A CONTRIBUTION TO THE THEORY OF PHAGOCYTOSIS.

By ERIC PONDER.

(From the Department of Physiology, University of Edinburgh, Edinburgh, Scotland.)

(Accepted for publication, May 4, 1926.)

In endeavours to reduce the phenomenon of phagocytosis to a physical basis, account has been taken of three principal factors. The first of these relates to the chance contact of cell and particle, which has been admirably dealt with by McKendrick from a theoretical point of view (1), and by Fenn from an experimental aspect (2). The second factor controlling the ingestion is that of the viscosity of the cell; the importance of this has been much emphasised by Loeb (3), although theoretical investigations are still to be made. The third factor is the nature of the surface conditions between cell and surrounding fluid, particle and surrounding fluid, and cell and particle, the consideration of which has led to information of much value, but unfortunately to a certain amount of controversy, the conclusions of Rhumbler (4) having been amplified by Tait (5), whose statement of the problem has been criticised by Fenn (6) and by Mudd and Mudd (7).

In this paper, we shall venture to add a note relating to this controversy, and thereafter to consider in outline a fourth factor, hitherto, so far as we are aware, unmentioned—the influence of electric charge of cell and particle on the phagocytosis.

1. The Surface Forces.

If we have a system composed of cell, rigid particle, and surrounding fluid, in which there are three interfacial tensions—that between the particle and the fluid, $S_1$, that between the cell and the fluid, $S_2$, and that between the particle and the cell, $S_{12}$—then when the system is in equilibrium, there must be a contact angle $\theta$ between cell and particle, which satisfies

$$\cos \theta = \frac{S_1 - S_{12}}{S_2}.$$
If there is equilibrium at the contact angle, then, from the nature of the action of surface forces, the whole system must be at equilibrium, and if so, the free energy is at a minimum. Further, $\theta$ must be single-valued, and the expression does not refer only to spheres, an important point, for phagocyted particles are not in general spheres.

The limits of the left-hand member of the equation are $(-1)$ and $(+1)$. The limits of the right-hand member are $(-\infty)$ and $(+\infty)$. There will accordingly be equilibrium at partial ingestion with a real value of $\theta$ when the value of the right-hand member falls within the limits of the left-hand member; if the value falls outside these limits, there will be no real angle of contact, and incomplete ingestion cannot satisfy the equilibrium. The condition for spreading of the cell on a rigid surface is the same as that for ingestion. We have $\cos \theta = \frac{(S_1 - S_2)}{S_2}$, and if the value of the right-hand member gives a real value of $\theta$, to do which it must lie between $(-1)$ and $(+1)$, incomplete spreading will satisfy the equilibrium conditions; if the value falls outside these limits, either no spreading at all, or spreading to infinity, will result. This follows simply from the fundamental expression when the particle is rigid and when the surface upon which spreading takes place is rigid; their curvatures, moreover, are not involved; if we have to treat the ingestion of a non-rigid particle, or spreading on a non-rigid surface, there is a little more difficulty. These cases do not concern us.

There are thus five possibilities. The value of $(S_1 - S_2)/S_2$ may be

(a) less than $(-1)$. This condition is not mentioned by Tait or by Fenn; it is possible, and the particle would flow on the cell. In the case of a rigid particle, no ingestion would result.

(b) equal to $(-1)$. The particle will not be ingested, but will be in equilibrium at the cell surface.

(c) greater than $(-1)$ and less than $(+1)$. In this case, $\theta$ has a real value, and there will be equilibrium at incomplete ingestion. Tait ignores this case, for which Fenn rightly criticises him. Fenn has shown by an admirable computation of a numerical example that such a condition results in minimum free energy at incomplete ingestion of the particle; we confess, however, without in any way wishing to underrate this excellent computation, that the result appears to us to
follow directly from the fundamental equation, and to require no laborious proof. Apart from this, there is the question as to the frequency of the occurrence of this condition in actual practice. For pure liquids, for example, it never arises, and whether it is of frequent occurrence in systems such as we are considering remains to be shown.

Fenn's appeal to experiment, in pointing out that incomplete spreading, which is associated with the same conditions as incomplete ingestion, does in fact occur and can be easily recognised, is not sufficient to settle the question, as incomplete spreading may be brought about by the operation of factors unconsidered in these equations. An apparent equilibrium may result, for instance, because the time allowed for spreading is not indefinitely great. It may thus well be that Tait has ignored a condition which does not occur in practice, or which is rare; had he been dealing with the engulfing of a drop of a pure liquid by another drop, we take it that he would have fallen into the same error, but he would have been, in fact, correct nevertheless, for the case omitted does not occur.

\(d\) equal to \((+1)\). This is a special case of \(e\).

\(e\) greater than \((+1)\). This is Tait's condition for complete ingestion, which is correct so far as it goes, although we note that Tait derives it by putting \(\theta = 0\). This would give \((d)\); \(e\) is given, not by \(\theta = 0\), but by unreal values of \(\theta\), associated with positive impossible values of \(\cos \theta\). This is the common, indeed invariable, case with pure liquids, and implies both complete ingestion of the particle by the cell, and also spreading to infinity by the cell, as Fenn points out.

Fenn's statement of the case is therefore perfectly correct, and his criticism of Tait justifiable. Tait's statement is correct as far as it goes, but omits all cases except \(d\) and \(e\). We add this note for two reasons, apart from recording our agreement with Fenn. First, we think it should be recognised that because case \(e\) is a mathematical possibility, it is not necessarily a common occurrence. Secondly, we feel that the conditions for spreading, incomplete ingestion, complete ingestion, etc., follow somewhat more simply from the initial conditions than has been indicated.
2. The Electrical Forces.

It is generally admitted that in many suspending fluids both the cells and the particles which undergo ingestion have a like charge. We have to consider how this charge affects phagocytosis.

The charge on a particle is generally looked upon as being due to a double layer at the particle boundary. The origin of the double layer is a matter of some doubt, but we propose to take it to be produced as follows. Owing to molecular configuration, the surface of the particle presents a series of point charges of one sign, say positive. Oriented by these is an equivalent number of charges of opposite sign at the surface of the surrounding fluid, while a second layer of positive charges, deeper in the surface of the fluid, surround this first layer. It will be understood that the particle is really surrounded by a kind of atmosphere made up of many layers, but that this division into two only is the result of a species of summation in the inner and outer parts of the atmosphere. The whole system is in kinetic equilibrium, and in the layers at least, if not on the surface of the particle, redistribution of charge is possible.

For the purposes of this problem, although we should hesitate to extend the idea in its simple form to other cases, the particle may be regarded as a solid sphere, bearing a charge, which may or may not be distributable, and surrounded by a spherical shell, in which distribution is possible. The charges on the surface of the sphere are in equilibrium with those on the inner surface of the shell; the charges on the outside of the shell are in equilibrium with all external space. Moreover, since these equilibria exist, the charge on the particle together with that on the inside of the shell may be removed entirely from the system, and do not affect in any way the distribution on the exterior of the shell, which is influenced only by the presence of neighbouring conductors in external space.

If the particle be regarded as such a system, then the cell bearing a like charge will be a similar system, and the influence of the one on the other will be on the distribution of charge on the external surface of the shells, and will in no way involve the internal surfaces or the surfaces of cell or particle, which may be regarded as being removed from the system.
Suppose a shell $B$, corresponding to the particle of radius $b$, is very small compared with $a$, the radius of a shell $A$, representing the cell. Let $A$ have potential $V$ and charge $E$, while $B$ has charge $e$. If the centre of $A$ is $O$, and that of $B$ is $P$, the effect of $e$ on the distribution on $A$ may be taken as being given very nearly by the single image at $P'$. Further, the induced density on $B$ due to the distribution on $A$ is very nearly that due to a charge $kV/a$ at $O$, and $-ea/c$ at $P'$, $c$ being the distance from $O$ to $P$. These vary with the cube of the distance of an element of $B$ from $O$ or $P'$, and so the distribution on $B$ may be taken as uniform.

The charge and potential on $B$ are thus

$$e \left( \frac{1}{b} - \frac{a}{c^2 - a^2} \right) k + V \frac{a}{c}$$

and that on $A$

$$E = kVa - ea/c,$$

$V$.

The energy of the whole system is thus

$$E = \frac{1}{2k} \left( \frac{E}{b} - \frac{a}{c^2 - a^2} \right) + \frac{V^2 ka}{2},$$

$$= \frac{1}{2k} \left( \frac{E}{b} + \frac{2Ec}{c} \right) + \frac{1}{2k} \left( \frac{1}{b} - \frac{a^3}{c^3 - a^3} \right).$$

If we separate the shells by a distance $dc$, $E$ will alter by $dE/dc \cdot dc$, and $-dE/dc$ is the repulsive force between the spheres in the line of centres. Now

$$-k \cdot dE/dc = \frac{E}{c^2} - \frac{a^2}{c^2 - a^2} \frac{2a^3 - a^5}{(c^3 - a^3)c},$$

or

$$kVe \frac{a}{c^2} - e^2 \frac{ac}{(c^2 - a^2)c}.$$

According as this expression is negative, zero, or positive, the force is an attraction, zero, or a repulsion. If $c$ is only a little greater than $a$,
that is, if the small shell is very near to the surface of the large one, \((c^2 - a^2)\) is very small, and the force is an attraction. More specifically, if \(E\) and \(e\) are of the same sign, there will be an attraction when

\[ E < \frac{ea^2}{c} \frac{2c^2 - a^2}{(c^2 - a^2)^2} \]

while if this expression is an equality, from which

\[ \frac{kV}{e} = \frac{c}{c/(c^2 - a^2)^2}, \]

there will be no force between the shells at all. Such will be an equilibrium position, but an unstable one.

Returning to the idea of the cell and the particle, both of the same sign, when these are at a distance, there will be a repulsive force between them; as the particle is moved up to the cell, this force becomes at first greater. It thereafter lessens, and, when the particle is near to the cell, vanishes. The particle passes through this point of unstable equilibrium, and is now attracted to the cell, until the two come into contact. At this moment the charges redistribute once more, and equalise. A removal of the particle to a very small distance from the cell surface reestablishes the attraction, and to remove the particle to the position of unstable equilibrium, work has to be done. There is thus a force, manifested as soon as the particle leaves the cell surface, which tends to draw it back towards the surface, and prevents its withdrawal into space. The particle will therefore tend to remain at or within the surface, and to be subject to the action of such surface forces as prevail. By the surface must, of course, be understood the surface of the hypothetical shell.

The action of these forces may be used to explain the frequently observed fact that the ingestion of a particle is preceded by a phase when the particle appears to be stuck to the surface of the cell. Fenn remarks that this preliminary stage is clearly a surface tension phenomenon, by which we take him to mean that it is a very early stage of ingestion, for he points out that it is due to the fact that the surface of the cell exposed to the plasma is decreased. We suggest, however, that the first stage is that the particle is attracted to the cell surface, and that the electrical conditions are such as will prevent its removal; hence it remains. Thereafter the early stage of ingestion takes place,
followed by more and more complete ingestion, until an equilibrium is reached.

This treatment may be objected to upon two grounds at least, quite apart from any criticism directed against the special conception of the Helmholtz double layer. The first objection may be that the values of $E$ and $e$ are small in the case of cells suspended in a fluid, and that therefore the resulting induction and forces will be small. This is true, but these small forces appear sufficiently large to produce very important effects—such as the stabilising of cell suspension and of bacterial suspensions—and so ought to be taken into account unless there is excellent reason for assuming that their effect is negligible. The second objection may be that the above treatment applies only if $b$, the radius of the particle, is very small compared with $a$, the radius of the cell. This is also true, and is a fundamental assumption; if the particle and the cell approximate to one another in size, a different treatment, in which not one, but an infinite series of images, are inserted, is required, and a different result is forthcoming, the attractive forces being scarcely manifest, if at all. This may account, or play a part in accounting, for the greater ease of ingestion of small particles than of large—a phenomenon remarked upon by Tait and also by Fenn. As we intend later to use the fuller electrostatic treatment in connection with another subject, we shall leave the matter as it stands for the present, content with pointing out that the above treatment applies only if the particle is very small compared with the cell.

In conclusion, it should be pointed out that the introduction of these electrostatic forces in no way affects previous work on chance contact, for now we require, not the contact of the particle with the cell surface, but its approach to the position of unstable equilibrium. The chance of the one happening in a mixture of cells and particles is governed by the same conditions as the chance of the other. Nor have the electrostatic forces any influence on the surface tension conditions as laid down by Fenn. Their only effect is to tend to retain the particle at the cell surface, and thus to render it under the influence of the surface forces for a longer period of time. In this way they may tend to reduce the importance of the viscosity factor, for, if the particle were in contact with the cell surface for only a very short time, the viscosity of the cell, determining the rate at which the cell could flow over the
particle, would be exceedingly important, whereas, if the particle were to remain at the surface, a slower flow of the cell substance would result in ingestion.

BIBLIOGRAPHY.