THE PHAGOCYTOSIS OF SOLID PARTICLES.

I. QUARTZ.

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Measurement of Phagocytosis.

The usual procedure for measuring phagocytosis is to incubate suspensions of leucocytes and solid particles or bacteria together in a test-tube for a given length of time; then to remove a sample and make a smear of the mixture on a slide which is stained, mounted, and counted at leisure. When bacteria are used as objects for ingestion, as in opsonic index determinations, the actual number of bacteria inside a given number of leucocytes is counted and comparisons are made on the basis of the average number of bacteria taken up per leucocyte per unit of time. It is impossible to do this with solid particles which are usually larger than bacteria and hence may almost completely fill the cell in a short time or become easily superimposed.

For this reason Hamburger (1), the author of the only extensive quantitative experiments on phagocytosis of solid particles, always counted the per cent of leucocytes containing solid particles per unit of time. On theoretical grounds this method possesses the objection pointed out by McKendrick (2) that it is not a measure of the amount of work done but a measure of the number of cells which have done the work. He shows, however, that if a normal frequency curve for the distribution of the bacteria in the leucocytes is assumed, the number of bacteria per leucocyte, i.e. the amount of work done, can be calculated from the per cent of empty leucocytes, the former being a logarithmic function of the latter, and he suggests that the determination of opsonic index may be simplified by counting only the empty leucocytes.
Madsen and Watabiki (3) have avoided this difficulty, in measurements of the rate of phagocytosis of bacteria at different temperatures, by measuring the time curves of the number of bacteria taken up per leucocyte at each temperature. By certain assumptions they succeeded in fitting their experimental curves to the formulas for mono- or bimolecular reactions, and thus determining a constant, $K$, which represented the rate of the reaction. There seemed to be in this case, however, no particular significance to the obedience of the curves to the laws for chemical reactions.

In the experiments on phagocytosis of solid particles to be described in this paper it was found possible to avoid the objections in former methods and to analyze the reactions quantitatively in terms of the number of collisions occurring between cells and particles. To accomplish this, particles of carbon or quartz of uniform size were incubated with leucocytes. At frequent intervals a sample was removed to an ordinary blood-counting chamber and the number of particles not taken up by the leucocytes was counted.

If the number of cells present is large and remains constant throughout the experiment, the number of collisions varies only with the number of particles, and it should be possible to calculate the rate of phagocytosis, $K$, from the equation for a monomolecular reaction, $K = \frac{1}{t} \log \frac{A}{A-x}$, where $A$ is the number of particles originally present and $x$ is the number ingested by the leucocytes in the time, $t$. The fact that $K$ is usually found to be constant shows that we are dealing with a process like a monomolecular reaction in which the collisions can be watched under the microscope. The leucocytes can take up so many particles that their capacity does not diminish sufficiently to limit the rate of the reaction. Certain uncontrollable complicating factors which cause $K$ to vary will be discussed with the experimental results and in a subsequent paper.

A constant $K$, calculated in this way, means that the same per cent of the particles present is being ingested per unit of time; i.e., $K$ is independent of the actual number of particles present. In other words, it is not the number of collisions but the chances of collision which determine $K$, other things being equal. Since the chances of collision
depend upon the size and density of the particles in relation to the size and density of the cells, large particles should be taken up more rapidly than small particles. This was found to be true, and, moreover, a direct proportionality was found to exist between the experimental constant, $K$, and the relative chance of collisions as calculated. It will be seen that this method presents great difficulties as a means of comparing the rate of phagocytosis of different particles and it was finally discarded in favor of a simpler method which will be described in a subsequent paper. The results by the first method possess considerable theoretical interest, however, and the consideration of the chances of collision should be of interest perhaps in opsonic index work where this factor has never been taken into consideration.

The technique of handling the quartz suspensions will first be described; then the method of calculating the chances of collision; and the experimental results, using three different sizes of quartz particles. Similar experiments with carbon and comparisons between quartz and carbon will be described in later papers.

**The Suspensions.**

The first requirement for these experiments is to have the particles of as nearly uniform size as possible. Suspensions prepared by Hamburger's method would not be sufficiently uniform to admit of a calculation of the chances of collision. Uniformity can be obtained by centrifugalization or settling. The former is quicker but the product is not so good nor so easily controlled. In both methods the principle consists in washing out the particles which are too small. The larger particles may be left in the suspension until a uniform sample is needed, when the suspension is shaken up and the large ones are either centrifugalized out or allowed to settle out by gravity. In the centrifuge, however, it is quite impossible not to set up currents which carry some excessively large particles to the top. The small particles can, however, be removed in the centrifuge and the sediment can thus be resuspended and allowed to settle out by gravity. It settles with a sharp line at the top from which a uniform sample can be withdrawn.

This combined method was the one usually used in these experiments. Suspensions of this sort of carbon and quartz have been
kept for nearly a year at room temperature, without sterilizing, and there has always been an abundant supply of uniform particles in each when shaken up and allowed to settle. Uniform suspensions cannot be kept, however, without the addition of acacia or some other protective colloid to prevent agglutination.

Chances of Collision.

If a suspension of cells of diameter $C$ and velocity (under the influence of gravity) $V_c$, and of particles of diameter $P$ and velocity $V_p$, is allowed to settle in a test-tube, the chances of collision, $R$, between them will be proportional to the velocity of the particle relative to the leucocyte and to the square of the sum of their diameters or

$$R = (V_p - V_c) \left( C + P \right)^2 \quad (1)$$

The last factor is derived by a consideration of Fig. 1. If an infinitely small particle is settling down, its chance of hitting a single cell $C$ is proportional to the cross-sectional area of $C$ or $\pi C^2$. If, however, the particle has a finite diameter $P$, its center may miss the edge of the cell by a distance $P/2$ and still collide. The effective cross-sectional area of $C$ as a target for a particle is thus increased to $\pi(C + P)^2$. The chance that $P$ will hit $C$ is, therefore, proportional to $(C + P)^2$.\(^1\)

In these experiments, however, the suspensions were not allowed to settle out in stationary test-tubes as the formula could no longer be applied to cells and particles which were resting on the bottom. Instead, the test-tubes holding 1 to 2 cc. of the mixture were placed horizontally on a drum revolving slowly about a horizontal axis. It can be shown, however, that the same formula applies to this case. Consider first a case where there is no air bubble in the tube. Each cell and particle in the mixture is settling at a constant rate in a uniformly rotating medium, and at the end of one revolution will have returned exactly to the original position in the tube, having

\(^1\) The writer is indebted to Dr. E. K. Carver, National Research Fellow in Chemistry, of the Wolcott Gibbs Memorial Laboratory of Harvard University, for assistance with this formula.
described a circle the circumference of which is equal to the distance which each cell or particle would have settled during the time of one revolution either in a straight line or otherwise.

\[ C = \pi D \]

**Fig. 1.** Diagram illustrating chance of collision between a particle, \( P \), settling down toward a cell, \( C \).

\[ \text{Fig. 2. Diagram illustrating orbits described by a particle at } A \text{ and cells at } b, c, \text{ and } d \text{ in a rotating medium. The figure represents a cross-section of the medium which is considered to be rotating clockwise. The relative direction of rotation of particles and cells settling by gravity is, therefore, counter-clockwise, as indicated by the arrows. Collisions occur at } B, C, \text{ and } D. \text{ Chance of collision is proportional to the circumference, } Abcd. \]

The circumference thus equals \( \frac{V}{n} \), where \( n \) is the number of revolutions per unit of time and \( V \) the velocity. Let the circle \( ABCD \) (Fig. 2) be the orbit described by a particle, \( P \), starting at \( A \) in the direction
of the arrow. Its radius equals $V_{b}^{2}/2\pi$. Then the cells of velocity $V_{c}$ which will collide with the particle at $B$, $C$, and $D$ after one-quarter, one-half, and three-quarters of a revolution, respectively, must have been originally located at the points $b$, $c$, and $d$. But all these points lie on a circle (Fig. 2) whose radius is $V_{b} - V_{c}$, and whose circumference is therefore, $V_{b} - V_{c}$. Obviously the chance of collision depends upon the length of the line upon which colliding cells may lie.

In some of the following experiments, however, these ideal conditions were not quite realized, especially at the beginning before the theory of the chances of collision had been thoroughly worked out. Instead, an air bubble was allowed to be present and in many cases traveled from one end of the tube to the other as it revolved, owing to the fact that the tube was not quite horizontal. As the bubble passes along the tube it leaves behind it little eddies. These may be seen in a thick suspension of quartz as layers of unequal concentration of particles caused by the piling up of particles in the eddies by centrifugal force. It was observed that counts of the number of particles from small amounts of samples (5 c.mm.), removed immediately after stirring, showed greater variations than from samples taken when these inequalities in concentration had disappeared. It is probable that both particles and cells are acted upon by centrifugal force when stirred up, and move, therefore, with exactly the same relative velocities as when settling under the force of gravity alone. However the mixture is stirred, there would be no collisions if all particles and cells had exactly the same velocities, for they would merely be carried along by the current. Any collisions, then, must be due to differences in velocities.

In the smallest of these particles brownian motion was just perceptible. Even if the particles were so small, however, as to have no velocity under gravity and therefore very active brownian motion, this fact would not affect the calculation of the chances of collision with a cell which is literally “sweeping up” the particles with a velocity
of 1.67 cm. per hour, since the number of particles which got in front
of the cell by their brownian motion would be just balanced by the
number which would get out of the way of the cell.

**Verification of the Theory by Varying the Speed of Rotation.**

According to the theory, then, the chances of collision determine
*K*, other things being equal. The fact that the chance of collision
is proportional to \( V_p - V_c \), i.e. the difference between the speeds of
settling of particles and cells, makes possible a verification of the
theory by varying the speed of rotation of the tubes in which the
phagocytic mixtures are incubated.

Returning to Fig. 2, it is evident that the orbits described by the
particles, being equal to \( V \), must decrease with an increase in \( n \), the
number of revolutions per unit of time. It should be possible by
increasing \( n \) to decrease the diameter of the orbits nearly to zero.
Under such conditions there should be less phagocytosis than when
the mixture is rotated more slowly. In a slowly rotated mixture the
particles have plenty of time to settle down to the neighboring cells.
To test this prediction under ideal conditions, six small tubes, 3 mm.
inside diameter and 1.5 cm. long, sealed at one end, were prepared.
At the beginning of the experiment all these tubes were filled with
the mixture of cells and quartz particles immediately after the cells
were added. The open ends of the tubes were then sealed by dipping
in melted paraffin, excluding all air.

Three of the tubes were then put on a drum rotating at 0.3 revolu-
tion per minute and three on one rotating at 19 revolutions per minute.
Counts were then made of the number of particles present in the
remainder of the original mixture. At intervals of about 1 hour one
tube was taken from each drum and the number of particles not yet
ingested was counted. From these data the phagocytosis constant,
*K*, was calculated by the equation for a monomolecular reaction.

As predicted, the particles are ingested nearly two and one-half
times as rapidly in the slowly revolving tube. Likewise, the cells
aggregate more rapidly in the slow tube, due to the greater number of
collisions between them.
The result was as follows:

<table>
<thead>
<tr>
<th>Hrs.</th>
<th>19 revolutions per minute</th>
<th>0.3 revolution per minute</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of particles</td>
<td>No. of cells</td>
</tr>
<tr>
<td>0</td>
<td>324</td>
<td>76</td>
</tr>
<tr>
<td>0.6</td>
<td>262</td>
<td>76</td>
</tr>
<tr>
<td>1.75</td>
<td>157</td>
<td>55</td>
</tr>
<tr>
<td>2.6</td>
<td>64</td>
<td>53</td>
</tr>
</tbody>
</table>

\[ K = 0.22 \quad K = 0.52 \]

(Counts refer to volumes of 0.02 mm³)

The diameter of the orbits of cells and particles has been calculated from the equation \( \text{Diameter} = \frac{V}{\pi n} \), and is compared in the following tabulation with the average distance between cells and particles calculated from the equation

\[
\text{Average distance} = \frac{1}{2} \left( \frac{\text{Total volume of suspension}}{\text{No. of cells} + \text{No. of particles}} \right) = 37 \mu
\]

<table>
<thead>
<tr>
<th></th>
<th>Cells</th>
<th>Particles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average diameter</td>
<td>9.0</td>
<td>2.9</td>
</tr>
<tr>
<td>Diameter of orbit at 19 revolutions per min.</td>
<td>4.6</td>
<td>7.3</td>
</tr>
<tr>
<td>&quot; 0.3 revolution &quot;</td>
<td>279</td>
<td>462</td>
</tr>
</tbody>
</table>

These results show that the diameters of the orbits of cells and particles at 19 revolutions per minute are small compared to the distances between them. It is, therefore, only due to the fact that the laws of chance prevent all particles from being equidistant from each other (i.e. 37µ) that there was any phagocytosis at all at 19 revolutions per minute. In this case, also, when the cells are practically stationary, brownian motion probably plays some part in bringing particles to the cells. Centrifugal force is another factor which would have a very slight effect in causing collisions. It was calculated that at 19 revolutions per minute 12 per cent of the particles and 8 per cent of the cells would be centrifugalized against the wall of the tube and that it would take 21 minutes for a particle to overtake a cell 37µ distant,
both moving under centrifugal force alone. Centrifugal force is not, therefore, an appreciable factor except in the case of those cells which become "plastered" against the wall of the tube and there ingest particles. This could have been avoided by rearranging the tubes on the drum at intervals.

This experiment was done under ideal conditions, however, in that there was no air bubble present in the tube. When such a bubble is included in a tube which is slightly inclined to the horizontal, it travels up and down in the tube and stirs the mixture. Under such conditions the rate of phagocytosis, $K$, should vary in direct proportion to the speed of rotation. This also is true. Thus $K$ was increased from 0.17 to 0.76 (4.5 times) by an increase from 7 to 27 revolutions per minute (3.9 times).

These two experiments offered a very satisfactory proof of the theory at the outset and the following predictions may be made with considerable assurance.

1. The rate of phagocytosis, $K$, may be calculated from the equation for a monomolecular reaction and will remain constant in any experiment as long as the concentration of cells remains constant.

2. For a given leucocyte suspension in a given medium, $K$ is determined by the chances of collision between cells and particles.

3. The chances of collision vary with the method of stirring (i.e. speed of rotation, etc.) but the relative chances of collision, $R$, are applicable however the cells and particles are kept in suspension.

4. The relative chance of collision of any particle is given by the formula $R = (C + P)^2 (V_p - V_c)$.

5. The equation $\frac{K_1}{K_2} = \frac{R_1}{R_2}$ should be true for any two sizes of particles, 1 and 2.

**Velocity of Particles.**

The relative chances of collision are, therefore, very simply calculated if the diameter and velocity of the particles and cells are known. The simplest way of determining the velocity of the particles is by direct observation of the speed with which the suspensions settle out in the centrifuge bottle. This can only be done accurately in a suspension of particles of such a uniform size that they all settle at
the same rate leaving a clear liquid above. In all the suspensions used in these experiments this was possible. From the velocity so obtained, the diameter can be calculated by Stokes's law.

For example, if a suspension has been allowed to settle for 1 hour until 5 cm. of clear water remain at the top, the upturned capillary tip of a siphon is now placed 7 cm. below the surface, and the upper 2 cm. of the suspension are drawn off, the velocity of these particles is somewhere between 5 and 7 cm. per hour. It is probably given most accurately by the formula

\[ V_{\sigma} = V_s - \frac{V_s - V_g}{\sqrt{2}} \]  

(2)

where \( V_s \) and \( V_g \) are the smallest and largest velocities, respectively. This may be seen from a consideration of Fig. 3. Here the abscissae represent velocities. Ordinates represent the number of particles of each velocity originally present; it is assumed that there were the same number, \( N \), of each in the original raw material. The area, \( AONB \), then represents the original suspension. The dotted lines \( DO \), etc., outline the parts of the suspension which were successively discarded with the supernatant liquid after being allowed to settle ten times until particles of velocity, \( V_s \), had just settled out.
per cent of the particles, which settle only half this distance, are removed each time.

The suspension as kept permanently is then represented by the area \( \text{ACDB} \). To prepare a uniform suspension the whole is then shaken up, allowed to settle, and the upper layer of the suspension pipetted off at such a point that only particles of velocity smaller than \( V_g \) are obtained. The final suspension is then represented by the area \( \text{VgCD} \), and the average velocity must be measured by the perpendicular bisector of this area. Since \( \text{VgCD} \) is small compared to the whole it may be neglected, particularly as \( V_g \) is necessarily more or less indeterminate in practice. The average \( V \) is, then, best given by the bisector of the triangle \( \text{VgV_D} \) or \( \text{Vat} \) which can be shown by elementary geometry to have the value given in equation (2) above.

Stokes’s law (4) gives the velocity, \( V \), in centimeters per second of a spherical body of radius, \( r \), and density, \( D \), in a medium of absolute viscosity, \( \eta \), and density, \( d \), as

\[
V = \frac{2 (D - d)}{9 \eta} g^2
\]

whence

\[
r = \sqrt{\frac{9 \eta V}{2g (D - d)}}
\]

Introducing values \( \frac{0.010 \text{ erg sec}}{\text{cm}^4} \) for \( \eta \) (5) and \( \frac{981 \text{ cm}}{\text{sec}^2} \) for \( g \), and changing \( V \) into velocity in \( \frac{\text{cm}}{\text{hr}} \) and \( r \) from centimeters to microns we have

\[
r = \sqrt{\frac{4.5 V \times 10^4}{981 (D - d) 3,600}}
\]

and

\[
\text{Diameter} = \sqrt{\frac{5.1 V}{(D - d)}} \quad (3)
\]

For the purposes of these experiments it is possible to use this formula directly without allowing for the differences in the density and viscosity of the phagocytic mixtures. The viscosity has no effect on the relative chances of collision because it modifies the
velocity of both the cells and particles equally. Since the density of the medium was only 1.01 this can also be disregarded as small in comparison with the density of the particles, 1.81 (carbon) and 2.68 (quartz). As far as the leucocytes were concerned with a density 1.11, this was really allowed for largely by the necessity of measuring their velocity in a medium of sodium chloride of density 1.007.

In the case of suspensions which are not uniform enough to settle with a sharp boundary it is impossible to determine anything but the velocity of their smallest particles by direct observation. For such cases a method was devised which is known as the "stop-cock" method. It was also necessary to use this method for determining the velocity of the leucocytes. The method consists in allowing the suspension to settle out in a glass tube provided with a specially made stop-cock, such that there was no constriction in the inside diameter; there was, thus, a straight uniform tube all the way through the stop-cock. After a given interval of time the stop-cock is turned. The change of concentration in the suspension above the stop-cock is determined by counts of the numbers of particles present before and after settling. If there are h centimeters of suspension above the stop-cock and if the concentration before and after is given by C and C1, the velocity is given by the formula

\[ V = \frac{h (C - C_1)}{C t} \]  

This method gives an average figure for the velocity because the small particles which settle too slowly compensate for the large ones, all of which may have passed below the stop-cock before it is turned. Obviously, however, the diameter which is calculated from the average velocity is not the average diameter because the velocity is proportional to the square of the diameter. The agreement between results by this method and measurements of the diameter of particles by microscopic methods was, however, sufficiently good for these purposes as shown in Table I. The agreement is in itself proof of the uniformity of the suspensions used.

In the table are also included some figures for the diameters of these suspensions by the method of evaporating to dryness a suspension containing a known number of particles, and calculating the

\footnote{This is the case with suspensions from which the large particles were removed by centrifuge instead of by settling.}
diameters on the assumption that each particle is a sphere. It is
doubtless due to the irregular sizes of the particles that this method
gives results which are uniformly lower than the other methods. At
the end of the table are also included some figures for the smallest
diameters of the particles as calculated from the velocity with which
the upper boundary of the suspension settles under gravity; i.e.,
the velocity of the smallest particles. This figure can readily be
obtained from the stop-cock method in addition to the figure for the

### TABLE I.

Measurements of Diameters in Microns of Three Sizes of Quartz Particles by Various Methods.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry weight</td>
<td></td>
<td>3.55</td>
<td>3.2</td>
<td>2.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.46</td>
<td>3.14</td>
<td></td>
</tr>
<tr>
<td>Microscopic</td>
<td></td>
<td>5.01</td>
<td>4.34</td>
<td>2.32, 2.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.63 ± 0.05</td>
<td>4.08 ± 0.05</td>
<td>2.93 ± 0.036</td>
</tr>
<tr>
<td>Counting</td>
<td></td>
<td>4.67</td>
<td>4.44</td>
<td>2.82</td>
</tr>
<tr>
<td>Stop-cock method</td>
<td></td>
<td>4.98</td>
<td>4.10</td>
<td>2.62</td>
</tr>
<tr>
<td>Colorimeter</td>
<td></td>
<td>5.05 ± 0.14</td>
<td>4.05 ± 0.08</td>
<td>2.7 ± 0.04</td>
</tr>
<tr>
<td>Smallest diameter</td>
<td></td>
<td>3.04</td>
<td>2.65</td>
<td>1.93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.26</td>
<td>2.16</td>
<td></td>
</tr>
</tbody>
</table>

average velocity and gives a good idea of the degree of uniformity
of the suspension. To avoid counting, the stop-cock method was
usually modified by the use of the colorimeter for comparing the con-
centration of the suspension before and after settling. All the meas-
urements in Table I were made on suspensions prepared separately

4 For this purpose underneath illumination only was used so that the intensity
of the transmitted, not the diffracted, beam was measured. The use of the color-
imeter is perhaps a doubtful measure on theoretical grounds unless the suspensions
are thoroughly uniform, because it assumes that the amount of light transmitted
is proportional to the number of particles without respect to size. After settling
there are of course relatively fewer large particles.
from the same original stock bottle, the large particles being removed by centrifugalization each time. It is not surprising, therefore, that the measurements should differ somewhat at different times even by the same method.

**Diameter and Velocity of Leucocytes.**

The leucocytes were obtained from rats. The animals were injected intraperitoneally with a suspension of aleuronat, and the peritoneal cavity was opened on the following day and washed out with 0.5 per cent sodium citrate in 0.9 per cent sodium chloride solution. The cells were then centrifuged once to get rid of the citrate and resuspended in salt solution. If the cells are not washed thus it is necessary to have so much citrate present to prevent clotting of the exudate that it prevents phagocytosis.

For these experiments it was of first importance to determine the diameter and velocity of the leucocytes. These determinations had to be done independently as the density could not be measured directly. The diameter was measured directly in the microscope on fresh specimens before they had had time to spread on the microscope slide. The velocity was determined by the stop-cock method. An average of five determinations was $1.67 \pm 0.05$ cm. per hour. The figures were as follows: 1.63, 1.56, 1.83, 1.47, 1.84.

Since, however, the leucocytes are not of the same size it is necessary, in order to calculate the chances of collision with any accuracy, to divide the cells into three groups of three average diameters and calculate the chances of collision for each group separately. These calculations were made as follows: The diameters, $D$, of the leucocytes were measured as already described. From these data a frequency curve was obtained which was divided into three parts (Table II). The relative velocities of the three groups were then calculated by the formula $V = D^2K$, where $K$ is a constant. From these velocities the average velocity may be calculated as shown by multiplying the velocity for each group by the percentage of total cells in that group and dividing the sum of the results by 100. But the average velocity was found by experiment by the stop-cock method to be $1.67 \pm 0.05$. Equating these we have

\[
1.67 \pm 0.05 = 81.0 K
\]

\[
K = 0.0206 \pm 0.00062
\]
Substituting this value for $K$ in the third column of Table II we have the true values for the velocity in the last column. These were the data used in calculating the chances of collision.

**TABLE II.**

<table>
<thead>
<tr>
<th>Cell group</th>
<th>Average diameter.</th>
<th>Relative velocity $\times$ per cent of total cells.</th>
<th>True velocity.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu$</td>
<td></td>
<td>cm. per hr.</td>
</tr>
<tr>
<td>1</td>
<td>$7.6 \pm 0.04$</td>
<td>$58.0 \times 0.32 = 18.6 \times K$</td>
<td>$1.20 \pm 0.035$</td>
</tr>
<tr>
<td>2</td>
<td>$8.9 \pm 0.016$</td>
<td>$79.5 \times 0.39 = 31.0 \times K$</td>
<td>$1.64 \pm 0.048$</td>
</tr>
<tr>
<td>3</td>
<td>$10.8 \pm 0.034$</td>
<td>$108.5 \times 0.29 = 31.4 \times K$</td>
<td>$2.24 \pm 0.066$</td>
</tr>
<tr>
<td>Average velocity</td>
<td></td>
<td></td>
<td>$81.0 \times K$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$1.67 \pm 0.05$</td>
</tr>
</tbody>
</table>

From these data the density of the cells may be determined by equation (3).

\[
D - d = \frac{5.1 \times 1.64}{8.9^2} = 0.105 \\
D = 1.007 + 0.105 = 1.11
\]

**Comparison of the Rates of Phagocytosis of Quartz Particles of Three Different Sizes.**

Experiments 1 and 2.—The three quartz suspensions were washed once with distilled water and were then allowed to settle at room temperature. After the sharp boundary of the suspension had settled 3 or 4 cm. from the top of the liquid, a definite amount, about 2 cm., was siphoned off the top of each suspension as already described. From the minimum and maximum velocities so obtained, the average velocity is calculated by equation (2) (Table III). The diameter is then given by equation (3), and the chances of collision with the leucocytes by the formula

\[
R_{total} = \frac{R_aP_a + R_bP_b + R_cP_c}{100} \quad (5)
\]

where $R_a$, $R_b$, and $R_c$ are the chances of collision with the three groups of cells $a$, $b$, and $c$; and $P_a$, $P_b$, and $P_c$, the percentages of cells in these groups. The velocity of the $2.55 \mu$ particles being most nearly equal to that of the cells, these particles have the smallest chance of collision, as shown in Table III. Under the microscope these suspensions appeared as absolutely uniform as any suspension of irregular particles could be (Fig. 4).
The phagocytic mixtures were prepared as follows: 0.2 cc. of washed leucocyte suspension in 0.9 per cent sodium chloride, plus 0.15 cc. of quartz suspension in 0.9 per cent acacia plus 0.1 cc. of fresh serum plus 0.05 cc. of M/10 phosphate mixture ([H]^+ = 3 × 10^{-9}) plus 0.05 cc. of 4.5 per cent sodium chloride. The acacia was added to prevent agglutination of the quartz. The phosphate helped to maintain the hydrogen ion concentration equal to that of blood serum. Frequent colorimetric measurements of [H]^+ at the close of the experiments showed no appreciable change in reaction from the original. Without serum there is very little phagocytosis.

TABLE III.
Comparison of Theoretical and Experimental Rates of Phagocytosis of Three Sizes of Quartz Particles.

<table>
<thead>
<tr>
<th>Average diameter of particles.</th>
<th>2.92 μm</th>
<th>2.55 μm</th>
<th>1.85 μm</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum..</td>
<td>2.51</td>
<td>1.89</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Maximum..</td>
<td>3.54</td>
<td>2.73</td>
<td>1.44</td>
<td></td>
</tr>
<tr>
<td>Average..</td>
<td>2.81</td>
<td>2.14</td>
<td>1.13</td>
<td></td>
</tr>
<tr>
<td>Chances of collision..</td>
<td>151.8</td>
<td>61.8</td>
<td>76.5</td>
<td>1:0.41:0.51 (Theoretical.)</td>
</tr>
</tbody>
</table>

Experiment 1 (Fig. 5)

<table>
<thead>
<tr>
<th>K</th>
<th>0.078</th>
<th>0.033</th>
<th>0.05</th>
<th>1:0.42:0.64 (Experimental.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average per cent of error</td>
<td>9.0</td>
<td>4.0</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>Assumed...</td>
<td>7.0</td>
<td>5.0</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>Expected...</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Experiment 2 (Fig. 6)

<table>
<thead>
<tr>
<th>K</th>
<th>0.38</th>
<th>0.33</th>
<th>0.22</th>
<th>1:0.87:0.58 (Experimental.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average per cent of error</td>
<td>7.0</td>
<td>3.1</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>Assumed...</td>
<td>8.0</td>
<td>7.6</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td>Expected...</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The above mixture was incubated at 37°C. in small glass-stoppered vials 6 mm. in outside diameter, which were rotated about their horizontal axis once in 2 minutes. The speed of rotation was kept constant in any one experiment. Since the tubes were so small and were held horizontal, there was very little stirring by the air bubble included at one end of the tube. Counts of the number of particles outside the phagocytes per 0.02 mm.³ of solution were made at intervals. The logarithms of these counts were then plotted as ordinates against time as abscissæ. If phagocytosis follows the laws of monomolecular reactions these points should lie on a straight line of slope K, where K is the velocity of the reaction. A summary of Experiments 1 and 2 is given in Table III and the experimental points are plotted in Figs. 5 and 6.
In Experiment 1, $K$ and logarithm $A$ were determined by the method of least squares; in Experiment 2, they were measured graphically. In the latter instance $K$ was nearly ten times as high as in the former. This may have been due to a difference in the cells or in the serum. The quartz suspensions used were identical.

![Figure 4](image)

**Fig. 4.** Photograph of quartz suspension 4.6 $\mu$ in diameter, to show uniformity in size of particles.

![Figure 5](image)

**Fig. 5.** Experiment 1. Ordinates represent logarithms of the number of particles not taken up by leucocytes plotted against time as abscissae. $K$ is equal to the slope of the graph. Quartz particles of three different sizes are compared, designated as in Fig. 6. Experimental ratios, 1:0.42:0.64; theoretical ratios, 1:0.41:0.51. $K$ determined in this experiment by method of least squares. See Table III.
In Table III is also given the average per cent of error which was assumed in the experimental points in order to fit them to the equation and the average probable error involved in counting the particles for the experimental points. The latter value was calculated by the expression $\frac{1.05}{\sqrt{N}}$, where $N$ was the number of particles counted. The assumption that the points lie on a straight line is justified by

![Graph](image)

Fig. 6. Experiment 2. Legend as in Fig. 5. Experimental ratios, 1:0.87:0.58; theoretical ratios, 1:0.41:0.51. $K$'s determined graphically. See Table III.

The error of a set of observations varies inversely as the square root of the number of observations (Tuttle, L., The theory of measurements, Philadelphia, 1916, 219). The constant $1.05 \pm 0.049$ was determined for counting particles from calculations of the dispersion of extensive counts made on fourteen different suspensions. The dispersion of the fourteen determinations was $\pm 0.19$. The constant was also determined from counts made on forty-eight different leucocyte suspensions and gave $1.07 \pm 0.05$, the dispersion of the individual determinations being $\pm 0.34$. 
the fact that the error assumed is in general no larger than the probable error. Reference to Table III will show that the ratios between the three experimental constants agree fairly well with the theoretical ratios.  

Experiments 3 and 4.—Two more experiments were tried in which only the largest and smallest particles were compared. New suspensions were prepared from the same stock and in the same way as before. The sizes are accordingly somewhat different. The rates of phagocytosis are compared with the chances of collision in Table IV, and the experimental points are plotted in Figs. 7 and 8.

### Table IV.

**Comparison of Theoretical and Experimental Rates of Phagocytosis of Two Sizes of Quartz Particles.**

<table>
<thead>
<tr>
<th></th>
<th>3.18 μ</th>
<th>1.73 μ</th>
<th>Ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average diameter of particles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>3.95</td>
<td>1.91</td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>3.07</td>
<td>1.21</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>3.33</td>
<td>1.41</td>
<td></td>
</tr>
<tr>
<td>Velocity per hr. in cm.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>286</td>
<td>51</td>
<td>1:0.18 (Theoretical.)</td>
</tr>
<tr>
<td>Minimum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chances of collision</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 3 (Fig. 7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial K</td>
<td>0.133</td>
<td>0.046</td>
<td>1:0.35 (Experimental.)</td>
</tr>
<tr>
<td>Later K</td>
<td>0.84</td>
<td>0.174</td>
<td>1:0.21 (&quot;&quot;&quot;)</td>
</tr>
<tr>
<td>Experiment 4 (Fig. 8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial K</td>
<td>0.265</td>
<td>0.1</td>
<td>1:0.38 (&quot;&quot;&quot;)</td>
</tr>
</tbody>
</table>

In Experiment 3 the reaction starts very slowly and for some unknown reason the cells become changed or the medium becomes more favorable so that they can ingest particles more readily. Each reaction is, therefore, made up apparently of two distinct parts. If the change in the cells in the two tubes is comparable the ratio of the K's should be the same for the later K's as for the initial K's. This turns out to be true and both ratios agree well with the theoretical ratio. The inclusion of the later K's is admittedly somewhat arbitrary and is given merely for what it is worth.

The graphs in Fig. 7 for the 3.18 μ particles show considerable irregularities but the agreement of the initial K's is very good and the ratio of the initial K's for the two sizes of particles agrees fairly well with the theoretical.

6 A discussion of the significance of a constant K in view of the gradual agglutination of cells is postponed to the next paper of this series.
Fig. 7. Experiment 3. Ordinates represent logarithms of numbers of particles not ingested. Since $K$ is not constant the initial $K$'s only can be used in comparisons. Experimental ratio, 1: 0.35; theoretical ratio, 1: 0.18. Ratio of $K$ from the end of the experiment is 1: 0.21. The two sizes of quartz particles correspond to the largest and smallest in Figs. 5 and 6. See Table IV.

Fig. 8. Experiment 4. Repetition of Experiment 3. Only the initial $K$'s give any basis for comparison. Experimental ratio, 1: 0.38; theoretical ratio, 1: 0.18. See Table IV.
Experiments 5 and 6.—In two more experiments on quartz particles the suspensions used were less uniform because the large particles were removed by centrifugalization. The diameters of these particles were obtained by direct microscopic measurement. Frequency curves so obtained are plotted in Fig. 9. Each frequency curve was then divided into a number of groups and the chances of collision calculated between each one of the quartz groups and the three groups of cells by equation (5). The chances of collision for any one suspension is, therefore, equal to the sum of the products of the chances of collision of each quartz group by the per cent of particles in that group, or

$$R_{\text{total}} = \frac{R_{a1}P_1 + R_{a2}P_2 + R_{a3}P_3 + \ldots + R_{ac}P_c}{100}$$

where $P$ is the per cent of cells or particles in the designated group, $R$ the chance of collision; the subscripts 1, 2, and 3 refer to groups of particles while $a$, $b$, and $c$ refer to groups of cells.

![Graph](image-url)

Fig. 9. Frequency curves where ordinates represent number of particles and abscissa represent diameters of particles in microns. Data from microscopic measurements of three quartz suspensions used in Experiments 5 and 6. Average diameters equal 2.44 μ, 4.08 μ, and 4.63 μ. Points plotted are experimental points smoothed by averaging each ordinate with the two adjacent ordinates. Plotted to such a scale that the areas subtended by each graph are equal. See Table V.

The calculation in this case is much more laborious and the results are not so satisfactory. Moreover, in these experiments, which were the first comparisons attempted, larger test-tubes (8 mm. inside
diameter) were used for incubation, and there was more irregular stirring by the air bubble which moved up and down the rotating tube. Under these circumstances the explanation offered for the different rates of ingestion of the three suspensions seems satisfactory.

The data used in calculating $R$ are given in Table V. The velocities obtained for each group were the weighted averages of all the separate velocities in the group, not the velocity corresponding to the average diameter. The group diameters of the two larger suspensions are the

<table>
<thead>
<tr>
<th>Average diameter of suspension</th>
<th>No. of group</th>
<th>Group diameter</th>
<th>Group velocity</th>
<th>Particles in group</th>
<th>Group chances of collision</th>
<th>Total $R$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu$</td>
<td>1</td>
<td>1.60</td>
<td>0.84</td>
<td>18.8</td>
<td>106</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.04</td>
<td>1.37</td>
<td>23.4</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.42</td>
<td>1.93</td>
<td>19.4</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2.83</td>
<td>2.64</td>
<td>24.8</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>3.68</td>
<td>4.46</td>
<td>13.3</td>
<td>413</td>
<td></td>
</tr>
<tr>
<td>2.44</td>
<td>1</td>
<td>2.82</td>
<td>2.69</td>
<td>11.6</td>
<td>132</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.05</td>
<td>5.23</td>
<td>71.5</td>
<td>602</td>
<td>697</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5.28</td>
<td>9.00</td>
<td>16.8</td>
<td>1,500</td>
<td></td>
</tr>
<tr>
<td>4.08</td>
<td>1</td>
<td>3.70</td>
<td>4.38</td>
<td>19.0</td>
<td>431</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.67</td>
<td>6.90</td>
<td>44.0</td>
<td>979</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5.45</td>
<td>10.00</td>
<td>32.0</td>
<td>1,745</td>
<td>1,299</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>7.40</td>
<td>18.5</td>
<td>5.0</td>
<td>4,560</td>
<td></td>
</tr>
</tbody>
</table>

average diameters of the group. For the smaller suspension they are calculated from the average velocity. The difference in the result is too small to have any appreciable effect. The latter method is more accurate because it is the square of the diameters which enters into the equation. The error in calculating must be larger for the smallest suspension because in this case the group velocities nearly coincide with the velocities of the groups of cells. The true chance of collision is, therefore, somewhat higher than the calculated figure. This could be avoided by the use of calculus, treating the frequency
curves in Fig. 8 as normal frequency curves, but the resulting expression would be too difficult to work with.\(^7\)

Since the group chances of collision are not all equal in the same suspension the chances of collision as a whole will tend to decrease as those particles with the greatest \(R\) are taken up first. It can be shown that \(R\) decreases most rapidly for the 4.63 \(\mu\) particles. The ratios, therefore, increase. This ratio has been calculated for the times in the experiment when the 4.63 \(\mu\) particles are 50, 75, and 90 per cent ingested (Table VI).

### TABLE VI.

*Comparison of Theoretical and Experimental Rates of Phagocytosis of Three Sizes of Quartz Particles.*

<table>
<thead>
<tr>
<th>Average diameter of particles</th>
<th>4.63 (\mu)</th>
<th>4.08 (\mu)</th>
<th>2.44 (\mu)</th>
<th>Ratios.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per cent of particles ingested</td>
<td>initial (K)</td>
<td>experiment 5 (Fig. 10)</td>
<td>total (K)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.76</td>
<td>0.70</td>
<td>0.33</td>
<td>(Theoretical.)</td>
</tr>
<tr>
<td>50</td>
<td>0.58</td>
<td>0.477</td>
<td>0.196</td>
<td>1:0.92:0.43</td>
</tr>
<tr>
<td>75</td>
<td>0.44</td>
<td>0.27</td>
<td>0.06</td>
<td>1:0.64:0.14</td>
</tr>
<tr>
<td>90</td>
<td>0.85</td>
<td>0.348</td>
<td>0.134</td>
<td>1:0.61:0.24</td>
</tr>
</tbody>
</table>

In Figs. 10 and 11 are plotted the experimental points in Experiments 5 and 6 respectively, and the results are summarized in Table VI. For comparison, the initial and the total \(K\)'s have been calculated. The latter are calculated by the method of least squares and the former directly from the experimental figures. The agreement between these experimental ratios and the expected ratios seems remarkably good when the many complicating factors involved in this type of experimentation are taken into consideration.

\(^7\) The writer wishes to express his gratitude to Professor R. G. Wilson, Massachusetts Institute of Technology, for information on this point and also for valuable criticism of this work.
Fig. 10. Experiment 5. Logarithms of number of particles not ingested plotted as ordinates against time as abscissae. See Table VI for comparison of theoretical and experimental ratios and values of initial $K$'s. Total $K$'s, as plotted, were determined by method of least squares.
SUMMARY.

1. A new quantitative method of measuring phagocytosis of solid particles is described.
2. A method of calculating the chances of collision between leucocytes and quartz particles of different sizes is developed.
3. The speed with which three suspensions of different sized quartz particles should be ingested by leucocytes is predicted from the calculated chances of collision, and the prediction is verified experimentally.
4. The formula for the chances of collision is also verified by varying the speed of rotation of the tubes in which the phagocytic mixtures are incubated.

The advice and assistance of Doctor C. K. Drinker and Doctor C. K. Reiman of this department are gratefully acknowledged.

BIBLIOGRAPHY.