THE PHAGOCYTOSIS OF SOLID PARTICLES.

III. Carbon and Quartz.

By WALLACE O. FENN.

(*From the Laboratory of Applied Physiology, Harvard Medical School, Boston.*)

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It has been shown by Haldane (1) and by Mavrogordato (2) that silicious dust when inhaled tends to remain in the lungs, causing phthisis. Coal dust, on the other hand, tends to move out of the lungs and is, therefore, harmless. The different behavior of silicious and carbonaceous dusts in the lungs is also the cause of the abnormally high mortality from tuberculosis among silicious miners and the abnormally low mortality among coal miners (3). Without going into a discussion of the mechanism of dust removal from the lungs, it is sufficient to state that the first step appears to be always the ingestion of the dust particles by phagocytic cells in the alveoli. It seemed probable, therefore, that a study of the phagocytosis of carbonaceous and silicious particles would show that the former are ingested more readily than the latter, in agreement with the clinical facts, and might throw some light on the cause of this difference. This was found to be true.

It became at once evident, in undertaking a comparison between the rates of phagocytosis of carbon and quartz particles, that the usual method of incubating them together with the leucocytes in a common suspension might yield nothing more than a comparison of the relative chances of collision of the two kinds of particles with the leucocytes. It has, in fact, been shown in two preceding papers on the phagocytosis of quartz (4) and carbon (5) particles that the more rapid ingestion of large particles by leucocytes compared to small ones can be accounted for quantitatively by the fact that a large particle moves faster when stirred up in a suspension with leucocytes, and therefore comes into collision with more cells in a given time. In this paper the same methods will be applied to a study of the comparative rates of phagocytosis of carbon and quartz particles to determine whether or not carbon is ingested by leucocytes more readily than would be predicted from the calculated chances of collision.

The method of calculating R, the chance of collision, and the experimental procedure are described in the earlier papers (4, 5). It must

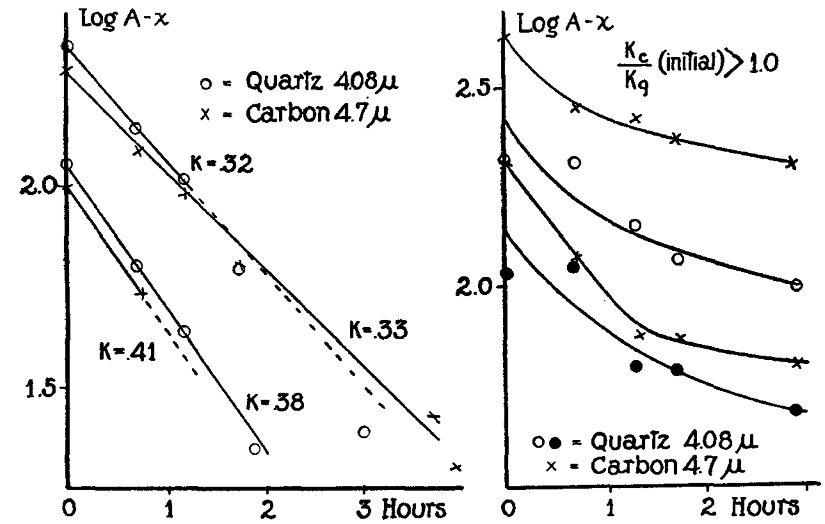

FIG. 1. FIG. 2.

FIG. 1. Ordinates represent logarithms of the number of particles outside the leucocytes in 0.02 c. mm. of the suspension. Curves show that the actual rates of ingestion of the quartz and carbon were nearly equal. Quartz, being, heavier, collides more often with the cells. The corrected ratio, $\frac{K \text{ carbon}}{K \text{ quartz}} = \frac{1.06}{0.36} = 3$, shows that carbon is taken up 3 times as readily as quartz. Two concentrations of both quartz and carbon were used, the higher concentration being twice the lower. See Table II.

FIG. 2. Ordinates and abscissae as in Fig. 1. Curves showing the rates of ingestion of quartz and carbon particles. If the experimental points for quartz are accurate the initial $K = 0$. Assuming that the first two points on the quartz curves are erroneous, as seems most probable, the initial ratio, $\frac{K \text{ carbon}}{K \text{ quartz}}$, is seen to be at least > 1. Even interpreting the curves thus in favor of the quartz, the corrected ratio is 2.8, showing that the carbon is taken up at least 2.8 times as readily. Two concentrations (in the proportion of 1 to 2) of both quartz and carbon were used.

suffice here to state that R is proportional to the difference between the velocities of leucocytes and particles, $Vp - Vc$, and to the square of the sum of the diameters of cells and particles, $(C + P)^2$. The latter factor allows for the fact that a larger particle makes a larger target. The rate of phagocytosis is measured by K of a monomolecular reaction and is equal to the slope of the straight line obtained by plotting against time the logarithms of the *number of particles not yet ingested*, as determined by frequent counts on an ordinary hemocytometer. The cell suspensions were obtained from peritoneal exudates in rats. The phagocytic mixtures were rotated slowly on a revolving drum during incubation to prevent settling out of the cells or particles.

TABLE I.

Calculated Chances of Collision, R.

Nature of Particle.	Diameter.	*R.*
	μ	
Carbon	3.2	144
	4.7	248
Quartz	2.4	130
	4.08	697
	4.63	1299

The results of one such experiment are plotted in Fig. 1. Since ordinates represent the logarithms of the number of particles counted at time, t (abscissæ), *outside* the leucocytes, the steeper the slope of the curve the more rapid the phagocytosis. A straight line in this figure, *i.e.*, a constant K, indicates that the same percentage of the number of collisions occurring between cells and particles is resulting in ingestion throughout the experiment. As has previously been pointed out, it is only the *initial slope*, in cases where K is *not* constant, which is expected to agree with the theoretical predictions. In Fig. 1, the initial K's of the two carbon experiments are 0.33 and 0.41, and of the quartz, 0.38 and 0.32 the averages being 0.37 and 0.35 respectively; *i.e.*, carbon is taken up $\frac{0.37}{0.35}$ or 1.06 times as fast as the quartz. Reference to Table I, however, in which are tabulated the chances of collision of the three quartz and two carbon suspensions used in

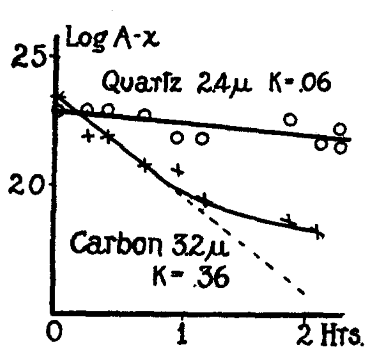

FIG. 3.

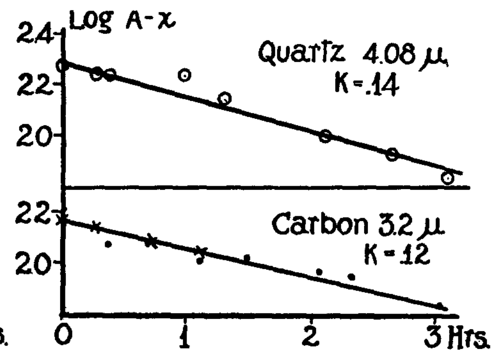

FIG. 4.

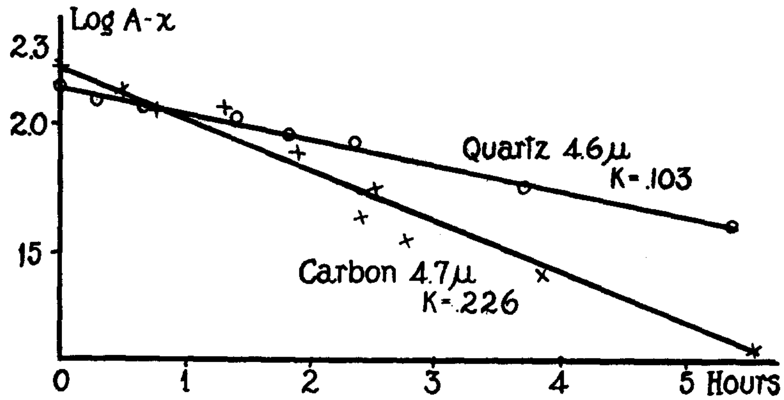

FIG. 5.

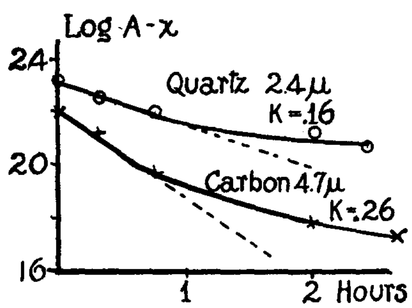

FIG. 6.

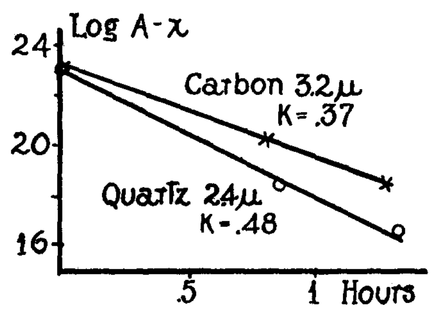

FIG. 7.

FIG. 3–7. Curves showing comparisons of rates of ingestion of quartz and carbon particles of various sizes by the suspension method. The logarithms of the number of particles in 0.02 c. mm. not yet ingested are plotted as ordinates against time in hours as abscissae. See Table II for the results of the comparisons when corrected for the chances of collision.

these experiments, shows that these carbon particles (4.7μ) should collide with the cells only $\frac{248}{697}$ or 0.36 times as often as the quartz (4.08μ). It may be concluded, therefore, that carbon is ingested $\frac{1.06}{0.36}$ or 3 times as readily as quartz.

Results of all the other available comparisons between quartz and carbon are plotted in Figs. 2 to 8 and tabulated in Table II. In each experiment the initial K only was used in comparison unless K was constant throughout. The last column in Table II shows the number of collisions necessary for ingestion of one quartz particle if every collision with a carbon particle results in ingestion; *i.e.*, the

TABLE II.

Comparison of Rates of Phagocytosis of Carbon and Quartz by "Suspension" Method.

Fig.	Diameter of particles.		Initial K.		Ratio* $\frac{K \text{ carbon}}{K \text{ quartz}}$.		
	Carbon.	Quartz.	Carbon.	Quartz.	Observed.	Calculated.	Corrected.
1	4.7	4.08	0.37	0.35	1.07	0.36	3.0
2	4.7	4.08	0.03	0.03−	1.0+	0.36	2.8+
3	3.2	2.4	0.36	0.06	6.0	1.1	5.4
4	3.2	4.08	0.12	0.14	0.87	0.21	4.1
5	4.7	4.6	0.226	0.103	2.2	0.19	11.5
6	4.7	2.4	0.26	0.16	1.6	1.9	0.8
7	3.2	2.4	0.37	0.48	0.77	1.1	0.7
							Average = 4.0

* The calculated ratios are the ratios of the chances of collision for the sizes of particles used as given in Table I. The corrected ratio is the quotient obtained by dividing the observed ratio by the calculated ratio.

value of the ratio $\frac{K \text{ carbon}}{K \text{ quartz}}$. In five out of seven experiments carbon is taken up 2.8 to 11.5 times as readily as quartz. In two instances only, due to some uncontrolled factor, quartz is taken up slightly more readily than carbon. The average of all shows that carbon is taken up 4 times as readily as quartz.

It should be observed that it is assumed that a large particle is taken up as easily as a small particle if the number of collisions are equal. That this is true, at least within limits of error of these experiments, was shown in the two previous papers (4, 5). It would appear from these figures that there is great variation in different experiments in the relative rates of ingestion of carbon and quartz, the ratio varying from 0.7 to 11.5. That this ratio may vary with the condition of the cells, even in the same experiment, will be shown later by another method.

Another contributing explanation is perhaps the agglutination of the carbon. In some experiments the carbon has been observed to be more stable than in others. This factor could always be controlled, however, by observation of the samples which were removed from the incubation mixtures at intervals for counting. Whenever any considerable agglutination was observed the experiment was discarded. With quartz, however, there was seldom any agglutination, and this is the most characteristic difference between the behavior of the two kinds of particles. Thus quartz can be thrown down repeatedly by centrifugalization, and when resuspended in water the particles are perfectly discrete. This cannot be done with carbon unless it is stabilized with acacia. This suggests that the cause of the more rapid agglutination may also be the cause of the greater speed of phagocytosis. One might suppose that if carbon agglutinates with carbon readily, it will also agglutinate readily with cells, whereupon ingestion promptly occurs.

Comparison of Quartz and Carbon by the "Film" Method.

Before proceeding to a discussion of this hypothesis, however, some experiments will be reported in which the rates of ingestion of carbon and quartz were compared by another method, which will be referred to as the "film" method. By this method, unlike the "suspension" method described previously, phagocytosis was allowed to proceed in a thin film between the slide and cover slip. Both cells and particles immediately settled out. The leucocytes crept about on the slide and ingested the particles.

A cover-slip was supported at its center and four corners by fragments of another fairly thick cover-slip. While held down with a small weight it was sealed at the corners with collodion. The weight prevented the cover-slip from floating up on the collodion, and made the distance from slide to cover uniform throughout. A thick suspension of cells with equal numbers of carbon and quartz particles was then allowed to run under the cover-slip and the edges were sealed with paraffin to prevent evaporation. The slide was at once placed on a warm stage at 37°C. and frequent counts were made of the number of free particles of quartz and carbon over a given area. The areas were measured by a disc micrometer in an ocular ruled in squares. It was customary to count sixteen such areas for each point.

The advantages of this method are:

1. The chance of collision depends merely upon the size which the particle offers as a target for the cells; i.e., $(C + P)$ which is so small a factor that it is almost negligible.[1]

2. Agglutination of particles is impossible.

3. Both quartz and carbon particles can be incubated together in the same film so that the number of active cells is identical at any one time.

The only disadvantage of the method is that the cells are injured by contact with the glass slide, so that the time curves do not follow the law for a monomolecular reaction as they otherwise would. Thus the cells are observed during the course of the reaction to spread out on the glass, becoming vacuolated and transparent and ceasing their activities.

Phagocytosis with the film method presents an interesting picture, as shown in the photographs (Figs. 8 and 9). Nearly all the cells are in active motion. The activities of one cell were recorded for 3 minutes during which time it refused one quartz particle and one carbon particle, and ingested three carbon particles, nearly reaching a fourth. No evidence was obtained that cells can sense particles from a distance, as reported by Commandon (6) for leucocytes ingesting starch grains, and by Schaeffer (7) for amebæ in proximity to carbon and glass. All the meetings seem to occur purely by chance. Sudden movements of leucocytes for a distance 2 or 3 times their own length have frequently attracted attention, as if the cell had been under

[1] C = diameter of cell, P = diameter of particle. This is merely the target factor from the formula for the chances of collision as used in the suspension method. In this instance, however, the chance of collision is $(C + P)$, not $(C + P)^2$, because motion is confined to two dimensions.

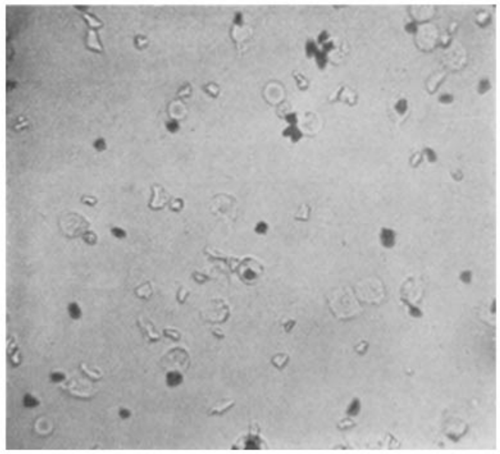

FIG. 8.

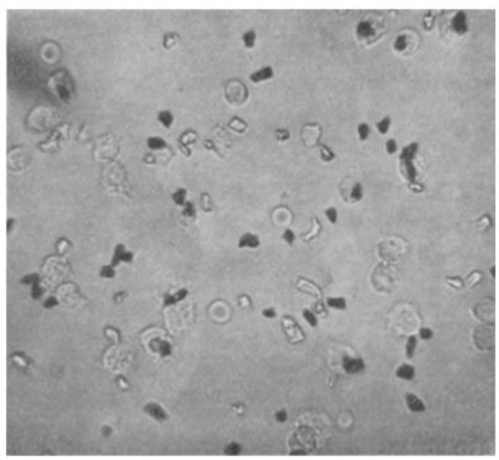

FIG. 9.

FIGS. 8 and 9. Photographs of quantitative comparison of the rates of phagocytosis of quartz and carbon particles by leucocytes of rats. The leucocytes are actively ameboid and many contain carbon particles. None of the available quartz particles, however, has yet been ingested. Attention is directed to the uniformity in the sizes of the quartz and carbon particles.

tension by some long contracting pseudopod[2] which had suddenly succeeded in pulling the cell loose from its contact with the slide. Toward the end of an experiment the cells are usually aggregated in groups, mostly heavily laden with particles. This clumping is also found, apparently to the same extent, in a control preparation from which particles are omitted. Often cells are so full of particles that close inspection is necessary to see protoplasm at all.

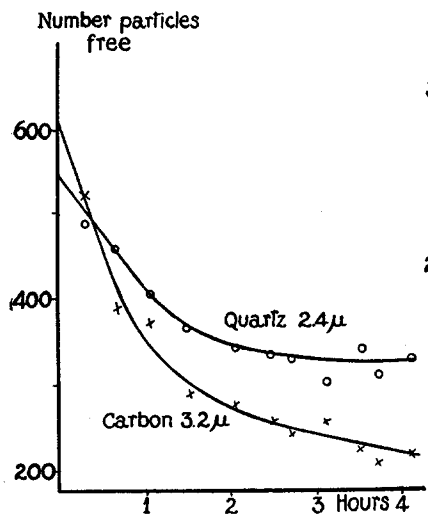

FIG. 10.

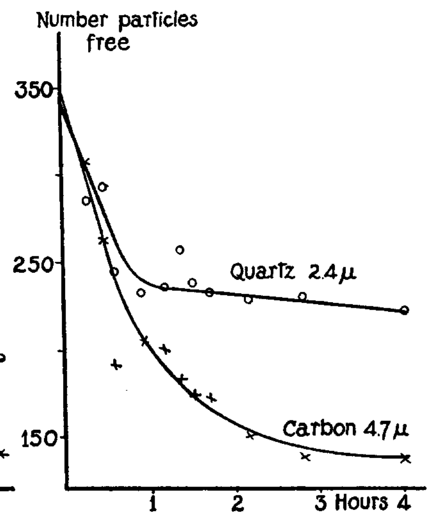

FIG. 11.

FIGS. 10 and 11. Comparison of phagocytosis of carbon and quartz by the film method. The numbers of particles not yet taken up in a given area are plotted as ordinates against time in hours as abscissae. Here no appreciable allowance need be made for the chances of collision, and carbon is obviously ingested more rapidly than quartz. See Table III for further analysis.

The results of two typical experiments of this sort are plotted in Figs. 10 and 11. Ordinates represent the number of particles of quartz or carbon counted over a given area which are still outside the cells. Time is plotted as abscissae. Inspection shows that the carbon is taken up more readily than the quartz. The question is how much more readily. Here the data can be analyzed with more

[2] Such pseudopods have been described by Kite (Kite, G. L., *J. Infect. Dis.*, 1914, xv, 319).

assurance than with the suspension method. From Figs. 10 and 11 the number of particles ingested during each hour of the experiment has been determined graphically. This value divided by the average number of particles present outside the cells during that hour gives the percentage of particles taken up during the hour. These figures are recorded in Table III.

TABLE III.

Comparison of Rates of Ingestion of Quartz and Carbon Particles by "Film" Method.

Hrs.	Particles ingested per hr.		Experimental ratio* = $\frac{\text{per cent } C}{\text{per cent } Q}$.	
	Carbon.	Quartz.		
	per cent	*per cent*		
0–1	54	29	1.9	Experiment 8.
1–2	22	19	1.1	Carbon 3.2 microns
2–3	15	4.5	3.3	Quartz 2.4 microns
3–4	13	1.6	8.1	Theoretical ratio 1.07
0–1	53	35	1.5	Experiment 9.
1–2	27	4.3	6.3	Carbon 4.7 microns
2–3	12	2.6	4.6	Quartz 2.4 microns
3–4	2.2	2.2	1.0	Theoretical ratio 1.20
0–1	57	27	2.2	Experiment 10.
1–2	27	5.9	4.6	Carbon 4.7 microns
2–3	3.5	1.2	2.9	Quartz 4.6 microns
				Theoretical ratio 1.01
0–1	44	15	2.9	Experiment 11.
1–2	22	11	2.0	Carbon 2.7 microns
2–3	10.5	4.9	2.1	Quartz 2.6 microns
				Theoretical ratio 1.01

* The experimental ratios are the ratios of the numbers of particles of carbon and quartz ingested per hour expressed in per cent of the average number present during that hour and $= \frac{\text{per cent Carbon}}{\text{per cent Quartz}}$. Data were obtained graphically from Figs. 10 and 11 for Experiments 8 and 9 and from similar unpublished figures for Experiments 10 and 11. The theoretical ratios are $\frac{R \text{ carbon}}{R \text{ quartz}}$. $R = C + P$ or the chance of collision, where $C = 9\mu$, the diameter of the leucocytes, and $P =$ the diameter of the particle.

The ratio of these values $\left(\frac{\text{per cent Carbon}}{\text{per cent Quartz}}\right)$ for carbon and quartz (last column) is then a measure of the greater speed of ingestion of carbon. Strictly these figures should now be corrected by dividing by the ratio $\frac{(C + P)\text{ carbon}^1}{(C + P)\text{ quartz}}$ which is a measure of the relative chances of collision. Since the value of this factor is small (1.07 and 1.2), this correction has not been applied, but the necessary factor for correction is given for each comparison.

It is a very significant fact that this $\frac{\text{per cent Carbon}}{\text{per cent Quartz}}$ ratio increases after the 1st hour. This means that in comparison to carbon it is relatively harder to ingest quartz toward the end of the experiment than at the beginning. Thus, in Experiment 8 (Fig. 10), if every meeting between a cell and a carbon particle results in ingestion throughout the experiment, we may conclude that at the beginning of the experiment two meetings with quartz are necessary for ingestion, and at the end of the experiment, eight meetings. Eventually, carbon also is refused and the ratio $\frac{\text{per cent Carbon}}{\text{per cent Quartz}}$ necessarily decreases again. This happens in Experiment 9 (Fig. 11). If the phagocytic activity of the cells remained the same throughout an experiment, the percentage of carbon particles ingested during each hour would be constant. The rate of decrease of this percentage may, therefore, be taken as a measure of the rate at which the activity of the cells decreases. Thus, in Experiment 8, the phagocytic activity of the cells after 3 hours is only $\frac{13}{54}$ or 24 per cent of the original. This may be due to contact with the glass, agglutination of cells, decreased capacity of cells, or other factors.

Data from Experiments 10 and 11 are also included in Table IV. The former is the only exception to the rule that the ratio $\frac{\text{per cent Carbon}}{\text{per cent Quartz}}$ increases after the 1st hour. In this experiment both quartz and carbon (particularly quartz) were taken up more slowly even during the 1st hour than in the other three experiments, and it therefore seems probable that the decrease in the ratio in

Experiment 11 may correspond to the decrease *following* the increase in Experiments 9 and 10.

The comparative rates of ingestion of carbon and quartz particles depend, therefore, upon the condition of the cells. Sometimes preparations of carbon and quartz have been made in which there was practically no ingestion of quartz. This may be seen in the photographs, Figs. 8 and 9, where no quartz is seen inside the cells though there is plenty available.

As far as the writer is aware the experiments in this paper constitute the first quantitative comparison of the effects of different kinds of solid substances on living cells. Even qualitative comparisons are limited. Aside from the effects of carbonaceous and silicious dusts in the lungs the only recorded observations seem to be the mere statement of Commandon (6) that starch is ingested by leucocytes more readily than carbon. Schaeffer (7) endeavored to compare the ingestion of carbon and glass particles by amebae but neither was ingested.

Phagocytosis of Carbon and Quartz by Sponge Cells

This experiment was done at the Marine Biological Laboratory, Woods Hole, with a small marine sponge, *Grantia*. The sponge was squeezed into a test-tube, and a thick suspension in sea water of active cells was obtained. 8 parts of this suspension were mixed with 4 parts of sea water concentrated to twice its normal strength by boiling, 1 part of 0.2 M borate mixture (pH 7.5), 1 part 10 per cent acacia neutralized with sodium hydroxide, and 2 parts of a suspension of 3.2 micron carbon particles and 2.4 micron quartz particles in distilled water. The result is a suspension of cells, quartz, and carbon in normal sea water plus 0.6 per cent acacia to stabilize the carbon and quartz, the alkalinity being approximately the same as that of normal sea water.

A sample of this mixture was allowed to run under a cover-slip supported as for the film method and sealed with paraffin. The cells were slowly ameboid and ingested the particles rather sluggishly compared to leucocytes. After 5 hours at room temperature, counts were made of the number of quartz and carbon particles found inside the cells. Only solitary cells were included in the count as it would be easy to overlook the colorless quartz particles in the aggregated cells. All the solitary cells in each field were examined. In all, 9 quartz particles and 59 carbon particles were found ingested by some 200 to 300 cells which were examined.

Since there were approximately twice as many carbon particles as quartz present, it may be concluded that carbon is ingested by sponge cells $\frac{59}{9 \times 2}$ or 3 times as readily as quartz. This agrees well with the results with leucocytes. The sponge cells were not considered sufficiently phagocytic to justify more quantitative experiments.

DISCUSSION

Whatever hypothesis is adopted to explain the more rapid ingestion of carbon, it must be concluded that there is for some reason greater attraction between the cell substance and the carbon than between the cell substance and the quartz. In view of the high adsorptive capacity of carbon this is not surprising, nor is this difference between different kinds of solid particles without its parallel in inorganic systems. Similar examples of selective "wetting" of solid particles are found in the flotation processes for the separation of different kinds of ores (8), which depend upon the fact that, in general, particles of the heavy metals have a greater affinity for oil and air phases than for water, and are thus floated to the surface while the worthless gangue particles such as silicates remain in the water and sink.

Rhumbler (9) remarks that the rhizopod *Euglypha,* in forming its shell of small particles, is able to distinguish between different materials. In imitating this he rubbed up coal and quartz particles in various oils and sprayed them into 70 per cent alcohol. In such instances the coal remains on the inside of the oil drops and the quartz collects in the surface. This observation being of considerable interest for these experiments has been repeated quantitatively with oil drops in 70 per cent alcohol and chloroform drops in water. In all cases the carbon collects in the interface between the phases more rapidly than the quartz.

The results with chloroform are plotted in Fig. 12. Similar results are obtained with cottonseed oil drops in 70 per cent alcohol or water. Moreover, if a mixture of equal numbers of quartz and carbon particles in water is warmed up with phenol above the critical solution tempera-

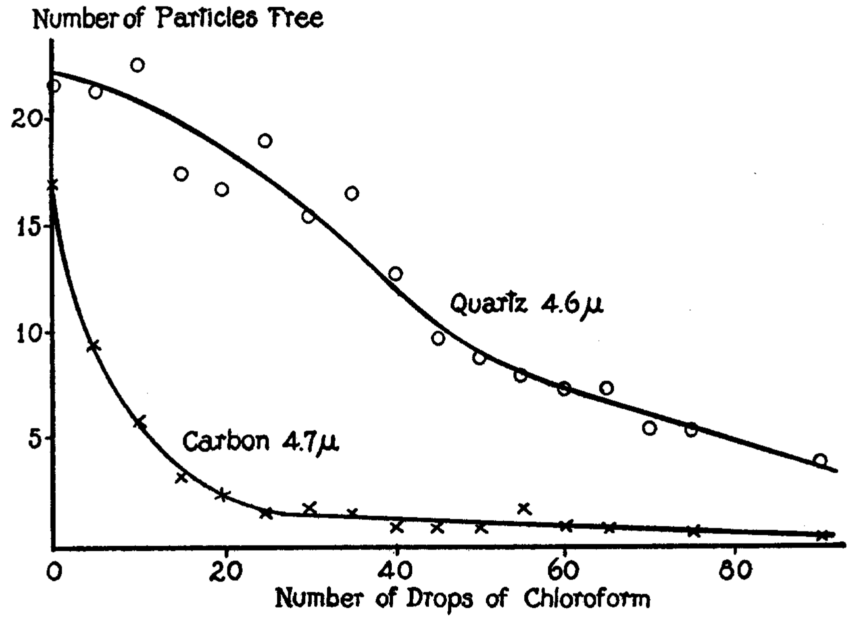

FIG. 12. Analogy to selective phagocytosis of carbon in a chloroform-water system. To 2 cc. of a suspension of carbon particles 4.7μ in diameter and quartz particles 4.6μ in diameter, chloroform is added 5 drops at a time. After each addition the mixture is shaken vigorously, allowed to stand a few minutes until the chloroform drops have settled to the bottom, and counts made of the number of carbon and quartz particles left. These figures are plotted as ordinates against the number of chloroform drops added as abscissae. Both particles accumulate in the chloroform-water surfaces, the carbon, however, much more rapidly than the quartz.

ture, on subsequently cooling the mixture, the majority of the carbon is found in the phenol phase and the majority of the quartz in the water phase, as shown by actual counts of the number of particles of each in the two phases, as follows:

	In phenol phase.	In water phase.
Carbon	44 60	5 20
Quartz	5 2	40 9

Extensive qualitative experiments of a similar nature have been carried out by Reinders (10) and by Hofmann (11). Both agree that particles of carbon are among those most easily adhering to droplets of oil, chloroform ether, etc., but neither used quartz particles for comparison.

The theoretical distribution of solid particles between two liquid phases according to the surface tension relations has been excellently summarized by Thompson (12). In a general way it may be said that in a carbon-oil-water system the lower the potential energy of a carbon-oil surface, the greater the chance of the carbon being in the oil, and *vice versa*. By collecting in the interface the oil-water surface is reduced. Therefore, the higher the oil-water tension, other quantities being equal, the greater the chance of the carbon being in the boundary. By collecting in the interface the carbon decreases the potential energy of the oil-water surface. This is presumably the reason for the emulsifying power of lampblack on water-kerosene systems reported by Moore (13).

Although the more rapid ingestion of carbon by leucocytes could not be predicted from the selective wetting of carbon by oil, phenol, and chloroform drops[3] the same principles of surface tension which explain the inorganic phenomenon can be applied to an explanation of the biological fact. It may, therefore, be argued that carbon would be taken up by leucocytes more rapidly than quartz, if the potential energy of a carbon-serum surface were greater than the potential

[3] The fallacy of predicting a surface tension of a particle in one medium from its surface tension in another is shown convincingly by the behavior of particles of manganese dioxide and manganese silicate. The former are taken up less rapidly than the latter by drops of chloroform and paraffin oil, but about twice as rapidly by sponge cells and in some experiments at least 20 times as rapidly by rat leucocytes.

energy of a quartz-serum surface, *other things being equal.* It is interesting that this is exactly the condition which should also cause more rapid clumping of the carbon than the quartz, since clumping involves a decrease of surface. It has already been mentioned that this difference in stability is the one outstanding distinction between quartz and carbon.[4]

According to Bredig (14) a low surface tension between a particle and a liquid should correspond to a high electric charge, thus reconciling electrical and surface tension theories of coagulation. If this theory is correct quartz should carry a higher charge than carbon, and the available evidence in the literature indicates that this is so. Thus, Whitney and Blake (15) measured the charge on quartz particles by kataphoresis and found a rather high negative charge of 0.042 volts. Rona and Michaelis (16) found that H and OH ions were equally adsorbed by carbon, and concluded, in consequence, that carbon must carry no electric charge. This opinion was, moreover, corroborated by a statement of Freundlich's (which they quote in a footnote) to the effect that this lack of a charge on carbon accounts for difficulty which he had experienced in trying to measure the kataphoresis of carbon. Rona and Gyorgy (17) were led to the same postulate concerning carbon by measurements of the effect of non-electrolytes on the speed of settling.

Some attempts were made to confirm this difference between the electric charges on quartz and carbon qualitatively by measuring the stability of the suspensions in various concentrations of sodium hydroxide and hydrochloric acid. Uniform suspensions, 2 to 3 microns in diameter, of quartz and carbon were used, and the degree of coagulation in the different solutions was determined by counts of the total number of particles, aggregate and single, in the case of carbon, and of the number of clumps in the case of quartz. The coagulation of quartz was so slight even in the most effective concentration of hydrochloric acid that there was scarcely a measurable decrease in the total number of particles. Both suspensions showed greatest stability in neutral and alkaline solutions and both showed an

[4] In agreement with this hypothesis is the fact that manganese dioxide is ingested by leucocytes with extraordinary rapidity compared to manganese silicate, and is also much less stable in suspension.

optimum coagulation between 0.0003 and 0.005 M hydrochloric acid (probably at 0.001 in the case of quartz). The results, therefore, neither prove nor disprove the hypothesis although they do indicate that carbon carries a negative charge contrary to the evidence from the literature. Also, the greater stability of quartz as compared to carbon in all concentrations of hydrochloric acid appears to prove that stability (and hence probably phagocytosis) is conditioned by some factor besides the electric charge, presumably the surface tension.

There are many valid objections (McClendón (18), Hyman (19), and Loeb (20)) to the explanation of ameboid movement by surface tension changes on account of the more or less rigid ectoplasm of the cell. In so far as phagocytosis is limited by ameboid movement, these objections also apply to the explanation of ingestion of particles by surface tension. The ingestion of quartz in these experiments, however, is not limited by the ameboid movement of the leucocytes which is sufficiently vigorous to cause ingestion of carbon at a more rapid rate. It is, therefore, a question of molecular attractions between particle and cell, and these are best expressed in terms of surface tension.

The analogy between this hypothesis and the sometimes parallel effects of opsonins and agglutinins has been suggested to the writer by Dr. W. B. Cannon. Thus, Bull (21) in a series of papers has brought out the fact that bacteria injected into the circulation are removed more rapidly, apparently by phagocytosis, when agglutinated by immune serum. Zinsser (22) quotes Ottenberg as authority for the statement that phagocytosis of foreign red cells in the circulation after blood transfusion occurs only when the patient's serum has an agglutinative action on the donor's cells. Likewise, Ledingham (23) has pointed out "that the opsonin-bacteria like the agglutinin-bacteria are probably in a more precipitable condition than non-sensitized bacteria. They are, in fact, as I have frequently observed, in a condition of extremely fine aggregation, and if the sensitizing fluid has marked agglutinating powers the sensitized organisms are frankly clumped." From this evidence it appears that bacteria as well as solid particles are more readily ingested when easily agglutinated.

SUMMARY

1. The rates of ingestion of quartz and carbon particles by leucocytes, when both are in suspension in serum, was compared with the availability of the two particles as predicted from the calculated chances of collision with the leucocytes, and it was shown that carbon is ingested about 4 times as readily as quartz.

2. The greater ease of ingestion of carbon was verified by a new method of measuring phagocytosis, described as the film method in which the cells ingest particles as they creep about on a slide.

3. The relative rates of ingestion of carbon and quartz depend upon the condition of the cells, the difference increasing as the phagocytic activity of the cells decreases.

4. Sponge cells also ingest carbon about 3 times as readily as quartz.

5. The hypothesis is suggested that the cause of the more rapid ingestion of carbon may be identical with the cause of the greater instability of the carbon suspensions.

6. An inorganic analogy to this selective phagocytic action is offered.

7. The application to opsonins and agglutinins is discussed.

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