CERTAIN INTERFACIAL TENSION RELATIONS AND THE BEHAVIOR OF BACTERIA IN FILMS.

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Phenomena exhibited by motile and by acid-fast microorganisms in two-phase liquid films are described in this communication. Considerations of interfacial surface tension which have proven successful in the hands of other workers with somewhat allied systems are applied to bacteria in simple and modified films. It is shown that theory and observation are in agreement, although in the absence of means of evaluating solid-liquid interfacial tensions a rigorously quantitative test is not possible. The technique employed is found to give an experimental means of discriminating between existing theories concerning the surface tension factor in phagocytosis.

Theoretical.

The principles involved are set forth in a comprehensive article on capillary phenomena by J. Clark Maxwell.[1]

> "If three fluids which do not mix are in contact with each other, the three surfaces of separation meet in a line, straight or curved. Let O [Text-fig. 1] be a point in this line, and let the plane of the paper be supposed to be normal to the line at the point O. The three angles between the tangent planes to the three surfaces of separation at the point O are completely determined by the tensions of the three surfaces.
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> . . . .
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> "When a solid body is in contact with two fluids, the surface of the solid cannot alter its form, but the angle at which the surface of contact of the two fluids meets the surface of the solid depends on the values of the three surface-tensions. If a and b are the two fluids and c the solid, then the equilibrium of the tensions at the point O depends only on that of thin components parallel

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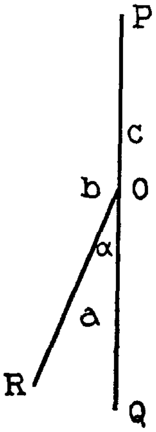
[1] Maxwell, J. C., Capillary action, in Encyclopædia Britannica, Cambridge, 11th edition, 1910–11, v, 262.

to the surface, because the surface-tensions normal to the surface are balanced by the resistance of the solid. Hence if the angle *ROQ* at which the surface of contact *OR* meets the solid is denoted by α,

$$T_{bc} - T_{ca} - T_{ab} \cos\alpha = 0$$



TEXT-FIG. 1.

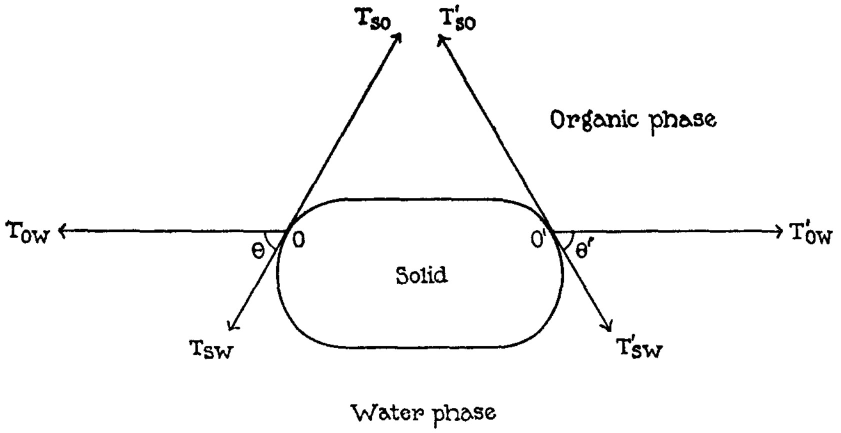
Whence

$$\cos\alpha = (T_{bc} - T_{ca})/T_{ab}$$

. . . .

"If the tension of the surface between the solid and one of the fluids exceeds the sum of the other two tensions, the point of contact will not be in equilibrium, but will be dragged towards the side on which the tension is greatest. If the quantity of the first fluid is small it will stand in a drop on the surface of the solid without wetting it. If the quantity of the second fluid is small it will spread itself over the surface and wet the solid. The angle of contact of the first fluid is 180° and that of the second is zero.

"If a drop of alcohol be made to touch one side of a drop of oil on a glass plate, the alcohol will appear to chase the oil over the plate, and if a drop of water and a drop of bisulphide of



TEXT-FIG. 2. Diagram of interfacial tensions acting at points *O* and *O'* in periphery of solid particle at equilibrium in interface between two immiscible liquids. The arrows by their direction and length indicate the direction and intensity of interfacial tensions at points *O* and *O'*. The solid-liquid tension at any other point could be indicated by arrows of proper length tangent to the solid at that point. θ is the contact angle made by the water phase with the solid.

carbon be placed in contact in a horizontal capillary tube, the bisulphide of carbon will chase the water along the tube. In both cases the liquids move in the direction in which the surface-pressure at the solid is least."

Let us consider now a bacterium in the interface between organic and aqueous phases (Text-fig. 2).

Let T_{so} be the tension in the solid (bacterial)—organic phase interface, T_{sw} that in the solid-water interface, and T_{ow} that in the interface between aqueous and organic phases.

At equilibrium,

$$T_{so} = T_{sw} + T_{ow} \cdot \cos\theta,$$

and similarly

$$T'_{so} = T'_{sw} + T'_{ow} \cdot \cos\theta' \qquad (1)$$

Suppose now other values of the several tensions, so that[2]

$$T_{so} > T_{sw} + T_{ow} \qquad (2)$$

Evidently the line of contact O will be dragged toward the organic side until the particle is completely wetted by and enveloped in the water phase. If

$$T_{sw} > T_{so} + T_{ow} \qquad (3)$$

the particle will be wetted and enveloped by the organic phase.

Thus the necessary conditions of stable equilibrium for the particle in the interface are that

$$T_{so} < T_{sw} + T_{ow}$$

and

$$T_{sw} < T_{so} + T_{ow} \qquad (4)$$

It follows then that

$$T_{sw} + T_{ow} - T_{so}$$

and

$$T_{so} + T_{ow} - T_{sw}$$

are in a sense measures of the stability of the particle in the interface. Explicitly, $T_{sw} + T_{ow} - T_{so}$ is the intensity of the force required to envelop the particle completely in the water phase and $T_{so} + T_{ow} - T_{sw}$ is the intensity of the force required to envelop the bacterium in the organic phase.

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[2] It is assumed in this discussion for simplicity that $T_{sw} = T'_{sw}$ and $T_{so} = T'_{so}$.

Only the liquid-liquid interfacial tensions, T_{ow}, can at present be given numerical values. Unfortunately means are not available for evaluation of the solid-liquid tensions. Certain relations of interest can be inferred indirectly, however, and they will be found in harmony with the observational data.

Behavior of Motile Bacteria in Films.

The method of studying the behavior of microorganisms in two-phase films described in the foregoing paper[3] may be altered by using as aqueous phase broth or hay infusion cultures of motile bacteria. The technique is otherwise the same. Under these circumstances the bacteria may be observed while subject simultaneously to the restraining and kinetic forces of the interfacial surface tensions, and the driving force of their own flagella; the former are, of course, however, somewhat modified by the presence of surface-active substances in the media.[4] The forces thus acting on the bacteria prove to be of the same order of magnitude, and capillarity or motility may prevail, successively, according to circumstances.

The protocol of an individual experiment may be given.

Experiment 1.—Film of cedar oil against a 20 hour broth culture of a virulent mouse typhoid bacillus.[5] The interface was seen as a bright line with the usual interference fringes; bacteria were swimming actively about in aqueous phase. When first viewed, a strong movement of inert particles along the interface was occurring. As bacteria swam into the interface their anterior ends projected a little into the oil, but they were promptly turned about so that their long axes lay parallel to the line of the interface. They then swam very swiftly "down stream" or "up stream" slowly against the interfacial "current," making progress, being carried backward, or remaining stationary, according to the balance between their own motility and the interfacial streaming. At one time, six bacteria were observed in the interface like so many salmon in a riffle, undergoing the vibratory movements characteristic of bacteria in rapid locomotion, but making no progress against the current. Dead bacteria and other inert particles in the interface passed them rapidly. Several bacteria were observed swimming out of the interface into the water phase. The rule was to stay in the interface when once there, however. None were observed leaving the interface to pass into the oil.

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[3] Mudd, S., and Mudd, E. B. H., *J. Exp. Med.*, 1924, xl, 633.

[4] Larson, W. P., Cantwell, W. F., and Hartzell, T. B., *J. Infect. Dis.*, 1919, xxv, 41.

[5] Webster, L. T., *J. Exp. Med.*, 1922, xxxvi, 97.

The angle of approach of bacteria entering the interface and the relative velocities of bacterial and interfacial movement determined whether the anterior end was directed up or down stream. Orientation down stream was naturally of more frequent occurrence. Bacteria swimming up to and apparently against the interface in this experiment and the others of the series were not always trapped there—often they swam immediately away.

Similar, though less striking results were obtained with *Vibrio percolans* and *Bacillus subtilis*. The following oils were used: cedar, peanut, sheep's foot, sperm, and mineral (liquid petrolatum Squibb) and melted vaseline (petroleum jelly, Chesebrough Co.). The vaseline was at first spread as a semisolid film against which the bacteria swam much as if against the glass. When the vaseline melted in the heat of the dark-ground illuminator, a liquid-liquid interface was formed in which the bacteria were trapped, along which they moved, and from which they escaped into the water much as with the oil-water interfaces of high tension.

Cyclohexane, cyclohexanol, oil of sassafras, oil of lavender, and 1 per cent phenol in albolene immobilized the bacteria near the interface, an ordinary non-motile experiment resulting.

A peculiarly vivid picture of the action of the interfacial trapping and kinetic mechanisms was obtained in these experiments. It is also noteworthy that when the bacteria threshed their way out of the interface, escape was always into the aqueous phase, and never, in the fourteen experiments performed, was escape into the oil noted. This would correspond to the condition that

$$T_{so} + T_{ow} - T_{sw} > T_{sw} + T_{ow} - T_{so},$$

or

$$T_{so} > T_{sw}$$

Behavior of Acid-Fast Microorganisms in Films.

Evidence has been adduced by Deycke and Much and by Leschke[6] to indicate that the acid-fast microorganisms are coated with a mixture of fatty acids and a neutral fat or fats. There is good reason to believe, on the other hand, that the membrane surface of ordinary

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[6] Leschke, E., *Beitr. Klin. Tuberk.*, 1911, xx, 400.

bacteria contains very many active or polar groups ($-COOH$, $-NH_2$, $-OH$, etc.).[7] Hence the ordinary bacteria should be more readily wet by water, the polar liquid, and the acid-fast microorganisms by the non-polar organic liquids. It therefore seemed likely that the behavior of the two groups of microorganisms in films might differ detectably. This has proved indeed to be the case. The acid-fast bacteria pass through the interface and into the organic phase with a readiness in marked contrast to the behavior of ordinary bacteria already described.

Acid-fast bacteria are present in much smaller numbers in the interfaces, for the reason that they are in an equilibrium of low stability there or not in equilibrium at all. Sliding movements up and down the interface are correspondingly reduced, and in the case of oleic acid-distilled water and some cedar oil-distilled water interfaces it may be almost entirely suppressed; interfacial forces may carry the acid-fast organisms into the oleic acid phase directly they enter the interface. A more typical behavior is for the individual bacterium in the water phase, when overtaken by the advancing interface, to slide along the interface for a greater or less distance before being completely imported into the organic phase with the aid of some mechanical work. This mechanical work is similar in kind, although very much less in degree, to that described in the foregoing paper in connection with ordinary bacteria.

When an organic phase is slowly encroaching on a water phase and the interface reaches a mat of acid-fast bacteria stuck to the glass, the interface usually runs ahead locally of the general line of advance and wets the group of acid-fast bacteria, enclosing many of them forthwith in the organic phase. The long bays and peninsulas drawn out behind an interface advancing on an aqueous suspension of ordinary bacteria, which the organic phase does not readily wet[8] (see Figs. 1 and 3), are correspondingly infrequent with the acid-fast preparations. Such peninsulas may be formed, however, when the interface is pulled out by some of the granular detritus found especially in old cultures of the acid-fast organisms.

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[7] Rideal, E. K., Colloid phenomena in bacteriology, *Brit. Assn. Advancement Sc., 5th Rep. on Colloid Chemistry*, 1923, 31.

[8] Mudd and Mudd,[3] Figs. 1 and 3.

Brownian movement in the interfaces has been noted as in general freer with the acid-fast than with the ordinary bacteria.

Acid-fast bacteria may be suspended in a number of the organic phases, *e.g.* mineral oil, cedar oil, olive oil, oleic acid, cyclohexanol. When such preparations were studied in films against distilled water, acid-fast bacteria were usually soon seen suspended in the aqueous phase also. Apparently they reached the aqueous phase with the aid of mechanical work in the same manner described for passage of ordinary bacteria in the reverse direction.[3]

In three of four experiments with mineral oil, no bacteria were observed to pass into the water, although much of the granular débris soon reached the aqueous phase.

Four strains of non-pathogenic Saranac Lake strains of acid-fast bacteria were used in these experiments, the smegma bacillus (*Mycobacterium smegmatis*), butter bacillus (*Mycobacterium berolinensis*), mist bacillus (*Mycobacterium stercusis*), and milk bacillus. They were grown on 5 per cent glycerol agar.

Olive oil, cedar oil, oils of lavender and sassafras, liquid albolene, mineral oil (liquid petrolatum Squibb), vaseline (petroleum jelly, Chesebrough Co.), isoamylic and isobutylic alcohols, cyclohexanol, cyclohexane, and oleic acid were used as organic phases.

Illustrative protocols may now be given.

Experiments 2 and 3.—Smegma bacillus and butter bacillus. Cultures had been kept sealed 3 months on 5 per cent glycerol agar. Films of oleic acid against distilled water suspension of acid-fast bacteria.

Throughout the observations, the organic liquid advanced slowly or rapidly on the aqueous bacterial suspension. Many bacteria in the water phase were adherent to the glass. As the interface reached and began to pass over such an adherent bacterium it was frequently torn loose from the glass and shot through into the organic phase. Floating bacteria were similarly swiftly imported into the organic phase. Floating granules were usually trapped in and underwent streaming movements along the interface. As the slowly moving interface advanced against a bacterium stuck to the glass in the aqueous phase, the interfacial line could frequently be seen to advance on the bacterium as a liquid does upon a solid it wets, and to accelerate the local advance of the organic phase. An occasional bacterium was observed to slide down the interface. Such were probably stuck to particles which were trapped in the interface.

Obviously, $T_{sw} > T_{so} + T_{ow}$. This expression represents the condition under which the organic phase tends to spread on the bacteria and displace the water.

Experiment 4.—Film of petroleum jelly against distilled water suspension of milk bacillus. When first observed, bacteria were to be seen in brownian movement in water, motionless in interface, and stuck to glass in vaseline phase. After a time the interface began to advance slowly toward the organic phase side. The bacteria in the boundary line moved slowly along the interface, for a short distance or through several low power fields, before passing by almost imperceptible degrees, floating through as it appeared, into vaseline phase. There was no brownian movement of bacteria in the interface. One droplet of vaseline was observed to approach a mat of bacteria adherent to the glass. The interface then ran out on the mat wetting it and engulfing some of the bacteria. The boundary line was observed to overtake floating mats of bacteria. These it scattered along the interface, some traveling in each direction from the site at which contact with the interface was first made. Stability in the interface seemed to exist, but the bacteria moved very readily into the organic phase.

The following conditions seem to obtain.

$$\left.\begin{array}{c} T_{sw} < T_{so} + T_{ow} \\ \text{slightly} \\ T_{so} < T_{sw} + T_{ow} \\ T_{sw} > T_{so} \end{array}\right\} \begin{array}{l} \text{The organic phase tends to spread on the bacteria} \\ \quad \text{and is prevented from so doing, if at all, only by} \\ \quad \text{the liquid-liquid interfacial tension.} \end{array}$$

Experiment 5.—Film of distilled water against suspension of butter bacteria in cyclohexanol. Bacteria were suspended in alcohol and water was allowed to run in from the other side of the cover-slip. Bacteria were swept in masses on the organic side of the retreating interface and held as against a wall except when an extremely active sweeping movement of phases or sliding up and down the interface shot bacteria over into water. When masses that stuck to the glass under alcohol were overtaken by the interface, they often became suspended in the water, gradually being dispersed to form an even suspension. Bacteria were seen trapped in and sliding up and down the interface but these were in relatively small numbers. When phases were nearly motionless, no bacteria were seen to pass from alcohol into water or *vice versa.* The general impression was of a strong trapping mechanism which prevented bacteria from passing from alcohol into water without violent work on them, or from water into alcohol without some work.

Bacteria in Modified Films.

It is clear that it should be possible, by adding substances which will decrease the liquid-liquid interfacial tension, to shift the picture presented by the group of liquids with high interfacial tension against water toward that of the low interfacial tension group. This again proves to be the case. As has already been indicated, preparations of mineral oil or cyclohexane against distilled water suspensions of ordinary bacteria show relatively straight interfaces, containing a high concentration of bacteria which reach the oil phase only with the aid of mechanical work of extreme degree. A suspension of bacteria in 0.06 per cent sodium oleate solution against mineral oil, on the other hand, shows interfaces more readily drawn out into peninsulas, containing fewer bacteria, and the microorganisms escape into the oil with the aid of less extreme mechanical work.

This effect of soap is, of course, not specific. A modification of the typical picture may similarly be occasioned by inadequately cleaned slides or cover-slips, and sufficient surface-active material from the bacterial culture may be added in making the suspension to cause a noticeable alteration from typical behavior. Differences in different cultures in the amount of surface-active constituents they contain seem to exist to detectable degree. It is to be noted, however, that neither by accident nor by design have we found preparations in which non-acid-fast bacteria pass by surface tension alone out of the interface. Some bacteria are always trapped in the interface, in equilibrium as regards the two phases, apparently, until acted upon by sufficient mechanical force to displace them into one or the other phase. And some sliding up and down the interface is almost invariably to be detected.

Modification of the preparation as a result of accidental contamination in such a way as to increase bacterial stability in the interface has also been noted. In one series of six experiments with distilled water suspensions of acid-fast bacteria against mineral, olive, and lavender oils, isobutylic alcohol and oleic acid (two experiments), the bacterial stability in the interface and the difficulty of ingress into the organic phase resembled preparations of ordinary bacteria and were unlike forty odd other experiments with acid-fast microorganisms. The difficulty was here traced to contamination of the slides used by a little dirty water in the bottom of the containing vessel.

Interpretation.

The possibility of explaining the observations on bacteria in interfaces by the theoretical interfacial tension relations may now be examined.

Suppose, first, that unequal diffusion of surface-active substances, unequal heating of the film, or other factor, decreases the water-organic phase tension on one side of the bacterium below that on the other, *i.e.* T_{ow} becomes greater or less than T'_{ow}. The bacterium will evidently tend to be dragged along the interface in the direction of the stronger interfacial tension. Thus if the free surface energy of the interface is not uniformly distributed, the particles trapped, "absorbed," in the interface will tend to accumulate in higher concentration in regions of greater interfacial tension than in regions of less tension. This mechanism was probably the chief means of effecting the streaming movements or "spreading" along the interface so evident in practically all systems studied in this and the foregoing paper, although we believe that movements due to evaporation from the margin of the film, mixing of the phases, etc. also played a part. Undoubtedly encroachment of one phase on another because of the first having greater wetting power for the glass, as described by Maxwell for carbon bisulfide and water, is partially responsible for the movements of the phases as a whole.

The interfacial kinetic mechanism just described was exhibited in greater or less degree in all the systems examined. The individual characteristics of the preparations will now be considered under several type cases.

Case A.—Ordinary non-motile or motile bacteria. The liquid-liquid tension, T_{ow}, is high.

Examples: *B. coli* or *B. subtilis* in interface between distilled water and cyclohexane or mineral oil. Motile *Vibrio percolans* or *B. pestis caviæ* in the interface between medium and mineral oil.

Since T_{ow} is large, the conditions of stability must obtain unless there is great disparity between T_{sw} and T_{so}. Such a disparity evidently does not exist in systems of this class, since the bacteria concentrate in the interface and only leave it when considerable mechanical work is done upon them. For this case, therefore,

$$T_{so} < T_{sw} + T_{ow},$$

and

$$T_{sw} < T_{so} + T_{ow}$$

Case B.—Ordinary bacteria. The liquid-liquid tension, T_{ow}, is low.

Examples: *B. coli* or *Vibrio percolans* in the interface between distilled water with cyclohexanol, isoamylic or isobutylic alcohols.

Stability should be much less than in Case A. As the limiting case is approached were $T_{ow} = O$, and the liquids become completely miscible, the bacterium can only be in surface tension equilibrium in the interface, provided T_{sw} practically equals T_{so}. In agreement with theory, the bacteria are found in much lower concentration in the interface than in Case A and they escape more readily into either water or organic phase than when T_{ow} is high.

The facts regarding behavior of bacteria in the systems of Case A and Case B were observed and recorded before we had an adequate explanation of them. The later cases were in part predicted from theory.

Case C.—Acid-fast bacteria.

Examples: Smegma, milk, butter, or mist bacteria in films of oleic acid, mineral oil, cyclohexane, cyclohexanol, etc. against water.

Bacterial surface layer of fatty material. Since mutual solubility or miscibility and low interfacial tension go hand in hand, and immiscibility is correlated with high interfacial tension, [9,10] T_{so} should be relatively small and T_{sw} large. Thus $T_{so} + T_{ow} - T_{sw}$ should be reduced to a low or even negative value.

Again, in agreement with expectation, the bacteria have extremely low stability in the interfaces, pass readily into the organic phases, and in oleic acid-water preparations at least are often thrust into the organic phase as soon as they come into contact with the interface.

Case D.—Ordinary bacteria. Liquid-liquid tension, T_{ow}, high in control, reduced by surface-active substance in experiment.

Examples: *B. subtilis* in interface between mineral oil and 0.06 per cent Na oleate solution. *Vibrio percolans* in interface between cyclohexane and 0.06 per cent Na oleate solution.

Theory requires shift of picture from that characteristic of Case A in the direction of that of Case B.

Experiment verifies expectation.

DISCUSSION.

Among the most important and fruitful attempts to explain phagocytosis in physical terms is that of Rhumbler, who attributed it almost wholly to the effect of interfacial molecular attractions at the cell surface. Although subsequent work has served to emphasize the importance of phenomena within the phagocytic cell, such, for

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[9] Harkins, W. D., Brown, F. E., and Davies, E. C. H., *J. Am. Chem. Soc.*, 1917, xxxix, 354.

[10] Coghill, W. H., and Anderson, C. O., Certain interfacial tension equilibria important in flotation, *U. S. Bureau of Mines, Techn. Paper 262*, 1923.

instance, as changes in the consistency of its protoplasm,[11–14] nevertheless the interfacial tension relations are of capital importance and deserve accurate statement. Rhumbler's formulation of the surface tension factor in phagocytosis, therefore, requires consideration. His *Importgesetz* and *Exportgesetz* state essentially that if a particle reaches the interface between two fluids it will be taken into that fluid for which it possesses the greater adhesion, provided that this adhesion is greater than the cohesion of the fluid in question.[15]

Consideration of Text-fig. 2 will show that this formulation is inadequate. It follows from the second law of thermodynamics that at equilibrium the particle will be so disposed as to give the system a minimal free energy, whether this equilibrium position is in one or the other liquid phase or in the interface between the phases. If the particle leaves the interface, the area of the liquid interface will be increased by some amount, y, and an increment of free energy, $y \cdot T_{ow}$, thereby made. Any formulation such as Rhumbler's which wholly leaves out of account T_{ow} is therefore inadequate on theoretical grounds. It is, in part, contradicted by our experiments, furthermore, which indicate a high degree of stability of the bacteria in the interface in many systems studied and correlation of that stability with the liquid-liquid tension, T_{ow}.

Tait[16] improved somewhat upon Rhumbler's formulation, but similarly supposed that the foreign particle must necessarily be carried by surface tension into one or the other phase.

Fenn[12] has criticized Tait's equations, and has himself formulated admirably the interfacial forces involved in the adhesion of a hypothetical fluid cell to a solid surface and in the ingestion of a solid particle by the cell. In this paper we are concerned only with the latter case. Fenn shows both from considerations of free interfacial energy and of interfacial tension that the cell will only completely ingest the

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[11] Loeb, L., *Am. J. Physiol.*, 1921, lvi, 160.

[12] Fenn, W. O., *J. Gen. Physiol.*, 1921–22, iv, 373.

[13] Barr, C. E., *J. Med. Research*, 1923, xliv, 79.

[14] Chambers, R., The physical structure of protoplasm as determined by micro-dissection and injection, General cytology, Chicago, 1924, Section V.

[15] Rhumbler, L., *Ergebn. Physiol.*, 1914, xiv, 577, 581.

[16] Tait, J., *Quart. J. Exp. Physiol.*, 1918–20, xii, 1.

particle unless other than capillary forces intervene, when the conditions of complete spreading of cell on particle are fulfilled. That is, if we call the cell the organic phase, the plasma the aqueous phase, and the particle *s*, ingestion is only complete if

$$T_{sw} > T_{so} + T_{ow}$$

If conditions of partial spreading obtain, the particle will be only partially ingested, *i.e.* will be in equilibrium in the plasma-cell interface.

It is clear then that our observations and formulation contradict those of Rhumbler and of Tait and substantiate the statement of Fenn.

For valuable data on and discussion of surface tension effects in somewhat allied systems, reference is made to papers by Harkins and Feldman,[17] Coghill and Anderson,[10] and the monograph of Willows and Hatschek.[18]

SUMMARY.

To account for the behavior of a solid particle in the interface between two fluids it is necessary to consider, as indicated by Clark Maxwell, three surface tensions: T_{so}, the tension in the interface between the solid particle and the organic phase; T_{sw}, the tension in the interface between solid particle and aqueous phase; and T_{ow}, the organic phase-water interfacial tension.

If $T_{so} > T_{sw} + T_{ow}$, (2), the stronger solid-organic phase tension should pull the line of intersection of the three phases around the periphery of the solid particle until the particle is completely enveloped in the *water* phase.

If $T_{sw} > T_{so} + T_{ow}$ (3), the solid-water tension should pull the line of meeting of the phases about the particle until it is enveloped in the *organic* phase.

If

$$\begin{aligned} T_{so} &< T_{sw} + T_{ow} \\ T_{sw} &< T_{so} + T_{ow} \end{aligned} \tag{4}$$

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[17] Harkins, W. D., and Feldman, A., *J. Am. Chem. Soc.*, 1922, xliv, 2665.

[18] Willows, R. S., and Hatschek, E., Surface tension and surface energy and their influence on chemical phenomena, Philadelphia, 3rd edition, revised and enlarged, 1923.

the particle should be stable in the interface, only leaving it when mechanical work overcomes the equilibrium due to the balance of interfacial tensions.

The ordinary bacteria used have been stable in the interfaces between water or aqueous solutions and all organic liquids tested; *i.e.*, condition (4) obtains. In preparations in which T_{ow} is large, stability has been found by experiment to be greater than when T_{ow} is small, as follows from condition (4).

The force, dependent upon condition (4), which holds bacteria in the liquid-liquid interface, and the force, dependent upon unequal distribution of tension in the liquid-liquid interface, which causes bacteria to glide along the interface, prove to be of the same order of magnitude as the force due to bacterial flagella. Interfacial tensions or its own motility may dominate the movement of the bacterium, according to circumstances. When bacteria thresh their way out of the interface, escape is into the aqueous phase.

Acid-fast bacteria possess very low or, in some cases, no stability in the interface, passing easily or even spontaneously into the organic phase.

Good evidence has been advanced by other workers to indicate that the surfaces of ordinary bacteria contain many polar radicals; on the other hand, the acid-fast microorganisms are coated with predominantly non-polar substances. It follows from known principles, therefore, that T_{so} should be greater than T_{sw} with ordinary bacteria, and T_{sw} should be greater than T_{so} with acid-fast bacteria. Consideration of relations (2) and (3) above will show that these conditions should result in the differences in behavior of acid-fast and ordinary bacteria actually found by experiment.

The theoretical and experimental data here developed contradict the theoretical formulations of the surface tension factor in phagocytosis advanced by Rhumbler and by Tait and substantiate those of Fenn.