatant fluid were then reinfected with staphylococci, on the one hand, and colon bacilli, on the other, and re-examined. It was found that the opsonic power for the staphylococcus had fallen to 20 and for the colon bacillus to 14.

To meet the possible objection that the 20 per cent. for the staphylococcus may have been referable to the presence of sensitized cocci which had not been thrown down by centrifugation the experiment was repeated, but the centrifugalized
serum treated with an abundant supply of washed corpuscles and incubated for fifteen minutes so as to remove the sensitized cocci as far as possible by phagocytosis. The corpuscles were then thrown down and the supernatant fluid examined as before; in this case the result was 16 for the staphylococcus and 20 for the colon bacillus.

These experiments were repeated again and again and variously modified. In one instance for example the serum was incubated with a large supply of colon bacilli and kept in the incubator for twenty hours. At the end of that time it was tested as described; six per cent. of the leucocytes showed phagocytosis for the colon bacillus and four per cent. for the staphylococcus. There was accordingly no indication whatsoever of any specificity of the opsonins; exhaustion for both organisms proceeded pari passu in all our experiments.

The same conclusion is suggested by our observation that the opsonic coefficient of extinction is practically the same for different organisms, and irrespective of the initial intensity of phagocytosis, as shown in Table V.

<table>
<thead>
<tr>
<th>TABLE V.</th>
<th>SHOWING THAT THE OPSONIC COEFFICIENT OF EXTINCTION IS THE SAME FOR DIFFERENT ORGANISMS (Pig Serum).</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1:10</td>
</tr>
<tr>
<td>Staphylococcus citreus</td>
<td>48</td>
</tr>
<tr>
<td>Spirillum Metchnikovi</td>
<td>24</td>
</tr>
<tr>
<td>Bacillus coli</td>
<td>44</td>
</tr>
<tr>
<td>Bacillus typhosus</td>
<td>52</td>
</tr>
<tr>
<td>Bacillus Pfeifferi</td>
<td>22</td>
</tr>
<tr>
<td>Bacillus anthracis</td>
<td>36</td>
</tr>
</tbody>
</table>

In a second class of experiments Bulloch reports that the serum of human beings was tested repeatedly both against the tubercle bacillus and against the staphylococcus. He finds that injections of tuberculin produced an increase in the “tubercular” opsonins, while leaving the quantity of “staphylococcus” opsonins unaltered and vice versa. It seems to us that experiments of this character are not well suited to settle the question under consideration. In such cases we are essentially
dealing with sera in which the entire immunizing mechanism of the body is without doubt thrown into activity and in which other factors besides the opsonins may be affected. We believe Wright and his collaborators have conclusively established the existence of opsonic substances in the blood serum, but we are not prepared at the present status of our knowledge of the subject to admit that no other factors enter into consideration in connection with the process of phagocytosis. It is quite conceivable to our minds that as the result of infection, viz. immunization, substances may be produced either de novo, or increased in amount, if pre-existent, which may so influence a given organism that under the subsequent action of a common non-specific opsonin it is taken up in larger or smaller numbers, as the case may be.

This possibility that a second substance besides the opsonins may determine the number of organisms phagocyted, also exists in the case of normal blood serum. It is strongly suggested by the fact that in some individuals a very small number of cocci only is taken up with a dilution of 1:20, for example, so small indeed that we would expect no phagocytosis at all with 1:30 and 1:40, reasoning by what we see in most cases; this dilution may nevertheless be borne without extinction of the phagocytic power. We have repeatedly observed that with a dilution of 1:20 not more than four or five staphylococci are taken up by the phagocytting cells, and that with a dilution of 1:30 we still find two or three and with 1:40 perhaps the same number of organisms. On the other hand we have found that with a fairly large number of cocci pro cell, with a dilution of 1:20, there may be practically no phagocytosis with 1:40. It would seem as though the quantity of opsonins in these cases was fairly uniform, but that there must be another factor which is variable.

This assumption receives a certain amount of support from the following observation:

To eliminate opsonic action blood serum of a pig was diluted one hundred times, infected with cocci and incubated for one half hour. Blood serum from an individual, whose phagocytic value with a 1:20 dilution was 50, was then diluted
Charles E. Simon, R. V. Lamar, and W. N. Bispham

with the diluted pig serum in the proportion of 1:20 and an increase in its phagocytic value to 80 noted. The diluted pig serum per se when treated with washed corpuscles showed no phagocytosis and a control specimen with 1.2 per cent. salt solution likewise was negative.

Even more suggestive is the following:

One of our rabbits has shown a remarkably low opsonic content, such that no phagocytosis was ever obtained with a 1:20 dilution and only rarely with 1:10 and then only to a slight degree, not exceeding 8. Pig's serum, which with a dilution of 1:20 gave a phagocytic value of 10, was diluted 100 times, infected and incubated for one half hour. The rabbit's serum by itself on this occasion showed 1:10 = 4 and 1:20 = 0, but when diluted 1:20 with the diluted pig serum phagocytosis was 47 and the cells contained a fair number of cocci. Pig serum was chosen for these experiments because the phagocytic value for staphylococci is exceptionally great, when used in concentrated form, although it is rapidly lost on dilution.

In order to invalidate the argument that the preceding incubation, previous to the 1:20 dilution, might be responsible for the increased phagocytosis salt solution was substituted for the 1:100 dilution of serum and this incubated with cocci for half an hour. The result showed no difference between the original 1:20 dilution of the serum and the subsequent 1:20 dilution with the incubated cocci; the phagocytic value in both was exactly alike, viz. 4.

It might further be argued that in some manner the opsonins in the 1:100 solution, even though they be insufficient in this dilution to bring about phagocytosis by themselves, become united with the opsonins of the serum, which is about to be diluted 1:20, and that the joint effect then becomes manifest. Upon a mathematical basis per se this would appear most unlikely; we have shown, however, by experiments of the following nature that this explanation is not valid. To this end the blood serum of one and the same person was used, instead of two different individuals. A 1:20 dilution was prepared as usual, as also a 1:100 dilution. The latter was incubated for half an hour after infection with cocci and a new dilution of 1:20 prepared using the hundredfold dilution in lieu of the salt solution as usual. The result shows conclusively that the 1:100 dilution is not capable of raising the phagocytic value of the same individual to a higher level than is obtained primarily. The values in a given case were 38 for the primary 1:20 dilution and 36 for the second dilution.

Experiments of this order have incidentally shown the interesting fact that on diluting the blood of one person with that of
another, or of one animal with that of another a higher phagocytic value may be obtained, but is not necessarily obtained, than in the case of either alone, when diluted with salt solution. This peculiar behavior we are not able to explain at the present time. The problem, however, is reserved for further investigation.

But even admitting the previously mentioned observations of Bulloch and Wright to consideration for the sake of argument, it appears to us that equally cogent reasons can be advanced to prove the non-specificity of the opsonins along similar lines. It is thus difficult to understand why the opsonins for Staphylococcus citreus should be increased in cases in which no evidence of a staphylococcus infection exists whatsoever. In a case of syphilitic cirrhosis of the liver, the phagocytosis for the staphylococcus was thus 100 with a dilution of 1:20 and 96 for 1:30. In a case of extensive miliary carcinomatosis the same values practically were found. The same was found in a case of pernicious anemia. In the trichinous rabbit similarly there is certainly no ground for belief that an associated staphylococcus infection existed. In a rabbit which had died as a result of a streptococcus infection and in which cultures from the various organs and from the heart blood revealed this organism only, the opsonins for Staphylococcus citreus were enormously increased, viz. at 92 for a dilution of 1:10. In a case of melanotic sarcoma further, in which an extensive excision had been done and in which the opsonic power was quite low, a marked rise, as tested by staphylococci, was observed as the result of Coley's mixed toxin injections, viz. the toxins of Streptococcus erysipelas and of Bacillus prodigiosus.

Our findings in cases of appendicitis also may be cited in this connection. Maximal values are here commonly observed and in accordance with the usual bacteriological findings can hardly be referred to a mixed infection of colon bacilli, streptococci and staphylococci.

We have further determined the opsonic content of the blood of tubercular individuals, with our method of dilution, both against Staphylococcus citreus and the tubercle bacillus, and have found the coefficient of extinction the same for both organisms.
In one instance of renal tuberculosis for example the value for the staphylococcus with a dilution of 1:20 was 3 and for the tubercle bacillus 2, while with 1:30 there was no phagocytosis with either organism.

Fig. 3, moreover, which illustrates the effect of tuberculin injections upon the opsonic curve, as tested simultaneously with the tubercle bacillus and the staphylococcus, shows that the two run essentially a parallel course and rise and fall together. The minor differences are unquestionably due to the difference between the organisms and their initial variability, corresponding to what we have demonstrated in the case of other organisms (vide supra). It is noteworthy also that, although in this case there was no evidence (by culture) of any staphylococcus infection, the staphylococcus curve was nevertheless unusually high and manifestly influenced by the tuberculin injections.

The good phagocytic values which were obtained in cold blooded animals, finally, in which staphylococcus infections hardly occur, would similarly argue against the specificity of the opsonins.

Summing up these various observations, we believe we are justified in concluding that specificity of the opsonins does not exist. Our experiments suggest the possibility that the opsonins may be a constant quantity and that the number of organisms which is taken up by a cell is influenced by a second factor which may be variable.