

## THE PHAGOCYTOSIS OF SOLID PARTICLES.

### IV. CARBON AND QUARTZ IN SOLUTIONS OF VARYING ACIDITY.

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In the preceding paper of this series (1) it was shown that carbon particles are ingested by rat leucocytes three or four times as readily as quartz particles of approximately the same size. Variations were found, however, in this ratio between the rates of ingestion of carbon and quartz during the course of a single experiment which suggested that significant variations might be produced by varying the fluid environment of the cells. The present paper includes experiments which indicate a reversal of this ratio with acidification of the solution, quartz becoming more readily ingested than carbon. It was hoped that a further study of this comparatively simple phagocytic system of leucocytes with quartz and carbon particles might yield results which could be interpreted in terms of physical chemistry and hence give some quantitative experimental clue as to the fundamental nature of phagocytosis.

#### *a. The Carbon-Quartz Ratio at Varying pH.*

The method used for these experiments was the "film method" previously (1) described. Briefly, it consists in mixing together suspensions of leucocytes obtained from a peritoneal exudate in the rat, quartz and carbon particles of uniform sizes, serum, and phosphate mixtures for the control of the hydrogen ion concentration. This mixture is allowed to run under a cover-glass supported on the slide by cover-glass fragments, which is then sealed with paraffin to prevent drying. A count is immediately made of the relative numbers of particles of the two sorts originally present. The preparation is then placed on the warm stage and, as the leucocytes creep about on the slide ingesting the particles, counts are made of the numbers of quartz

and carbon particles which appear inside the cells. Later in the experiment, when phagocytosis becomes more complete, a better measure of the relative rates of phagocytosis is obtained by counting the numbers of particles still free; *i.e.*, neither inside nor stuck to the cells.

TABLE I.  
*Increase of the Ratio of Ingested Carbon to Ingested Quartz Particles with Increase in pH.*

Experiment No.	Serum.	pH	Per cent carbon Per cent quartz	Experiment No.	Serum.	pH	Per cent carbon Per cent quartz
	<i>per cent</i>				<i>per cent</i>		
1	17	7.2	0.88	6a	10	7.1	2.28
		7.6	1.8			7.3	2.6
2	7	6.4	0.43	6b	10	7.1	0.87
		7.3	1.39			7.3	4.0
		7.6	2.93	7	10	6.6	0.58
6.5	0.84	7.0	0.73				
3	10	7.0	2.03			7.2	1.5
		8.0	1.49			7.4	1.8
4	10	7.2	1.32	8a	36	6.6	1.3
		7.5	1.56			7.3	2.2
5	17	7.0	1.21	8b	36	6.6	0.8
		7.2	1.90			7.3	2.1

pH was measured electrometrically. Ratios were calculated from counts of particles inside the cells except in Experiments 6b and 8b where the particles outside the cells were counted and those inside calculated by difference. pH was controlled by phosphate buffers except in Experiment 8a where the acid reaction was obtained by equilibrating with 4 per cent CO<sub>2</sub> in room air (see text).

The results of a series of eight experiments with this method are collected in Table I. Each experiment involves so much counting that it is difficult to compare more than two or three solutions at once. The pH was measured electrometrically in most cases, but in a few it was estimated accurately enough from that of the phosphate mixtures used. In this table all the ratios are based on the percentages of particles found inside the cells except as noted. In a number of other experiments the ratio between the numbers of quartz and carbon

particles free at the close of the experiment was determined, and it was always found that there was relatively more carbon free in acid solutions and relatively more quartz free in alkaline solutions. This confirms the results in Table I which show that the percentage of carbon particles inside the cells increases relatively to the quartz with increasing alkalinity. If the pH at which the ratio equals 1 is determined by interpolation from the results in Table I, the points indicated are pH 6.93, 7.17, 7.07, 7.25, 7.11, and 6.71, the average being 7.04. These figures are, of course, far from accurate, but the agreement among them is good enough to warrant the general conclusion that *quartz is ingested more readily than carbon in acid solutions, but carbon is ingested more readily than quartz in alkaline solutions*. It should be particularly emphasized that this conclusion does not contradict the fact that the optimum pH for phagocytosis of both carbon and quartz particles by this method is at or near neutrality. This optimum is best interpreted as due to the physiological effect of the pH on the leucocytes rather than on the particles.

Special mention should be made of Experiment 8a in Table I where the acid reaction was obtained by equilibrating with room air containing 4 per cent CO<sub>2</sub>, the resulting pH being measured electrometrically using 4 per cent CO<sub>2</sub> in hydrogen for the gas. The method of performing this experiment is simple in principle but rather difficult technically.

A cover-slip was supported as usual on a slide and under its opposite edges two fine capillary tubes were inserted. One of these was drawn from the bottom of a small 1 cc. test-tube in which the mixture of cells and particles was placed. The edges of the cover-slip were then sealed with paraffin. Gas was then introduced through one capillary tube passing under the cover-slip and escaping by bubbling up through the suspensions in the test-tube. This was continued until the system was in equilibrium. The particles were not added until just before turning off the gas in order to minimize the clumping which otherwise occurs. Finally the gas is shut off, a little of the mixture is sucked quickly back under the cover-slip, the capillary tubes are broken off, and the ends sealed over with paraffin.

One point, possibly of some theoretical significance, and which came to light in these experiments, is the fact that when carbon was prepared with the use of 0.4 per cent acacia as a stabilizer, the addition of acid failed to prevent the carbon from being taken up better than the quartz. Acacia also affects the surface of the carbon in such a way

that the negative charge on the particle cannot be reversed by the addition of acid. The ratios observed (always  $> 1$ ) between the numbers of carbon and quartz particles ingested were 2.4, 2.45, and 2.2 at pH 6.0; 1.5, 1.46 at pH 6.2; 1.57 and 1.56 at pH 6.7; and 3.5 at pH 7.6. Figures in Table I, it will be remembered, showed ratios as low as 0.5 in acid solutions, indicating that the carbon was taken up only one-half as well as the quartz.

*b. The Phagocytosis of Manganese Dioxide and Manganese Silicate.*

On account of experiments from this laboratory by Drinker and Shaw (2) on the distribution of manganese dioxide and manganese

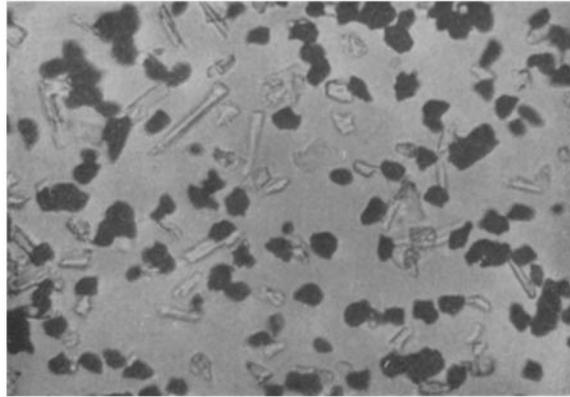


FIG. 1. Photograph of a mixture of the suspensions of manganese dioxide and manganese silicate used in the phagocytosis experiments. Leucocytes are attracted toward the black  $MnO_2$  particles which they ingest leaving the silicate. The average diameter of the particles was somewhere between 3 and 5 microns.

silicate after intravenous administration in the cat and the relative rates of their removal from the blood stream, it became important to determine the relative rates of phagocytosis of these two kinds of particles. For this purpose uniform suspensions were prepared by fractional centrifugation in 0.4 per cent acacia. The acacia prevents the manganese dioxide from clumping and is indispensable. A photograph of the mixture of the two products is shown in Fig. 1. The diameters were not measured but were between 3 and 5 microns. The method used was the same as that described for carbon and

quartz. *The rate of ingestion of MnO<sub>2</sub> was astonishingly rapid.* In the first experiment the number of free MnO<sub>2</sub> particles fell from 105 to 22 per unit area in 12 minutes, during which time the silicate count over the same area had not perceptibly changed from 106. In the second experiment 78 MnO<sub>2</sub> particles were ingested per unit area in 10 minutes while 31 silicate particles were being ingested, approximately equal numbers of each being present originally. In a third experiment the free particles of MnO<sub>2</sub> decreased from 93 to 7 in 8 minutes while the silicate count decreased only from 99 to 67. In another experiment the rate of ingestion of quartz was compared with that of MnO<sub>2</sub> at pH 6.3, 7.0, and 7.6. The results are summarized in Table II. A change in pH from 6.3 to 7.6 causes therefore no reversal in the relative rates of phagocytosis of these two kinds of

TABLE II.  
*Phagocytosis of Manganese Dioxide and Quartz with Varying pH.*

pH	No. of particles per unit area at start.		No. of particles still free after 20 min.		No. of particles ingested after 10 min.	
	Quartz.	MnO <sub>2</sub>	Quartz.	MnO <sub>2</sub>	Quartz.	MnO <sub>2</sub>
6.3	80	60	54	4	4	65
7.0	76	53	59	8	7	86
7.6	69	47	67	9	15	62

particles, like that found for carbon and quartz. The ingestion of the manganese dioxide, however, was so rapid that there was not sufficient time to make the counts accurate enough to exclude the possibility of some effect.

*c. Are Meetings between Cells and Particles Fortuitous?*

It has been shown by Commandon (3) by means of moving pictures that leucocytes are attracted by starch grains. It was of interest therefore to determine whether the preference shown for carbon and for manganese dioxide exhibited by leucocytes is due to a greater ease of ingestion or to an attraction between them resulting in more frequent meetings.

For this purpose a preparation was made under the cover-slip as for the "film method." One field was chosen which was divided into

squares by a suitably ruled ocular disc. While the cells were still inactive, having just come from the cold room, the positions of each carbon and each quartz particle in the field were plotted on a duplicate chart in the note-book. The heating current was then passed through the warm stage and as ingestion began each square was rapidly inspected in turn. As soon as any particle was seen in contact with a leucocyte a circle was drawn around it in the note-book and the time noted. Later collisions with this same particle were disregarded. It made no difference in the result, therefore, whether the particle was ingested or whether the leucocyte crept away again and left it. Thus the experiment closely resembled a game of tag in which the leucocytes were "it," the particles being out of the game when tagged. By working rapidly it was just possible to keep a fairly accurate record of 30 to 40 particles of each kind but there was considerable room for errors of judgment. The method was nevertheless more than accurate enough to detect the large difference between  $MnO_2$  and quartz.

Seven experiments of this sort have been tried with carbon and quartz particles. The results are difficult to tabulate briefly without exaggerating their significance, but they can be quite accurately summarized by two perfectly representative examples. In one experiment a field was selected for observation containing 44 carbon and 38 quartz particles and 77 leucocytes. After 24 minutes on the warm stage there were 1 quartz particle and 12 carbon particles to be seen inside the leucocytes. Yet the number of observed collisions between leucocytes and quartz had been 36 as compared to 37 between the leucocytes and the carbon. In another experiment a field of 38 carbon and 42 quartz particles showed after 28 minutes only 4 quartz to 20 carbon particles ingested, while 34 quartz and 33 carbon particles had been seen at one time or another in contact with the leucocytes. By careful observation of such experiments leucocytes can frequently be seen in contact simultaneously with both quartz and carbon particles, finally ingesting, however, only the carbon. Likewise in other experiments where the acidity is such that quartz particles are more readily ingested, there is ample evidence to show that cells are unable to keep hold of the carbon particles although the quartz is tenaciously retained. In no cases, however, did the rates of collision

of leucocytes with the two kinds of particles show variations which could not be attributed to unwitting errors of judgment or similar causes.

In some earlier experiments it was found that carbon particles which had been centrifuged out of 0.4 per cent acacia solution and then resuspended in distilled water before mixing with the leucocytes were ingested much more rapidly than untreated carbon. The cause of this result has not been ascertained but it suggested the possibility that leucocytes might be attracted toward carbon so treated but not towards normal carbon. Four experiments were therefore tried using carbon which had been centrifuged from 0.4 per cent acacia in which they had been kept (unsterile) for  $2\frac{1}{2}$  years. It was estimated that the

TABLE III.

*Rate of Collision of Leucocytes with Manganese Dioxide and Manganese Silicate.*

Experiment No.	No. of collisions per min.	
	MnO <sub>2</sub>	MnSiO <sub>3</sub>
1	3.3	0.73
2	2.5	0.85
3	4.0	1.8
4	2.9	1.3
5	2.0	1.1
Average.....	2.9	1.2

carbon was ingested 1.8 times as readily as the quartz but the rates of collision, though slightly in favor of the carbon (1 to 1.2 times as fast as the quartz), could not be regarded as significant.

From these experiments there is reason to believe that *the encounters of cells with quartz and carbon particles are purely fortuitous*. In this connection it should be mentioned that Schaeffer (4) asserts that amebæ are attracted toward particles of glass.

The rates of collision of leucocytes with MnO<sub>2</sub> and quartz particles were also investigated by this same method. Five such experiments are readily summarized in Table III, in which the average number of collisions per minute is recorded. *The rate of collision of the leucocytes with the manganese is seen to be  $\frac{2.9}{1.2} = 2.4$  times as great as with the*

*quartz.* This difference indeed was almost obvious from mere inspection. Pseudopods often seemed to be put out in the direction of the manganese particles and in one case, recorded in Fig. 2, a leucocyte seemed obviously to be attracted from a distance of perhaps 30 microns toward a group of two manganese and one quartz particle. From its unusually rapid movements the leucocyte appeared to be "in haste" and its path was directly blocked by the quartz particle. Two pseudopods were put out "straddling" this obstruction, each of which promptly ingested a manganese particle. After this feat the leucocyte rounded up and wandered away with its twin load leaving the quartz particle undisturbed. The whole performance could not have lasted over 2 minutes. Similar but less striking occurrences



FIG. 2. Diagram of an actual case in which a leucocyte was apparently attracted toward two particles of manganese dioxide which it ingested with such precision that it seemed almost "purposeful," an intervening particle of quartz being completely neglected. Time about 2 minutes.

were frequently observed. The acacia used in preparing the manganese could hardly have been responsible for this attraction since it was in large measure removed by 2 or 3 washings on the centrifuge before using the suspension. Moreover, carbon particles similarly treated showed no comparable attraction for leucocytes. It seems probable, therefore, that the attraction of the  $MnO_2$  is due to its slight solubility which is particularly noticeable in the presence of carbonic acid and organic matter (Drinker and Shaw (2)).

It has been mentioned in a foot-note in a previous paper (1) that when a mixture of manganese silicate and manganese dioxide particles is shaken up with chloroform or oil the particles are found collecting in the interfaces between the drops of chloroform or oil and the water. If counts are made of the numbers of the two kinds of particles still

free after unit additions of chloroform or oil, it is found that the silicate particles collect in the interfaces more rapidly than the manganese dioxide, leaving an increasing proportion of the latter in solution. Thus it may be concluded that artificial leucocytes of chloroform or oil ingest the silicate more rapidly than the dioxide, the result being thus exactly contrary to the findings with live material.

*d. Penicillium Spores versus Quartz.*

One experiment was tried with *Penicillium* spores and quartz particles. Only single spores and double spores were counted, longer chains being totally disregarded. In spite of the fact that the quartz particles were somewhat larger than the spores, the latter were carefully picked out by the leucocytes so that after 2 hours 58 per cent of the spores and only 9 per cent of the quartz had been ingested. The corresponding ingestion after 4 hours was 65 per cent for the spores and 25 per cent for the quartz.

*e. Relative Ingestibility of Large and Small Particles.*

In the course of some experiments on the relative rates of ingestion of various kinds of lead dusts<sup>1</sup> it became necessary to use some of the dusts in very small particles, and the question arose whether such extreme variations in the size would affect the rate of ingestion. It had already been ascertained (5) that a variation from 2 to 5 microns in diameter had no measurable influence on the rate of phagocytosis except in so far as the chances of collision were increased by increase in the size of the particle. This chance of collision could easily be calculated, it being equal to the sum of the diameters of the leucocyte and particle, provided all movement of the leucocytes was confined to two dimensions, as on a microscope slide. It was expected that the large particles would be taken up slightly less rapidly than would be predicted from the chances of collision alone because of the greater mechanical deformation of the cell necessary for their ingestion. It was a surprise to find that the small particles, approximately 0.9 micron in diameter were apparently ingested with considerable difficulty. This was determined by comparing the rates of ingestion of four sizes

<sup>1</sup> These were undertaken with the cooperation and at the suggestion of Dr. Alice Hamilton, and with the assistance of Mr. Jacob Fine.

of quartz particles of diameters 0.9, 2.8, 4.0, and 7.8 microns respectively. The results are shown in Table IV where the "expected" and "observed" ratios of the rates of phagocytosis of the small to large particles are compared. The figures show a fairly definite tendency for the smaller particles to be taken up less rapidly than the calculations predicted, especially where the 0.9 micron particles are concerned. In other cases (7.8 *versus* 2.8 microns) it is in fact doubtful

TABLE IV.  
*Ratios of Ingestion Rates of Large and Small Quartz Particles.*

Diameters of particles. <i>microns</i>	Expected ratio.	Observed ratio.
2.8 vs. 0.9	0.85	0-0.19
	0.85	0.35-0.54
7.8 vs. 0.9	0.61	0.06-0.28
	0.61	0.22-0.66
7.8 vs. 2.8	0.72	0.72
	0.72	0.48-0.63
4.0-0.9	0.7-0.8	0.32
		0.1-0.22
		0.55-0.66
		0.35
		0.29-0.45

Expected ratios are the ratios of the chances of collision  $\left(\frac{\text{small}}{\text{large}}\right)$  calculated from the diameters of the particles and leucocytes. Observed ratios are the ratios of the percentages of small to large particles ingested in equal times. Each line in the table represents a separate experiment. Where the observed ratios are smaller than the expected, the smaller particles are taken up less readily than expected.

whether the differences between expected and observed ratios are greater than the experimental error. There is no doubt, however, that under the conditions of these experiments *some factor other than surface tension* becomes significant in the phagocytosis of the 0.9 micron particles. In some experiments almost all the larger particles were found inside the cells before the ingestion of these smallest particles became greater than the experimental error of the counts.

Three possible explanations of this result are: (1) The small particles are excreted as rapidly as they are ingested. This obviously could not be true if phagocytosis were merely a matter of surface tension. (2) The small particles, being almost at the limit of microscopic visibility and in brownian movement, may be merely pushed aside by the leucocyte which may be regarded as experiencing the same difficulty which a child finds in "bobbing for apples" in a tub of water. (3) Small particles may give the cell less of a "stimulus" than the large particles, the response being correspondingly less.

It is regretted that time has not permitted a more thorough investigation of the rates of ingestion of different sizes of particles. These experiments, though preliminary in nature, are reported because they illustrate one definite quantitative method of testing the surface tension hypothesis. By this test it is found incomplete.

#### DISCUSSION OF RESULTS.

What seems to be the most interesting and significant fact which has come out of these experiments on the phagocytosis of carbon and quartz particles is the reversal of preference at neutrality, carbon being taken better than quartz in alkaline solutions, quartz better than carbon in acid solutions. It would seem as if this fact might provide a means of discovering just what property of a particle it is which favors its ingestion by a leucocyte. Obviously the limiting factor in these experiments is something which concerns the surface of the solid particle. Either it may be related to the interface between the particle and the solution or to the interface (not necessarily surface tension) between the particle and the leucocyte. It cannot be due to the effect of a change in acidity on the leucocyte-solution interface, for the ingestion of the two different particles is differently affected by the same change.

To explain this fact a series of measurements on the particles themselves was undertaken. The most obvious property to investigate from this point of view was the electrical charge on the particle which is known to vary characteristically with the hydrogen ion concentration. On account of the ready agglutination of the carbon particles by acid it was thought easier to measure the charge by making membranes of the carbon and observing the electrical endosmosis through

the membrane. A rapid and simple method was devised which was accurate enough for this purpose.

A glass tube of about 6 mm. inside diameter open at both ends was fixed by means of a rubber stopper upright in a centrifuge tube and just off the bottom. A thick suspension of the particles to be investigated was then sedimented out in the centrifuge tube so that the open end of the small central tube was completely embedded in the sediment. A difference of potential of 50 to 90 volts was then established between the inside and the outside of the central tube by means of small platinum electrodes dipping in the supernatant liquid. The movement of the meniscus in the central tube was then observed before and after reversing the current and the average of the two readings was taken as a measure of the charge on the particle. A useful modification is to have the level of solution in the central tube higher than that outside. If the membrane leaks, the difference in the rates of filtration before and after reversal of the current gives a measure of the charge. In experiments lasting only 5 minutes there is no serious danger of products of electrolysis in the supernatant liquid affecting the charge on the particles embedded in the sediment. A more serious error, inherent in any membrane method, is the change of the fluid inside the membrane due to solubility of the particles or absorbed electrolytes.

By this method a considerable number of measurements were made of the charges on quartz and various types of carbon in solutions of varying pH. It is unnecessary to give these in detail since they add nothing essential to the observations of Gyemant (6) by a more difficult method which came to our attention afterwards. They showed, however, an isoelectric point for carbon at about  $C_H 10^{-5}$  and a negative charge for quartz which diminished toward zero in normal or 0.1 normal HCl but never reached an isoelectric point. In the presence of acacia carbon behaved like quartz, there being no isoelectric point. Likewise it was found, as already stated, that in the presence of acacia quartz was never preferred to carbon even in acid solutions. It is probable, however, that no difference between the endosmotic charges of quartz and carbon existed in the actual phagocytosis experiments, for Davis has found in experiments from this laboratory (unpublished) that in the presence of minute amounts of serum, glass particles assume the isoelectric point of the serum proteins. This he established by the use of the method described above as well as by cataphoresis. If it is true that quartz becomes thus coated with the serum proteins, one would expect it to be even more true of carbon with its high adsorptive capacity. The measurements therefore make

it probable that both carbon and quartz have isoelectric points corresponding to those of the serum proteins, under the conditions of the phagocytosis experiments, and it becomes difficult to explain the observed differences in their behavior toward leucocytes by means of their endosmotic charges. Moreover, even if carbon and quartz did not have the same isoelectric points, one would expect that carbon would be taken up relatively better in acid solutions as it approaches its isoelectric point where the repulsion between it and the similarly charged leucocyte becomes zero, whereas experiment tells us that the reverse is true.

The charge on the manganese dioxide particles used for phagocytosis has been investigated by this same method and found to behave much like quartz having a negative charge in all solutions of HCl. Here again these endosmotic potentials offer no explanation of the differences detected by the leucocytes.

Freundlich (7) has pointed out in a recent series of papers that two different potentials can be measured across interfaces such as that between glass and water. These are (*a*) the endosmotic potential which he interprets as existing between the body of the solution and the fixed layer of liquid which remains adhering to the solid wall, and (*b*) the Nernst electrode potential which he believes to be the potential between the solid itself and the body of the solution. Whether this interpretation of the facts be valid or not, it seems not impossible that these electrode potentials might be concerned in phagocytosis instead of the endosmotic potentials. The impossibility of measuring the electrode potential of a leucocyte makes it difficult to test this hypothesis completely. Both carbon and glass, however, show increasing positive potentials with increasing hydrogen ion concentration so that there is no obvious qualitative difference between them which might explain the behavior of leucocytes in ingesting them.

A carbon electrode was prepared from the same material from which the suspensions were made by fixing the largest piece available, about the size of a pea, in a pair of tweezers. The whole electrode was then paraffined except for the tip of the carbon which was in contact with the solution, the tweezers being connected to the potentiometer. The potential of the carbon electrode was then measured against the saturated calomel electrode in solutions of varying pH.

Such carbon electrodes when first immersed in the solution have a potential of about 0.1 volt higher than the saturated calomel electrode (*i. e.* when measured

against an H electrode they give 0.1 volt higher p.d. than the saturated calomel electrode). In acid solutions this p.d. increases quite rapidly with time and after a day or two comes to approximate equilibrium at about 0.3 or 0.4 volt. In alkaline solutions the p. d. falls with time to 0 or  $-0.05$  volt. Using buffered phosphate solutions between pH 6 and pH 10 it was found that there was no change with time in solutions between pH 8 and pH 9 approximately. Distilled water shaken up with carbon suspensions also gives pH readings between these limits. It would appear therefore that carbon is in equilibrium with slightly alkaline solutions. Pieces of a carbon rheostat used as electrodes gave confirmatory results.

Using Palmaer's (8) value of 0.5732 for the absolute potential of the 0.1 normal calomel electrode it may easily be calculated that the absolute potential of the carbon electrode at pH 5, *i.e.* at its isoelectric point, is between 0.6 and 0.7 volt. The difference between the endosmotic and the electrode potentials thus becomes evident.

The potential of the glass electrode was first measured by Haber and Klemensiewicz (9). Their findings were confirmed by Freundlich and more recently by Dr. W. T. Bovie and Mr. W. S. Hughes (unpublished), who have plotted the potential against the hydrogen ion concentration and find the charge increasingly positive with increasing acidity.

In conclusion it must be admitted that these experiments have not led to any explanation of the relative rates of ingestion of carbon and quartz particles by leucocytes which can be proved experimentally. The search for such an explanation was perhaps manifestly hopeless from the start in the present state of our knowledge of the physical chemistry of solid surfaces. Yet in comparison with any phagocytic system involving bacteria, where previous explanations have been attempted, this problem was extraordinarily simple. One great simplification is that there are two kinds of particles, each one serving as a control for the other and eliminating to a large extent the physiological variations in the leucocyte itself with change of pH. If an explanation could be found it seems certain that we should have made a substantial advance not only in the study of the mechanism of phagocytosis but in the study of the fundamental nature of living protoplasm. Progress in this direction is certainly to be made by the intensive study of comparatively simple cases such as that here discussed.

#### SUMMARY.

1. Leucocytes ingest quartz particles more readily than carbon in acid solutions, and carbon more readily than quartz in alkaline solutions.

2. In the presence of acacia carbon is always preferred to quartz even in acid solutions.

3. Manganese dioxide particles are ingested by leucocytes with extraordinary rapidity as compared with manganese silicate or quartz.

4. Leucocytes are not attracted toward carbon or quartz particles but manganese dioxide exerts a distinct attraction for them.

5. Spores of *Penicillium* are ingested more readily than quartz.

6. Very small quartz particles, 1 micron in diameter, are not ingested as readily as larger particles of the same material. This result being contrary to the predictions of surface tension indicates that some other factor is involved in the ingestion of these small particles.

7. Measurements of the carbon electrode potentials and the cathoretic charges on the particles have failed to supply an explanation for the varying relative rates of ingestion of carbon and quartz with varying hydrogen ion concentration.

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