THE THEORETICAL RESPONSE OF LIVING CELLS TO CONTACT WITH SOLID BODIES.

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(Received for publication, December 23, 1921.)

Recent discussions of the behavior of living cells in contact with solid bodies have been published by Loeb (1) and Tait (2). Both authors admit that two factors are involved in the reactions of the cells—changes in consistency of the protoplasm and surface tension forces. Tait lays particular stress on the latter, while Loeb believes that changes in consistency are more important. The latter has, perhaps, taken a somewhat safer position in his interpretation of the phenomena described. It would seem, however, that the relative importance of these two factors must vary with the particular type of cell chosen and the conditions of observation. Thus, invertebrate blood cells are more at the mercy of surface tension forces on coming into contact with a solid body than Amoeba or mammalian leucocytes. It is certainly true, nevertheless, that all blood cells are subjected to the abnormal forces of surface tension when they strike a foreign body. Whether or not these forces are sufficient to determine the behavior of the cell depends upon its fluidity. If it is only to enable us to discuss intelligently the relative importance of consistency changes and surface tension forces as applied to cells, it is necessary to formulate these hypotheses as accurately as possible.

There is, unfortunately, little to be said about such an indefinite factor as changes in the consistency of protoplasm, but surface tension lends itself readily to quantitative treatment. Tait has endeavored to predict the behavior of cells in contact with flat surfaces and small solid bodies from principles of surface tension. His discussion, however, was both incomplete and, in respect to phagocytosis of small bodies, erroneous. It seems important, therefore, to amplify and correct his discussion of the theory.
To begin with a simple case, we may consider the various positions which a perfectly fluid hypothetical cell would assume on a flat surface of glass in terms of the surface tensions between the cell and the plasma, the plasma and the glass, and the glass and the cell. In Fig. 1 let \( a \) represent the cell suspended in plasma, \( P \), before coming into contact with the glass, \( G \), and let \( b \) represent the same cell in an equilibrium position with respect to \( G \). In taking this position or any other position in contact with any solid body, \( G \), the interface, \( x \), has been increased (or decreased), and an area, \( s \), of the \( gp \) interface has been exchanged for an equal area of the \( gc \) interface. The surface energy, \( E \), at \( b \) is expressed by the equation

\[
E = xcTp + sgTc - sgTp
\]

where \( cTp \), \( gTc \), and \( gTp \) represent the surface tensions of the \( cp \), \( gc \), and \( gp \) interfaces respectively.

The problem is to calculate the height, \( h \), of the cell above the glass at equilibrium in terms of \( gTc \), \( gTp \), and \( cTp \), the volume of the cell remaining constant. Now by definition the surface energy, \( E \), at equilibrium must be at a minimum, and \( \frac{dE}{dh} = 0 \). To find \( \frac{dE}{dh} \) let us
express $x$ and $s$ in equation (1) in terms of $h$ when the radius, $r$, of the cell (when spherical as at $a$) is equal to 1. In order to do this it is necessary to neglect the effect of gravity and assume that $C$ always has the shape of a sphere or spherical segment. This is quite legitimate since the effect of gravity would be practically negligible where the dimensions of $C$ are those of leucocytes and where the difference in density between $C$ and $P$ is small.\footnote{Gravity would merely shift the equilibrium point without altering the principles involved.} By familiar formulas we may write

$$x = \pi \left( a^3 + h^3 \right)$$  \hspace{1cm} (2)

$$s = \pi a^2$$  \hspace{1cm} (3)

where $a$ is the radius of the base of the cell.

The volume of the spherical segment at $b$ equals the volume of the sphere at $a$ (Fig. 1) or

$$\frac{1}{6} \pi h \left( h^3 + 3a^2 \right) = \frac{4}{3} \pi a^3$$

Putting $r = 1$ and solving

$$a = \sqrt[3]{\frac{8}{3h} - \frac{h^3}{3}}$$  \hspace{1cm} (4)

Substituting the value of $a$ from equation (4) in (2) and (3) and introducing the resulting values of $x$ and $s$ in equation (1) we have

$$E = cT_p \left( \pi h^3 + \frac{8\pi}{3h} \frac{h^3}{3} + (gT_c - gT_p) \left( \frac{8\pi}{3h} \frac{h^3}{3} \right) \right)$$  \hspace{1cm} (5)

Putting $n = cT_p$ and $m = gT_c - gT_p$, differentiating with respect to $h$, and simplifying, we find

$$\frac{dE}{dh} = \frac{\pi h^4 \left( 2m - \frac{2}{3} (m+n) \right) - \frac{8\pi}{3} (m+n)}{3h^2}$$  \hspace{1cm} (6)

At equilibrium $\frac{dE}{dh} = 0$. Hence, putting the right hand member of equation (6) equal to 0 and solving for $h$, we have
With this equation, knowing \( m \) and \( n \), we can calculate \( h \) in terms of the original radius of the cell and from \( h \), in turn, we can calculate by equation (4) the radius of the base of the cell, \( a \).

Another formula for the equilibrium is given by Tait from considerations of the contact angle in Fig. 2.

\[
\begin{align*}
\gamma_{Tp} & = \gamma_{Tc} + \gamma_{Tf} \cos A \\
\gamma_{Tp} - \gamma_{Tc} & = \gamma_{Tf} \cos A \\
- m & = n \cos A
\end{align*}
\]  

(8)

(9)

Substituting the value of \( h \) from equation (7) in (10) and reducing, we have \( \cos A = -\frac{m}{n} \), which is the same as equation (9), and proves that the same equilibrium results either from considerations of the contact angle or considerations of the surface energy.

Now Tait's argument is incomplete because in using equation (9) he assumes that \( A = 0 \), i.e., that the cell spreads to infinity, whence \( \cos A = 1 \) and \( m + n = 0 \) or, in his terminology, a cell will spread to infinity when

\( ^2 \) The cell is, of course, assumed to be a mathematical sphere without structure. Even a pure liquid could not spread out in a layer less than one molecule thick.
But equation (7) as well as equation (9) proves that a cell may spread without spreading to infinity; indeed, any position is possible. Thus in equation (7) if \( n = +1 \) and \( m = +1 \), then \( h = 2 \), i.e., twice the radius of the cell when spherical, and the cell will not spread at all on \( G \). If \( n = +1 \) and \( m = -1 \), then \( h = 0 \) and the cell will spread to infinity. If, however, to take an intermediate case, \( n = +1 \) and \( m = 0 \), then \( h = \sqrt{2} = 1.26 \), which means that the cell will take the position of a hemisphere, having the same volume as the original sphere. At this point \( \cos A = -\frac{m}{n} = 0 \) and \( A = 90^\circ \).

When \( m = 0 \) there is neither gain nor loss of energy when an area of the interface between \( G \) and the plasma is replaced by an equal area of the interface between \( G \) and the cell. The explanation of the hemisphere as the equilibrium shape under these circumstances (when \( m = 0 \)) is that in this position the surface of the cell exposed to the plasma is at a minimum. This brings out the significant fact that the area of the exposed surface of a liquid sphere of diameter, \( d \), which is spreading to infinity on a flat surface, first decreases, passes through a minimum when the apparent diameter (diameter of the base) is \( 1.26d \), then increases until, at an apparent diameter of \( 1.86d \), it is again equal to the original surface area, and finally increases to infinity. Exactly comparable changes in the surface area of the cell occur during the ingestion of a small particle, except that the final increase is limited by the size of the particle instead of by infinity. This is clearly the reason why adhesiveness is such a familiar property of blood cells. We have thus been led to a definition of what we mean by adhesiveness or stickiness of cells. A cell which is stuck to a slide is one that is incompletely spread out by forces of surface tension. The energy necessary to detach the cell is stored up as surface energy on the newly formed surfaces. If the cell tears, leaving a layer of protoplasm still clinging on the slide, we have an exception in which the energy expended is merely a measure of the cohesion of the protoplasm (surface tension between protoplasm and protoplasm). It is of course possible that the natural rigidity of a cell will prevent it from spreading out on a solid surface so far and, therefore, from
sticking as hard as it otherwise would, but it is always true that the
force which holds a cell to a glass slide, indeed which holds any liquid
to any solid surface, is the force of surface tension. 3

In extending this discussion to cover cases where the surface of G is
curved and G becomes a small sphere, Tait makes a fundamental
error. He argues that a particle of G will be ingested by C if the
surface energy can thereby be decreased. In other words, G will be
ingested if the decrease in energy, due to exchanging \( sgT_p \) for \( sgT_c \)
(-sm), more than compensates for the increase in energy due to the
enlargement of the \( cp \) interface (\( \Delta xn \)) after ingestion of G. Thus
a particle will be ingested if \(-sm > \Delta xn\) or if \( sm + \Delta xn < 0\).

For the comparison with the condition necessary for the spreading
of a cell on a flat surface, that \( m + n = 0 \) or \( < 0 \), Tait puts this in-
equality into the form \( m + \frac{\Delta x}{s} n < 0 \) and reasons that since \( \frac{\Delta x}{s} \) is
"as a rule" 4 less than 1, \( m + \frac{\Delta x}{s} n \) will be more likely to be less than 0
than will \( m + n \). Hence he predicts that if a cell ingests a small
particle of G it will surely spread on a flat surface but that the reverse
may or may not be true.

This prediction is erroneous, because, as we shall attempt to show,
even though the surface energy may be less at complete ingestion than
before ingestion, it is always at a minimum (still less) at incomplete
ingestion, unless \( m + n = 0 \); i.e., unless the cell would spread to
infinity on a flat surface. This means that no particle of G can ever
be completely ingested by C unless C will spread to infinity on G.

The truth of this statement becomes evident from considerations
of the contact angle between C and G. As G becomes more and more
nearly ingested the angle of contact approaches 0. It can never reach 0
unless \(-m = 1\) for from equation (9)

3 Some writers (Mathews, A. P., Physiol. Rev., 1921, i, 553) would restrict the
term surface tension to the free energy of cohesion on liquid surfaces. It is
used here to denote the intensity factor of the free surface energy on either solid
or liquid surfaces regardless of the nature of the forces involved.

4 Actually it is always less than 1. It approaches 1 as a limit as the diameter
of the ingested particle approaches infinity.
Since this subject has apparently never been worked out from the point of view of surface energy it seemed desirable to be satisfied that, when the forces of surface tension at the contact angle are in equilibrium, the surface energy is also at a minimum. Unfortunately, we have not been able, even with the expert assistance of a professional mathematician, to express \( x \) and \( s \) of equation (1) in terms of the degree of ingestion of a particle as measured by the length of the line \( y \) in Fig. 3, and thereby to obtain the value of \( \frac{dE}{dy} \) and finally an equation like (7) for a surface of any degree of curvature. Theoretically this procedure is not impossible, but the necessary equations are too difficult to solve. A test case has been taken, however, where the radius of the particle, \( g \), is one-quarter of the radius of the cell \( (r = 1) \) and the values of \( \Delta x \) (the change in surface area of the cell in contact with the plasma), and of \( s \) (the surface area of \( G \) in contact with \( C \)), have been calculated for the different values of \( y \), the height of the spherical segment inside the cell (Fig. 3). These values are given in Table I together with the cosine of the corresponding angle of contact. By assigning various values to \( m \) and \( n \) and using the calculated values of \( \Delta x \) and \( s \) in the equation \[ sm + \Delta xn = E, \] curves can be plotted showing how the surface energy, \( E \), varies as \( y \) increases to 0.5 or twice the radius of the particle, \( G \); i.e., as ingestion approaches completion. These curves, for different values of \( m \) when \( n = 1 \), are shown in Fig. 4. The values of \( m \) used were calculated from the equation for the contact angle equilibrium, \( m = -n \cos A \) where \( A \) is the angle of contact corresponding to the chosen value of \( y \) and \( n \)

The complication arising from the increase in the radius of the cell to \( r + \Delta r \), as the particle is more and more completely ingested is responsible for the difficulty of the calculation. In order to evaluate \( \Delta x \) and \( s \) it is necessary to solve for \( s \) (Fig. 3) in an equation involving \( s^3 \) and \( s^2 \) by trial and error. This must be done to four significant figures in order to calculate \( \Delta x \) and \( s \) accurately enough for the purpose.

This equation is the same as (1), replacing \( gTc-gTp \) by \( m \) and \( cTp \) by \( n \) as in equation (6) and omitting the constant \( 4 \pi r^2 n = 12.566 n = (x - \Delta x)n \) representing the surface energy of the cell when spherical.
Theoretical Response of Cells to Contact

= 1. On each curve the point corresponding to the value of \( \cos A \) (Table I) used in calculating \( n \) for that curve is marked by a circle.\(^7\)

The fact that the surface energy is always at a minimum at the marked point proves that, when the forces of surface tension are in equilibrium at the contact angle, the surface energy of the whole system is also at a minimum. That part of each curve beyond the minimum is dotted to indicate that it is imaginary, because the cell would continue to ingest a particle only as long as the surface energy was decreasing.

\[ \cos A = \frac{(r + \Delta r)^2 + g^2 - (r + \Delta r + g - z - y)^2}{2g(r + \Delta r)} \]

This equation was used in calculating \( \cos A \) as given in Table I.
Fig. 4. Graphs showing how free surface energy (expressed in relative not absolute units) varies during the passage of a solid particle of radius 0.25, inside a hypothetical cell of radius 1.0, for various values of $m$ where $n$ and $m$ represent the gain in free surface energy caused by a given increase in the area of the cell plasma and cell glass interfaces respectively. Abscissa, $y$, vary with the position of the particle with respect to the cell, half inside at $0.22y$ and completely inside at $0.5y$. Points marked by large circles are points of equilibrium where surface energy is at a minimum, and represent also positions of cell and particle where the values of $m$ and $n$ used in calculating that curve would be in equilibrium at the angle of contact. The curves are dotted beyond these points to indicate that work must be done upon the particle to complete its ingestion since the surface energy is increasing. Values of $-\frac{m}{n} = \cos \theta$ are given in Table I. See text for further discussion.
reached at complete ingestion until the surface tension is such that the cell will spread to infinity. In the lower five curves in Fig. 4 the surface energy is less at complete ingestion than before ingestion; i.e., Tait's condition is fulfilled, but the particle is nevertheless only partially ingested except in the lowest curve.

From this point of view the surface of every particle, however small, must be regarded as an infinite surface, just as a flat surface may be regarded as a curved surface with a radius infinitely large. The only predictions which we can make from principles of surface tension are:

1. If a cell spreads to infinity on a flat surface of G, it will completely ingest a particle of G.
2. If a cell does not stick to a flat surface of G, a small particle of G will not stick to the cell.
3. If a cell is partially spread out on a flat surface of G, a particle of G will be partially ingested.

Tait has predicted from his discussion that: “If a substance, C, is unstable on a slab of G, then C will ingest G.” This is true if “unstable” means spreading to infinity; but when he tries to prove this prediction experimentally by observing that hyaline thigmocytes are unstable on glass and ingest small particles of glass, it may fairly be objected that he could not have observed the cells spreading to infinity and that the prediction consequently is not fulfilled by the facts. The explanation of the experiment is either that the cell was too rigid to spread to infinity on a flat surface though it could do so readily on the curved surface of a small particle, or that the equilibrium observed on a flat surface was a true surface tension equilibrium and that the small force of surface tension which prevented the small particle from being completely ingested was easily overcome by the ameboid movement (changes in consistency) of the cell.

A similar explanation may be given when Tait predicts that: “If C ingests a particle of G, then C (may or) may not be unstable on a slab of G,” and adds in consequence that “the fact that stable cells such as mammalian polymorphonuclears do not spread extensively on glass is no evidence that they ingest by other than physical means.” The true prediction from principles of surface tension is that if C ingests G, it will spread to infinity on a slab of G, and the ob-
served behavior of leucocytes means either that the rigidity of the cell prevents its reaching a true equilibrium on a flat surface, or that it ingests, by its ameboid movement, a small particle of G which otherwise would be merely stuck on the outside. Both alternatives seem equally probable. Obviously such tests of the surface tension hypothesis are worthless.

TABLE I.  
Areas of Contact Surfaces during Ingestion of a Particle.

<table>
<thead>
<tr>
<th>( \gamma )</th>
<th>( z )</th>
<th>( \Delta z )</th>
<th>( \cos \theta )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.785</td>
<td>+0.131</td>
<td>1.00</td>
</tr>
<tr>
<td>0.475</td>
<td>0.746</td>
<td>+0.092</td>
<td>0.926</td>
</tr>
<tr>
<td>0.425</td>
<td>0.667</td>
<td>+0.024</td>
<td>0.772</td>
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<td>0.375</td>
<td>0.588</td>
<td>-0.028</td>
<td>0.613</td>
</tr>
<tr>
<td>0.312</td>
<td>0.490</td>
<td>-0.082</td>
<td>0.393</td>
</tr>
<tr>
<td>0.250</td>
<td>0.393</td>
<td>-0.118</td>
<td>0.149</td>
</tr>
<tr>
<td>0.210</td>
<td>0.196</td>
<td>-0.130</td>
<td>-0.384</td>
</tr>
<tr>
<td>0.025</td>
<td>0.039</td>
<td>-0.037</td>
<td>-0.870</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-1.0</td>
</tr>
</tbody>
</table>

\( \gamma \) expresses the degree of ingestion as shown in Fig. 3. Ingestion is complete at 0.5\( \gamma \), one-half complete at 0.22\( \gamma \). \( z \) is the area of the cell in contact with the particle; \( \Delta z \) the change in the area of the cell plasma interface; \( \theta \) is the angle of contact.

Is it easier to ingest a small particle than a large particle, and if so, why? Tait says: "If in the case of a given phagocytic cell we have a certain volume of material which can just be ingested as a spherical piece, that cell will ingest the same volume more readily when the material is either sub-divided or distorted from the spherical form for by this means \( z \) is increased without change in \( x \) [\( \Delta x \) in our terminology]." It is true from principles of surface tension that a large particle will be less completely ingested than a small one for the same values of \( m \) and \( n \), i.e., for the same contact angle, but the same limiting values of \( m \) and \( n \) (i.e., \( -\frac{m}{n} = 1 \)) are necessary for complete ingestion of any particle of any shape or size. It is true, however, that a small particle could be more easily pulled inside against the force of surface tension from some equilibrium position in the surface than
a large particle which made the same angle of contact with the cell. Also, it is easier for a cell to spread around a small particle than around a large one because the necessary mechanical deformation is less.

Phagocytosis has been described repeatedly as taking place in two stages (Kite and Wherry (3)), the actual ingestion being preceded by a phase in which the object is merely stuck on the outside. The preliminary stage is clearly a surface tension phenomenon. The frequency of its occurrence is due to the fact that the surface of the cell exposed to the plasma is thereby decreased. When objects seem to be permanently stuck on the outside of a cell this may be a true surface tension equilibrium or, more likely, it may be that the rigidity of the structure of the interior of the cell prevents the further deformation necessary to reach a true surface tension equilibrium with the object completely inside.

In conclusion emphasis may be laid upon the significance, from the point of view of surface tension of Tait’s general proposition which states that only unstable cells tend to be phagocytic.

**SUMMARY.**

The theoretical behavior of a hypothetical fluid cell in contact with flat and curved solid surfaces is discussed from the point of view of surface tension.

An equation is derived for calculating the equilibrium position of the cell on a flat surface in terms of the surface tensions between the cell and the plasma, the plasma and the solid surface, and the solid surface and the cell. It is shown that the same equilibrium is predicted from consideration of the contact angle between the cell and the solid body.

The relative surface energy has been calculated at various stages in the ingestion of a solid particle by a fluid cell four times as large in diameter, and it is thus shown that no particle will be ingested until the surface tensions are such that the cell would spread to infinity on a flat surface of the same substance. Here again the same equilibrium is predicted from considerations of the contact angle.

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*This decrease is evidenced in Table I by the negative values of Δx when y is 0.375 or less.*
The adhesiveness of blood cells to solid substances is shown to be a pure surface tension phenomenon, but in most reactions between living cells and solid bodies the fluidity of the protoplasm is also a factor of prime importance.

The frequent occurrence of adhesiveness as a property of cells in contact with solid bodies is due in part to the fact that, by so adhering, the surface area of the cell not touching the solid is decreased.

Careful criticisms of this manuscript by Dr. W. T. Bovie and Dr. E. K. Carver are gratefully acknowledged.

BIBLIOGRAPHY.