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THEORIES OF AMOEBOID MOVEMENT

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INTRODUCTION

IN ADDITION to the rhizopods, many other organisms exhibit movements of protoplasm designated as "amoeboid." Among these are Foraminifera, Radiolaria, and Heliozoa; the plasmodia of myxomycetes; the egg cells of sponges; the spermatozoa of Monoblepharidaceae; the zoospora of many green algae; blood cells, fibroblasts, macrophages, and epithelial cells in tissue cultures; nerve fibers growing out in tissue cultures, and others. The "common denominator" of this heterogeneous group is the fact that in these organisms the movement of protoplasm is accompanied by changes in shape and by progressive motion. The protoplasmic streaming which occurs in plant cells (cyclosis) lacks these characteristics and so such movement is not classified as amoeboid. The protoplasmic movement of Heliozoa and Radiolaria also is frequently set apart as a different type. In the filamentous pseudopodia of these organisms the protoplasmic streaming somewhat resembles that in plant cells, and has been termed by Engelmann (1879) "filament streaming" ("Fädchenströmung").

Even when protoplasmic movements are divided into these three groups, the types of movement classified as amoeboid remain very heterogeneous in character. This diversity is well illustrated by the motion of certain cells in tissue cultures. It has been shown that in tissue cultures of white blood cells, different cell types exhibit different types of movement. These differences are constant and characteristic for the cell type involved, and are most simply expressed in terms of polarization of protoplasmic activity (De Bruyn, 1944, 1945). For example, the movement of the lymphocytes is progressive and the activity of the protoplasm is directionally polarized, i.e., pseudopodia form only in a localized area of the moving cell. In contrast, the protoplasmic activity of the macrophage is not polarized; the pseudopodia arise from all sides of the cell body, and there is no significant progressive motion. However, despite the profound differences in the character of the protoplasmic movement of various organisms, the essential mechanism in all cases may be the same.

Our knowledge of the visible phenomena of the movement of *Amoeba*, myxomycete plasmodia, and white blood cells is well founded. The movement of these organisms is believed to be identical in essential details, and the theories about amoeboid movement have been based almost entirely on observations of these organisms. Other types of protoplasmic movement are considered in this review only as they have a direct bearing upon the several theories.

Our knowledge of amoeboid movement does not go much beyond those phenomena which are microscopically visible. As to what causes such movement, we have only hypotheses. Several theories have been advanced in the course of time. They are all speculative in nature, but with the accumulation of data and with improved methods of study, plus the application of the knowledge produced by the advancing physical sciences, these theories have gradually become more meaningful

and better founded. From now on, important progress is to be expected only from a more experimental approach than has been taken in the past. Efforts to verify the proposed theories experimentally have been few, and not enough physical or chemical data, requisite to an understanding of the mechanism of protoplasmic movement, have been obtained. For instance, it is obvious that any theory of amoeboid movement will ultimately have to take into account the processes of energy production and metabolism. At present, nothing is known about these processes insofar as they are involved in protoplasmic movement. Such investigations will be of special interest because many investigators believe that protoplasmic movement is related to muscular contraction or is even identical with it. The application of new microchemical and microphysical techniques may open the way to the solution of these important problems.

The various theories of amoeboid movement have been greatly influenced by the prevailing concepts of protoplasmic structure. This is not surprising; it has always been evident that the final explanation of the mechanism of protoplasmic movement must ultimately be based on the structure or structural elements of protoplasm. Such other functional cellular phenomena as metabolism, respiration, and secretion may or may not depend on protoplasmic structure, but the phenomenon of protoplasmic movement *must* be so based. For this reason concepts of protoplasmic movement must take into account protoplasmic structure, just as theories of protoplasmic structure cannot ignore protoplasmic movement.

EARLY CONTRACTILITY THEORIES OF AMOEBOID MOVEMENT

The first efforts to explain the mechanism of protoplasmic movement arose from the controversy between Ehrenberg and Dujardin on the organization of Infusoria. Ehrenberg (1830, 1832) maintained that Infusoria, a term which at that time included the rhizopods, possessed organs comparable to those of higher organisms. He based his views on the motility of *Amoeba* entirely on this belief. To him, the pseudopodia were hernia-like protrusions caused by the local weakening of the "body, " through which the contents of the "body" were forced by (muscular) contractions. Dujardin (1835, 1838) opposed the view that Infusoria were differentiated into cellular organs, and found that they consisted of a homogeneous jelly-like mass, for which he proposed the name sarcode. He claimed that his sarcode possessed the properties of extensibility and contractility.

Dujardin thought these inherent properties of the sarcode to be responsible for the changes in form and for the locomotion of *Amoeba*. As he described the phenomenon, the inherent forces of extensibility cause a protusion to be formed and to advance. This protrusion becomes attached to the substratum and subsequently contracts, thus pulling up the rest of the organism.

The role of extensibility in the formation of a pseudopodial protrusion was denied by Ecker (1849), who showed that pseudopodia could also be formed by a process of contraction. He observed that when a pseudopodium forms, a stream of granular material is forced into the growing protrusion. To Ecker this indicated that a pressure was exerted against the growing pseudopodium by that part of the sarcode not taking part in the formation of the pseudopodium. It was his opinion that locomotion resulted from a contraction of the body, by which its contents were forced into the pseudopodium; the pseudopodium then became "body" and in this manner the organism progressed.

After M. Schultze (1861, 1863) identified the sarcode with the protoplasm of other cells, contractility was universally accepted as a general property of living substances, a property upon which was based the ability of protoplasm to move. The mechanism by which the general process of protoplasmic contraction produced protoplasmic flow, change of shape, and progressive movement was later defined more closely by observations which indicated that in cells showing these phenomena the protoplasm was differentiated in an outer cortical layer, which was contractile, and a passive centrally located mass which was brought into motion by the contraction of the cortical layer. This mechanism was first described somewhat vaguely by Brücke (1861, 1862) in his observations of the protoplasmic streaming in the hairs of *Urtica urens*. However, Brücke seemed to believe that the flowing mass was not true protoplasm but a non-living intracellular fluid. Wallich (1863 a, b, c) stated definitely that in *Amoeba*, motion is effected by the contraction of the external sarcode layer (ectosarc), with the internal mass (endosarc) participating only passively in the movement. Wallich also came

to the conclusion that ectosarc and endosarc were not permanent structures but were "mutually convertible one into the other."

The studies of de Bary (1864) on the movement of myxomycete plasmodia led him to a similar view. However, in addition to the movement arising from the contraction of the cortical protoplasm, de Bary believed that there was a movement produced by another force. He thought this force arose from an active expansion at the ends of the plasmodium branches which "sucked" the fluid protoplasm in this direction. To prove the existence of these two forces, de Bary decided whether the driving force in certain branches of a plasmodium was contraction or expansion ("suction"), on the basis of the character of the protoplasmic flow, and then cut these branches. Where he had assumed contraction to be the driving force, protoplasm flowed out at the surface proximal to the cut; the distal surface remained even. When he cut a branch of which he assumed the driving force to be expansion, both cut surfaces remained even. The value of his experiments, however, is weakened by the fact that regardless of whether he thought a contractive or an expansive force to be present, an outflow of protoplasm sometimes occurred at both surfaces.

To give further weight to his theory that contractility produced a protoplasmic flow, de Bary cited experiments by Kühne (1864). This investigator had shown that electric stimulation would cause part of the plasmodium to contract and that this contraction pushed the more fluid granular mass into the neighboring, uncontracted parts of the plasmodium. De Bary also produced experimental pseudopodia in a plasmodium by chemical action (K_2CO_3), an effect which he thought resulted from expansion brought about by swelling. But these experiments do not conclusively prove the existence of an expansive force; pseudopodia may form as a direct result of the chemical action, but the outgrowth does not necessarily occur because of swelling or expansion. De Bary's experiments seemed to produce, primarily, evidence for a contractile force; they were less conclusive as to the existence of an expansive force.

Greeff (1874) ascribed the motility of *Pelomyxa* to the outer layer of protoplasm, contraction of which displaced the centrally located protoplasm, which remained passive. In addition, he noted a difference in consistency, terming the central protoplasm more fluid, or less viscous, than the cortical protoplasm.

F. E. Schulze (1875) was the first to make a detailed study of the currents in the protoplasm of a moving *Amoeba* (*Pelomyxa*). On the basis of his observations, integrated with the idea of protoplasmic contractility, he developed a concept of the mechanism of the progressive movement of *Amoeba*. His observations on the movement of protoplasm in *Pelomyxa* he summarized in a diagrammatic drawing which is reproduced in Fig. 1. In this figure the arrows indicate the direction of movement, their length the rate of movement.

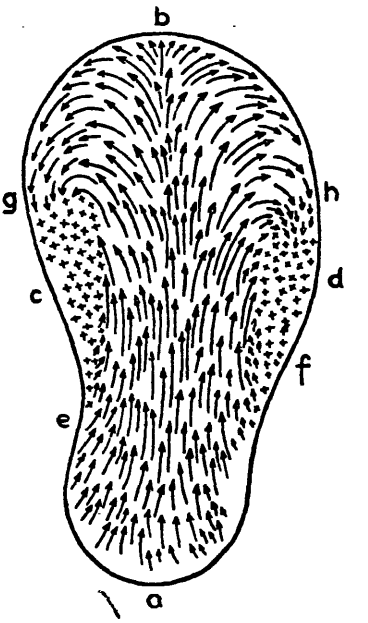

FIG. 1. CURRENTS IN PELOMYXA PALUSTRIS, GREEFF
After F. E. Schulze (1875).

The crosses represent places of rest. It can be seen from this figure that while the organism itself progresses, a part of the protoplasm is not in motion. This mass of protoplasm at rest is located at the sides. From the posterior part of this resting mass, material is continually released and then is taken up into the central forward-moving stream. At the anterior zone of the resting mass, material from the streaming mass is continuously deposited. Thus, material previously released from the posterior part of the resting mass comes later to rest at its anterior part. Schulze observed this displacement and replacement not only at the lateral sides of the moving

organism but also at the upper half. He could not determine whether it also occurred along the lower half, which was in contact with the object glass, but thought this very probable.

On the basis of these observations, Schulze formed his hypothesis regarding the manner of movement and driving force in *Pelomyxa*. Agreeing with Greeff that the cortical layer of the protoplasm is more dense and more viscous than the centrally located protoplasm, he said that the contraction of the cortical layer of the posterior part of the organism at *e*, *a*, *f*, pushed the central fluid mass forward. The resting zone at and before *e* and *f* does not expand by the pressure originating from this contraction at the posterior end, since here the cortical layer also is contracted. But at the anterior part the cortical layer is thin and offers little resistance to the forward-streaming material. The important question which arises if one accepts this explanation is: Why does the forward-streaming movement continue once the posterior part of the cortical layer is contracted? This Schulze explained by assuming that there is a continuous transfer of contracted material of the posterior part of the cortical layer into the central streaming mass, and its simultaneous replacement by material from the cortical layer at the sides.

Schulze's observations have been proved essentially correct. Perhaps the most important part of his theory was his explanation of the continuity of the protoplasmic processes involved in progressive movement. All theories based on contractility were temporarily eclipsed by surface tension theories, but after these became untenable, Schulze's ideas emerged again, although in a different form, in Mast's work (1926).

A. Gruber (1882, 1885, 1886) without going into a detailed analysis of the mechanism of amoeboid movement, also was of the opinion that the contraction of the cortical layer at the posterior end is the actual locomotor force. He believed this contraction to be associated with a dehydration of the protoplasm.

The weakness of these early contractility theories was the absence of any accurate knowledge regarding the physical basis of the process of contraction. To most investigators, "contraction" was no more than a somewhat more definitive term for the broad concept of protoplasmic motility, and did not necessarily imply a relation with muscular contraction. M. Schultze, in his work on the protoplasm of rhizopods and plant cells (1863), stated that there is no other expression than the term "contractility" to characterize the inner cause of active motion, and that one was completely in the dark as to the relationship of protoplasmic movement to the contractility of other elements, as, for example, muscle tissue. In contrast, Ecker (1849) believed the motility of sarcode and muscle contraction to be definitely linked. He maintained that sarcode ("undifferentiated contractile substance") was a primitive form of the muscle tissue of higher organisms ("differentiated contractile substance"). He also thought that muscle tissue develops embryologically from undifferentiated contractile substances present in the egg.

Heitzmann's theory (1873) of the structure of protoplasm was an attempt to explain contractility on a morphological basis. To him, protoplasm was not a homogeneous mass, as it was considered by Dujardin, but consisted of a (visible) living contractile three-dimensional reticulum which was imbedded in a non-living non-contractile fluid. It was his view that the fibers increased in thickness at their points of contact and that at these places granular formations occurred (Fig. 2A). Contraction, he said, took place by a transfer of material to the granules from the filaments, shortening the filaments and increasing the size of the granules. This caused the meshes of the network to become narrower, and contraction was the result (Fig. 2B). The obviously homogeneous appearance of certain parts of the protoplasm he explained by assuming that here the reticular framework was stretched to such an extreme that it became invisible (Fig. 2C).

Fromann (1880) also believed in a reticular structure of protoplasm, but he did not try to explain contractility on this ground. Others gave the reticular theory of protoplasmic structure considerable attention, and many accepted Heitzmann's general idea of a contractile framework However, very few investigators expressed themselves as favoring his theory of the mechamism of the contraction.

Leydig (1885) and Schäfer (1883, 1891) held that it was not the reticular framework which was contractile but rather the fluid material in which the framework was embedded (hyaloplasm). Schäfer observed the movement of the white blood corpuscles of the newt (*Triton cristatus*). He succeeded in fixing these in the amoeboid condition with their pseudopodia extended, by instantly

applying a jet of steam to the surface of the cover glass. In such preparations he noted that the protoplasm of the cell body and that of the pseudopodia differed in appearance. In the cell body

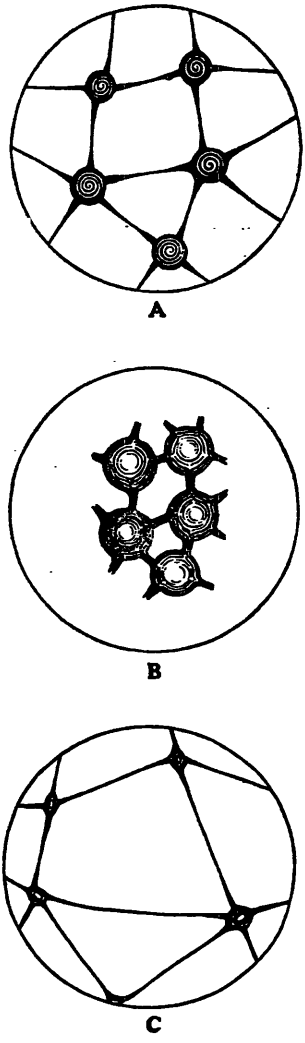

FIG. 2. RETICULAR STRUCTURE OF PROTOPLASM ACCORDING TO HEITZMANN

A, protoplasm at rest; B, contracted protoplasm; C, stretched protoplasm. After C. Heitzmann (1873).

the protoplasm appeared finely reticulated, while in the pseudopodia it was entirely homogeneous. From these observations he concluded that protoplasm did have a reticular structure as claimed by Heitzmann and others, but that the mobility was inherent in the fluid material which surrounded the reticulum. It was his view that this fluid material could actively leave the reticulum and so form pseudopodia.

Engelmann (1879) considered protoplasm to be an aggregate of contractile particles of molecular dimensions, which he called "inotagmen." According to him, protoplasm moves as the result of changes in shape of these elements. He assumed that the particles at rest were rod-like in shape, while when stimulated they contracted into the form of a sphere, these changes in form being accompanied by changes in water content (swelling). He discarded the idea that pseudopodia were formed by a contraction of the posterior cortical layer, and thought such formation resulted primarily from the relaxing of a group of contracted "inotagmen"; that is, by changing from the sphere shape to the rod shape they produced extensions of protoplasm. In essence, this is a mechanism which Dujardin, in his extensibility concept, thought to be in part responsible for the movement of sarcode.

Deficient as were the contractility theories of that time, relatively few dissenting opinions were expressed, of which the more important will now be mentioned. Such criticism as there was arose largely from the lack of certainty regarding the site of origin of the protoplasmic stream in moving cells. The contractility theories were based on the assumption that the protoplasmic stream was created by a vis-a-tergo, and so originated at the end opposed to the direction of movement. De Bary's (1864) observations on the movement of myxomycete plasmodia led him to the belief that other currents arose at the end which was moving forward, as the result of a suction created by an "expansive" force at the end of the pseudopodia. De Bary is not very explicit as to the nature of this expansive force, but from the interpretation of his experiments to prove its existence, he appeared to regard it as a swelling process (see page 3).

Hofmeister (1865) specifically denied that contractility causes protoplasmic movement. According to him, the currents in plasmodia arise only at the advancing end of the pseudopodia; from here the movement of the protoplasm gradually spreads backwards. He rejected de Bary's conception regarding the cause of the currents because they occurred without the changes in shape which he thought should be present if they were produced by an expansive force. To Hofmeister, the protoplasmic movements were based

on differences in water absorption. He assumed protoplasm to be composed of submicroscopic particles whose imbibition of water was continously changing. A transfer of water from one group of particles to another group would result in movement, since an increase of water content would be accompanied by an increase in the volume of the particles.

Sachs (1882) tried to explain the origin of the currents at the end of a pseudopodium by a local disturbance in certain forces which he believed to be active in holding the molecules in a state of labile equilibrium. Such a local disturbance would then spread to places further removed from the site of origin, and a movement would be created which would be directed backward. Just how such a disturbance of equilibrium would produce movement and why such a movement would be directed toward the site of origin, Sachs did not explain.

SURFACE TENSION THEORIES OF AMOEBOID MOVEMENT

When improvements in microscopic technique toward the end of the nineteenth century failed to verify the presence of a reticular framework in protoplasm, the reticular theory of protoplasmic structure had to give way to other concepts. The idea of a continuous framework was abandoned. According to the new theories, the essential structural elements of protoplasm were minute isolated particles dispersed in a homogeneous fluid. These particles were thought to be filaments (Flemming, 1882), granules (Altmann, 1890), or droplets (Bütschli, 1892). Contraction was more difficult to visualize on the basis of these dispersed structural elements than on the basis of a continuous reticulum. Influenced by the new concepts of protoplasmic structure, the theories of amoeboid movement acquired an entirely different aspect. The responsible forces were no longer sought primarily in the protoplasm itself, but outside the cell, or rather, at its boundary.

Quincke's studies (1870, 1877) on the conditions governing the spreading of liquids in contact with other liquids or solids furnished grounds for a new approach to the problem. If three liquids, 1, 2, and 3, are in contact with each other, and the surface tensions at the three interfaces are represented by $\alpha_{1,2}$, $\alpha_{1,3}$, $\alpha_{2,3}$, then it follows from considerations regarding the angles formed by the intersecting interfaces that, for instance, fluid 2 will spread over the interface between fluid 1 and fluid 3, if

$$\alpha_{1,3} \geqq \alpha_{1,2} + \alpha_{2,3}.$$

The same is also true if one of the substances 1 or 3 is a solid.

Berthold (1886) considered protoplasm as a liquid. He believed that the spreading phenomena of liquids in contact with a solid substances are essentially of the same nature as the amoeboid movement of a mass of protoplasm on a solid substrate. The fact that a mass of protoplasm on a solid surface does not spread the same on all sides, Berthold explained by the influence of local differences of chemical nature. According to him, the spreading of a moving amoeba is greatest at the anterior pole, least at the posterior pole. While the spreading at the anterior end continues, the adhesion to the surface toward the posterior end gradually lessens, until it becomes free from the substrate and rounds off under the influence of the surface tension. According to this concept, the formation of pseudopodia is a passive process; the pseudopodia are pulled out, not pushed out.

Berthold produced movement in drops on liquid and solid surfaces by the same physical conditions which he thought to be responsible for the movement of amoebae. For example, he brought into motion a drop of water on a glass plate by applying ether vapor locally. According to him, this motion resulted when the adhesion was locally lowered by the condensation of the ether vapor on the surface of the water drop. By placing drops of oil on tap water, previously soiled with oil, he obtained movements similar to those of amoebae. This motion he believed to be caused by locally varying rates of exchange of the fluids by mutual solution, resulting in local changes in the forces governing spreading.

Berthold also studied the currents in drops in which the surface tension was locally altered. He noted the similarity between the currents in such drops (Fig. 3) and those in moving amoebae (Fig. 1). However, he did not believe that the currents in both amoebae and drops were caused by the same mechanism, since the currents in the medium surrounding the drop are not present in the fluid around the moving amoeba. It was his conclusion that the "fountain streaming" in moving amoebae was caused simply by a more intensive spreading in the axis of the cell body than at the lateral parts.

That drops in a fluid medium and on a solid substrate change form and can be brought into motion by local changes in surface tension was also shown later—and more conclusively—by Quincke (1888) and Bernstein (1900). However the theory of amoeboid movement based on such considerations is markedly deficient in that it does not explain the extension of pseudopodia which are not in contact with a solid substrate. To explain the formation of such free pseudopodia, Berthold was forced to assume an entirely different mechanism—a pressure from behind by a contraction of the peripheral protoplasm. Thus he proposed two different mechanisms for essentially the same process.

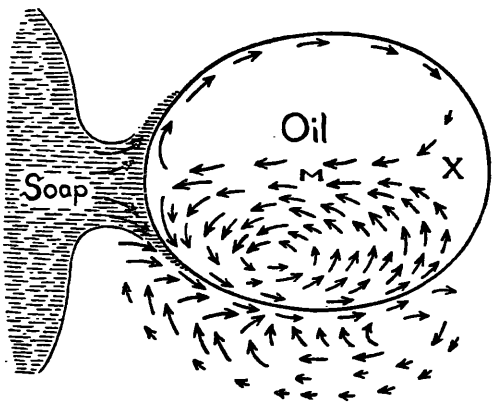

FIG. 3. CURRENTS IN A DROP OF OIL IN WATER AND IN CONTACT WITH A SOAP SOLUTION
After O. Bütschli (1892).

Bütschli (1892) agreed with Berthold that local differences in surface tension were the primary cause of amoeboid motion, but he did not accept that part of Berthold's hypothesis concerned with the mechanism of the movement. Bütschli's views are best understood in the light of his ideas of protoplasmic structure. Studying protoplasm with the finest optical equipment then available he came to believe that it was "foam-like" in structure, meaning that it consisted of minute droplets suspended in another fluid. In other words, he thought protoplasm to be what now is termed an emulsion. He imitated this "foam-like" structure of protoplasm with emulsions prepared with olive oil, potassium carbonate, and water. Thus he obtained a water and oil emulsion of which the watery phase contained, besides potassium carbonate, also potassium soap. When he placed drops of such emulsions in water, or, better, in dilute glycerin, he found that they moved much as an amoeba moves. He noted also that the streaming phenomena in these drops were similar to those described in amoebae, in that there was an axial current in the direction of movement which spread out toward the sides at the anterior end. Currents of this nature (observed earlier by Berthold, 1886, and Quincke, 1888) arise when the surface tension of a drop is locally lowered, as happens when one side of a drop of oil in water is touched by a soap solution (Fig. 3). The drop moves in the direction of the soap solution. Bütschli explained this movement in the following way. Some of the soap solution is swept toward the posterior end of the drop by the currents at the surface of the drop. As this material is removed, it is then replaced by the soap solution, by the water, and also by the oil. Since the oil drop tends to keep its form, the drop itself moves forward.

According to Bütschli, the movements of emulsion drops in water or in dilute glycerin can be similarly explained. The surface tension would be lowered locally by soap solution that leaves the watery phase of the emulsion by diffusion or by the bursting of superficial alveoli. As a result, currents are set up which produce the movement of the drop.

Applying these ideas to the movement of amoebae, Bütschli assumed that protoplasm was an emulsion of droplets of a watery solution in a fluid not soluble in water. This fluid he held to be identical to the plastin of Reinke and Rodewald (1881). He thought it probable that the droplets contained soap-like compounds which poured out on the surface of the amoeba with the bursting of superficial alveoli. Just as in a drop of oil emulsion, the surface tension would be locally lowered, currents would be created, and movement would occur. Bütschli believed this explanation to hold not only for simple monopodal movement (*A. limax*, *Pelomyxa*, etc.) but to be equally tenable for more complicated changes in form involving several pseudopodia.

In an appendix to his book, Bütschli reported his observations on the currents in the water around a moving *Pelomyxa*. As has been mentioned, Berthold had rejected the idea that the currents in amoebae were caused by the same factors as those in moving drops, because he could not observe currents in the water around moving amoebae such as he saw around the drops.

Bütschli did find currents around *Pelomyxa*, but they seemed to run in a direction opposite to what he expected on the basis of his experiments with drops. As a tentative explanation for this difference, he suggested that the superficial layer of the protoplasm might be more viscous than the inner mass, and that there might be a current running toward the anterior end. In other words, two currents would occur at the surface: one in the internal zone, running backward as in moving drops, and one in the external zone, running forward. Bütschli did not observe superficial forward-running currents, but they were later reported by Blochmann (1894), who agreed with Bütschli as to the forward current in the medium outside the moving amoeba. However, Blochmann's observations regarding forward currents inside amoebae have not been confirmed by other investigators, although detailed studies have been undertaken. Whether or not such currents exist, the fact remains that the currents in the medium *around* moving amoebae are different from those around moving drops. Since the nature of the currents in the medium is an essential factor in Bütschli's explanation of the forward movement, it must be concluded that his theory is not adequate.

It is interesting to note that according to Bütschli's own description the currents in moving amoebae are not entirely the same as those in the moving drops. In the drops he found that the currents under the surface continue backward and then bend toward the axial stream (Fig. 3), while in moving amoebae he observed that these currents move backward only a short distance and then come to rest. This agrees with Schulze's observations (Fig. 1). Bütschli used the similarity of these currents as his basic evidence that the mechanism of the movement of amoebae was the same as that of drops of which the surface tension is locally lowered. The fact that the identity he alleged for the currents is not in agreement with his observations further weakens his theory.

The surface tension theory was again modified by Rhumbler (1898a, b). This investigator agreed with Berthold and Bütschli that protoplasm is essentially a fluid, but he emphasized that in an amoeba the surface (ectoplasm) is more dense and more viscous than the interior (endoplasm). According to Rhumbler, the ectoplasm is of tougher consistency because of contact with the external medium. Further, he assumed that amoebae secrete a sticky substance at the place of contact with the substrate and so create the friction between the cell and the substrate necessary for progressive movement. The mechanism of amoeboid movement he described as follows (Fig. 4): For an unknown cause, which may originate in the medium or in the cell body itself, the surface tension is locally reduced at some place on the surface of the cell. As a result, the protoplasm bulges out at the point of lowered surface tension (from A to A_1), bringing about an axial current in the endoplasm. Because of the increase in surface area at the anterior end, protoplasm which previously was under the surface is now located at the surface, where contact with the external medium transforms it from endoplasm

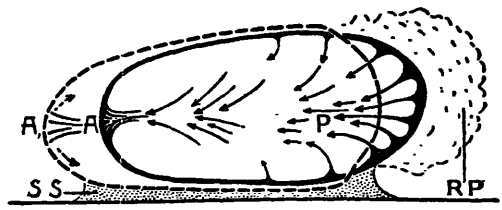

FIG. 4. RHUMBLER'S CONCEPT OF AMOEBOID MOVEMENT

SS, sticky substance; RP, resting protoplasm; A, anterior end; P, posterior end. For explanation see text. After L. Rhumbler (1898a).

to ectoplasm. This newly formed ectoplasm attains only gradually the viscosity of the older ectoplasm, and, according to Rhumbler, the surface tension remains lower here than at other parts of the surface of the cell body, and further bulging results. Since by this bulging more new ectoplasm is formed, the ectoplasm formed earlier takes up a position relatively further back. As endoplasm streams forward in the axial current, ectoplasm at the posterior end moves forward too, becoming thicker in mass. Here the inner portion of the ectoplasm, because it is farther removed from the surrounding medium, is transformed into endoplasm. Thus, as the amoeba moves forward, endoplasm is continuously transformed to ectoplasm at the anterior end, and at the posterior end this transformation is reversed. The essential features of this so-called "ectoplasm-endoplasm process" had previously been described by Wallich (1863 a, b, c), A. Gruber (1882, 1885, 1886–87), and particularly by F. E. Schulze (1875).

According to Rhumbler's papers of 1898, he did not attach particular significance to the occurrence of "fountain currents." He believed that a true fountain current, i. e., a forward-directed current which spreads to the sides and then turns backward, did not always occur at the anterior end of a moving amoeba. If such a current occurred, he explained it rather vaguely as being caused by the transformation of endoplasm into ectoplasm at the advancing end, which would be accompanied by a change in density, which in turn would push material backward. Just exactly what the force was which directed the current backward he did not explain. His opinion differed markedly from that of previous investigators, who held that the fountain currents resulted directly from a local lowering of the surface tension, and who based their evidence for the presence of local changes in surface tension on the occurrence of fountain currents.

As a result of Jennings' observations (1904) which will be discussed in detail later, Rhumbler changed his views in 1905. After Jennings had denied the presence of any fountain currents in moving amoebae, Rhumbler emphasized their occurrence in various amoebae (*A. blattae, Pelomyxa penardi, A. proteus, A. limax*) and apparently regarded their existence in these cases as proof for the activity of surface tension forces.

For movements without typical fountain currents, Rhumbler accepted Jennings' explanation, according to which the amoeba moves forward by a rolling motion. His views regarding the forces bringing about the movement in this type of motion are about as follows. At the anterior end the forces would be the same as those which determine spreading of a drop in a liquid medium and in contact with a solid surface. This is identical with the theory earlier put forth by Berthold. However, Rhumbler believed that at the posterior end there was another force. This force he assumed to originate from a gelation process, which by means of dehydration produced contraction ("Gelatinierungsdruck"). He believed the contraction to serve the same function as the surface tension forces which constituted the moving agent in amoebae moving with fountain-currents.

Rhumbler is not quite clear regarding the relative importance of the surface tension forces at the anterior end and the contraction at the posterior end. It is obvious, however, that he believed in the existence of two different mechanisms in amoeboid movement, one of them no different in essence from the mechanisms proposed by the adherents of the earlier contractility theories. It is remarkable, however, that in a later paper (1907) he still seemed to think that the mechanism of amoeboid movement could be fully explained by the activity of surface tension forces.

The forces at the surface were somewhat more accurately analyzed by Jensen (1901, 1902). He considered these forces to consist (1) of a normal pressure (the resultant of the forces of attraction by the molecules in the fluid), and (2) of a pressure due to the curvature of the surface $\left(\frac{=2T}{R}\right)$. The sum of these forces he designated as surface pressure. A decrease of either of these two forces would result in a bulging out of the fluid, since the pressure elsewhere would remain the same. As this happened, the radius of the curvature at this place would become smaller and the pressure due to the curvature of the surface would increase. If the reduction of the pressure at the surface were not too great, an equilibrium would be established. A withdrawal of the protrusion would occur if the surface pressure increased again. Jensen so explained the formation and retraction of short blunt pseudopodia, and said that long filamentous pseudopodia would result whenever the surface pressure at the tip continued to be less than at the rest of the surface.

Jensen's analysis did not introduce a new concept of amoeboid motion. The factors determining the force, which he termed surface pressure, are essentially the same as those which determine the force to which the previous authors refer as surface tension. Moreover, his analysis insufficiently explains amoeboid movement, since he did not consider continuous progressive movement.

There have been several attempts to explain the mechanism which would bring about the changes in surface tension resulting in amoeboid movement. Most of these explanations are purely hypothetical. As mentioned before, Bütschli (1892) believed that saponaceous compounds were released from the cell and, by contact with the surface, locally reduced the surface tension. Other investigators thought that the changes in surface tension resulted from metabolic changes in the

cell. For example, Verworn (1892, 1894) associated them with the chemical activity of oxygen. He based this opinion largely on experiments by Kühne (1864), who had shown that in an oxygen-free atmosphere amoebae stopped moving and remained fixed in the amoeboid shape they had assumed during their movement.

The hypotheses advanced by Hirschfeld (1909) and McClendon (1911) were founded on the fact that the surface tension is dependent on the interfacial potential. According to Hirschfeld the CO_2 produced by the amoeba gives its proteins an electric charge, by which the amoeba is able to react with ions or other charged particles, with resultant changes in surface tension. McClendon was more specific. He believed that the permeability of the surface membranes of amoebae was greater for cations than for anions, and that therefore HCO_3^- and $CO_3^=$ ions would accumulate in the cell, while the H^+ ions would penetrate the membrane. This would cause an electric polarization of the membrane, which would vary in proportion with the CO_2 production. Local differences in CO_2 production could thus cause local differences in polarization, which in turn would result in local changes in surface tension. McClendon experimentally showed Bütschli's thesis, according to which saponaceous substances lowered the surface tension and thus produced movements, to be incorrect. By means of a fine pipet he brought a soap solution near an amoeba and found that the amoeba moved away from the soap solution instead of toward it, as would be the case if Bütschli's hypothesis were correct. McClendon explained this by assuming that the soap solution increased the permeability for anions.

Still another explanation for a mechanism to lower the surface tension was proposed by Fürth (1922). He believed that lactic acid was produced during amoeboid movement. Since lactic acid lowers the surface tension of protein suspensions, Fürth supposed that the local production of lactic acid in amoeba would similarly result in a local reduction in surface tension. He believed also that swelling of protoplasmic particles, as a result of lactic acid production, plays a role in amoeboid movement.

That surface tension forces are active in the movement and changes in form of amoebae and white blood cells was a view accepted by many other investigators, including K. Gruber (1912), Haberlandt (1919), Tait (1918–1920), and others. A discussion of their work is omitted, since their views do not differ materially from those presented above.

Criticism and Discussion of the Surface Tension Theories of Amoeboid Movement

The surface tension theories of amoeboid movement rested mainly on the assumption that protoplasm is fluid, and hence its behavior is governed by the same physical laws which determine the behavior of a liquid in contact with a solid and (or) another liquid. That protoplasm actually follows these laws was thought to be proved by the similarity of the currents in a moving amoeba to the currents in a drop set in motion by a local lowering of the surface tension.

The currents which occur in a drop moving under the influence of a locally lowered surface tension are termed "fountain currents." They are characterized by an axial current in the direction of movement, which at the anterior end flows outward toward the sides and then moves backward along the surface. At the posterior end the current bends inward to form the axial current (Fig. 3). According to Bütschli (1892), the backward current in moving amoebae comes to rest with respect to the substrate soon after it has turned backward. In this he supported F. E. Schulze's (1875) descriptions (Fig. 1). Although these observations showed that the currents in a moving amoeba are not entirely identical with those in a moving drop, Bütschli believed that they were enough alike to substantiate his theory of amoeboid movement. The difference he explained as due to the greater viscosity of protoplasm. He also stated that in drops moving very slowly, such short backward currents sometimes occur.

Rhumbler (1898a, b, 1905) and also K. Gruber (1912) believed that true fountain currents occur in moving amoebae, but only occasionally. For amoebae moving without fountain currents, Rhumbler proposed a different mechanism, in which contraction caused by gelation replaced at least partially the surface forces. However, he still seemed to believe the surface tension theory to be generally applicable (Rhumbler, 1907).

Jennings (1904) was the first to claim that the currents in a moving amoeba differ essentially from those in a drop of fluid whose surface tension

is locally lowered. On this basis he rejected the theories which explained amoeboid movement as caused by a local decrease in surface tension. This investigator made a careful analysis of the currents in *Amoeba limax*, *A. proteus*, *A. angulata*, *A. verrucosa*, and *A. sphaeronucleolus*, by studying the movements of their granules. He concluded that there is no backward marginal current; that in reality the protoplasm here is at rest. He said that the impression that such a current exists is created by contrast with the forward axial current. Jennings' account of the currents in amoebae in locomotion is as follows (Fig. 5A).

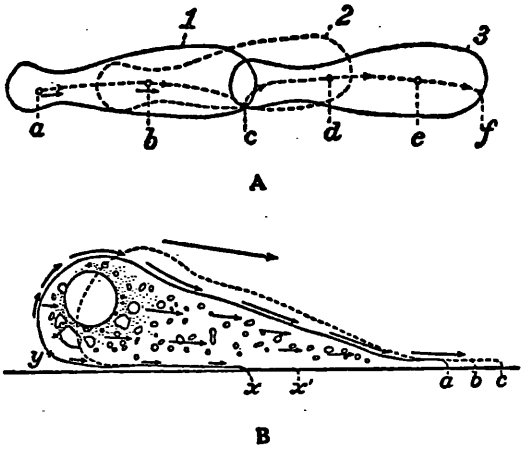

FIG. 5. A, DIAGRAM OF THE MOVEMENT OF A PARTICLE IN A MOVING AMOEBA. B, "ROLLING MOVEMENT" IN AMOEBA ACCORDING TO JENNINGS

A, As seen from above (Jennings, 1904). B, Seen in side view. For explanation see text (Jennings, 1904).

In the direction of movement there is an axial current which spreads out fanlike at the anterior end, so that some of the granules closely approach the lateral margin of the amoeba. Here the granules stop, while the central current goes on. The rest of the substance of the amoeba advances, but these granules, located at the lateral border of the cell, remain at rest with respect to objects in space. Finally the posterior end of the main body of the amoeba reaches these granules and they are then taken up in the central current, pass to the anterior end, to come again to rest. Jennings said that at no point of their course do the granules move backward with respect to the substrate.

Jennings also studied the movements at the surface of a moving amoeba. He mixed soot with the water in which the amoebae were present and observed the behavior of the soot particles which adhered to them. By this method he was able to determine that in *Amoeba verrucosa* and in *A. sphaeronucleolus* the upper surface moves forward, rolls over the advancing edge, and then comes to rest. At the posterior edge the surface moves upward and then at the upper surface again moves forward. In other words, the surface of these species of *Amoeba* rotates in the direction of movement; they progress by means of a rolling motion. It is as if a rubber sac filled with fluid were rolling over a solid support, the rubber sac representing the ectosarc and the fluid contents the endosarc.

Jennings found that at one important point the movement of such an object differs from that of an amoeba. This is best made clear with the aid of Jennings' diagram (Fig. 5B). The amoeba is attached to the substrate at its anterior end (from a to x). The upper surface moves forward as indicated by the arrows. A similar current is also present inside the amoeba. As the amoeba moves forward, the upper surface rolls over and the anterior edge moves from a to b and subsequently to c. The posterior edge of the area attached to the substrate (x) becomes free and the posterior margin is now at x^1. The material at the surface in contact with the substrate is at rest with respect to points in space. But the lower surface posterior to x and not in contact with the substrate is not at rest, as would be expected if the movement were a true rolling movement. Rather, it moves slowly forward. Jennings said this forward movement is caused by a contraction of the unattached posterior portion of the endosarc. Here not only the upper surface moves forward, but also particles on the sides and under surface. He held that this contraction does not occur in that part of the amoeba which is in contact with the substrate but begins at once when the attachment ceases.

The essential features of the movement of amoebae as described by Jennings are the same as those of drops which move as a result of one-sided adherence to a solid substrate. This seemed to strengthen the theory of amoeboid movement proposed by Berthold in 1886. However, Jennings did not believe that amoebae moved by a one-sided adherence caused by spreading on a solid substrate, for this explanation did not account for the free extension of pseudopodia into the water—the same objection which made Berthold's

theory inadequate. While Jennings concluded that surface tension forces were not responsible for the locomotion of amoebae, he did not propose a new theory and said the physical factors involved in the mechanism of amoeboid movement were as yet unknown.

It is rather curious that Jennings did not attach more significance to his observations on the contraction of the ectosarc. He did not doubt that the outer layer of *Amoeba* had the power of contraction, and he reported several observations giving evidence for this. Also, he observed that during locomotion a contraction of the protoplasm took place by which the posterior half of the cell body was pulled forward. However, he seemed to believe that this contraction played no active role in the progressive movement of amoebae, but was an inessential secondary phenomenon.

The surface tension theories were rejected also by Dellinger (1906), this time on the basis of the fact that an amoeba does not have the shape nor the behavior of a drop of fluid in contact with a solid substrate. By means of an ingenious technique Dellinger observed the movement of *Amoeba* and *Difflugia* from the side. Viewing them this way, he found that *Difflugia* moved in the following fashion (Fig. 6A). A pseudopodium is extended free in the water and becomes attached at the tip. This pseudopodium contracts and draws the shell up to the point of attachment. While this happens a new pseudopodium arises at the base of the first one and increases in length. This new pseudopodium subsequently becomes attached and then begins to contract, and the process is repeated. According to Dellinger, *Amoeba verrucosa* moves with the rolling motion described by Jennings, but other forms move in essentially the same fashion as *Difflugia*. For example, he found that in *Amoeba proteus* (Fig. 6B) the anterior end is stretched out into the medium and then becomes attached at the tip. Meanwhile, the posterior end contracts. Then a new protrusion is formed at the anterior end, which again becomes attached to the substrate, while the contraction continues at the other end.

It is obvious from Dellinger's observations that an amoeba cannot be regarded as a drop of fluid, and that the physical laws governing the behavior of fluid cannot be applied to the locomotion of amoebae, as is required by the surface tension theories. Dellinger did not make a detailed study of the protoplasmic processes involved in the mechanism of this "walking movement." He concluded that the movements of *Amoeba* and *Difflugia* are caused by a contractile substance, which is distributed through the cell as a coarse reticulum, the physical nature of which he did not define. In this respect his theory did not differ from the earlier contractility theories.

Hyman (1917) confirmed Dellingers's observations and brought forward further evidence that amoeboid organisms have properties which indicate that their protoplasm is not in a fluid state.

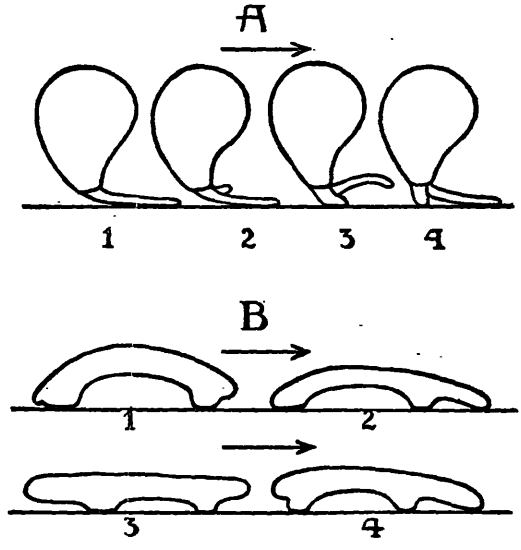

FIG. 6. "WALKING MOVEMENT" IN DIFFLUGIA SPIRALIS (A) AND IN AMOEBA PROTEUS (B), AS SEEN IN SIDE VIEW

For explanation see text. After O. P. Dellinger (1906).

As a result, this investigator also decided that considerations regarding the surface tension at liquid interfaces are not applicable to amoeboid movement. The theory she did evolve is presented in the following section on sol-gel changes.

That the surface tension theory could not explain the movement of the pseudopodia of amoebae feeding on paramecia was demonstrated by Mast and Root (1916). They observed that the pseudopodia of *Amoeba* could develop enough force to cut paramecia in half. When they calculated how much force was needed to cut through a paramecium, they found that the necessary reduction in surface tension would exceed by many times the surface tension of protoplasm.

Beers (1924) came to the same conclusion regarding *Amoeba* feeding on *Frontonia*.

However, the surface tension theories of amoeboid movement were well entrenched. Although the criticism of them was well founded, they were not immediately abandoned. Even after their primary postulate—that protoplasm was a fluid and behaved physically as a fluid—was proved incorrect, they were relinquished only reluctantly. The endurance of the surface tension theories in the face of observations indicating their inadequacy perhaps is explained by the desire to interpret vital phenomena in terms of simple physical or chemical processes. The surface tension theories seemed to have a physical exactness which was lacking in the early contractility theories, in which the term "contractility" stood for a phenomenon which could not be further analyzed on a physical or chemical basis. However, in this respect the expression "protoplasmic contractility" does not differ from many other terms used in biology to describe concepts of which the physical nature is obscure. As will be seen, the present views on the mechanism of amoeboid movement are closer to the early contractility theories than to the surface tension theories proposed later.

SOL-GEL TRANSFORMATIONS IN AMOEBOID MOVEMENT

At the beginning of this century, colloidal concepts began to be applied to the study of protoplasm. The recognition that protoplasm was a colloid not only provided a suitable terminology to describe the state of protoplasm but also gave a better insight into some of its properties. The study of the mechanism of amoeboid movement profited greatly from this development, and an accurate description of the protoplasmic processes, based on definite physical concepts, became possible.

Hyman (1917) was one of the first investigators to refer to differences in the consistency of the protoplasm of *Amoeba* in distinct colloid chemical terms. She pointed out that the ectoplasm has the properties of a gel whose degree of rigidity varies in the different species; she considered the endoplasm to be a sol of a varying viscosity. She explained amoeboid movement in the following way. Local metabolic changes in the superficial gel layer cause a solation. Because of the tension exerted by the ectoplasmic gel, the endoplasm flows out at this point, forming a pseudopodium. As the pseudopodium so formed comes in contact with the water, its surface gelates again; the metabolic change causing the solation is continuous in order to explain the continuous production of a pseudopodium. The tension of the ectoplasm which forces the pseudopodium forward is, according to Hyman, nothing else than the elastic tension characteristic of a colloidal gel.

In Hyman's view, the withdrawal of pseudopodia is essentially a process of gelation, during which the protoplasm develops the property of contractility, which she holds to be inherent in the gelation process: "I should therefore state emphatically that the property of protoplasm, which we call contractility, is nothing more or less than the gelation of an emulsoid colloidal solution; or, as we like to say in science, gelation is the 'cause' of contractility."

From this it may be concluded that Hyman believes that two forces are active in amoeboid movement: (1) a force which results from the elastic tension of ectoplasm and is active in the production of pseudopodia, and (2) a contractile force, which results from the gelation of protoplasm and which produces the withdrawal of pseudopodia. It is not quite clear whether Hyman believes this contraction to take place during the process of gelation, or after the gel is formed. If the latter, the contraction could be compared to the phenomenon of syneresis of gels. From the above quotation it appears that she thinks that a contraction accompanies the gelation process itself, but with regard to this contraction she refers to literature in which the syneretic contraction of gels is discussed. As to the force which results from the elastic tension of the ectoplasm, she does not state what force causes the stretching necessary to produce the elastic tension. Furthermore, her theory fails to account for that continuous action of either the elastic tension or the contraction which is necessary to explain progressive locomotion.

The continuity of these processes of gelation and solation involved in amoeboid movement was a particular concern of Pantin (1923), who further defined the nature of the locomotor force and the continuity of its action. Observing marine amoebae of the limax type, Pantin thought of a moving amoeba as a tube of gelated ectoplasm closed at its hind end and filled with fluid endoplasm (Fig. 7). He gave the following descrip-

tion of the processes of gelation and solation during amoeboid movement. As the endoplasm streams forward through the ectoplasmic tube, it gelates at the sides of the anterior end, thus adding to the gelated ectoplasm. In the middle the endoplasm continuously advances, because of a contraction of the ectoplasm. The endoplasmic stream originates at the closed end of the ectoplasmic tube, where solation is continuous, in pace with the gelation process at the open end. These protoplasmic processes are essentially the same as those described by Schulze, and the sol-gel changes correspond to Rhumbler's ectoplasm-endoplasm process. Pantin did not observe the "rolling movement" described by Jennings, nor Dellinger's "walking movement."

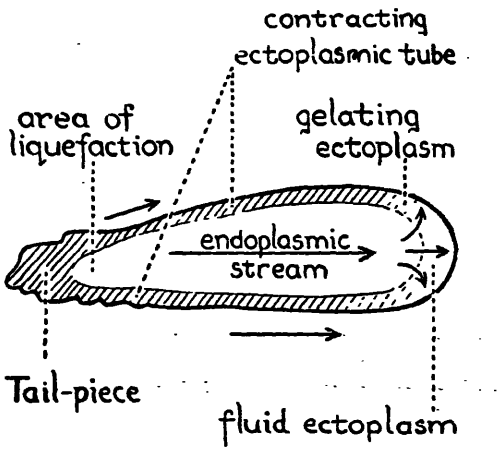

FIG. 7. SOL-GEL CHANGES IN MOVING AMOEBA
For explanation see text. After Pantin (1923).

Pantin also studied the effect on amoebae of osmotic pressure and came to certain conclusions regarding the mechanism of the contraction of the ectoplasm. He observed that in a hypertonic solution the entire amoeba came to look like the tail piece looks under normal conditions. From this he concluded that during locomotion water is abstracted from the posterior part. Because of the fluid appearance of the advancing pseudopodia, he assumed that water is here adsorbed. So, during locomotion, water would move from the posterior to the anterior part of the amoeba. By staining amoebae with neutral red, Pantin found that in an active pseudopodium there is a rise in the hydrogen ion concentration, which he believed to be the cause of the adsorption of water, resulting in swelling.

On the basis of these observations, Pantin suggested this hypothesis: A pseudopodium is extended as a result of a local acid-swelling of the protoplasm. This is accompanied by adsorption of water, which is abstracted from the posterior end. As this pseudopodium advances, a gelation process results in the forming of new ectoplasm. At the posterior end of the gelated tube of ectoplasm, water is lost by syneresis and a contraction takes place, driving forward the fluid endoplasm. During this contraction, gelated protoplasm is converted into fluid endoplasm, which is added to the endoplasmic stream. These processes result in continuous movement, since the transformation of ectoplasm into endoplasm is continuous at the posterior end, and at the anterior end the ectoplasmic tube is continuously built up by the gelation of endoplasm.

Mast (1926, 1931), who analyzed the structure of *Amoeba proteus* and the function of the different parts in locomotion, came to essentially the same conclusions as Pantin regarding solation and gelation during amoeboid movement. In addition, he described the character of the movement so as to explain the "rolling movement" observed by Jennings, as well as Dellinger's "walking movement." According to Mast, the endoplasm (plasmasol) is entirely surrounded by gelated ectoplasm (plasmagel) (Fig. 8). The amoeba is covered by a thin membrane (plasmalemma), which is separated from the plasmagel by a thin layer of fluid, except where it is in contact with the substrate. Here the plasmalemma adheres directly to the plasmagel. In Mast's theory the plasmasol is hypertonic and the plasmagel is semi-permeable and elastic, with the result that the plasmagel has an elastic tension. In locomotion, the plasmagel is locally weakened. Owing to the elastic contraction of the rest of the plasmagel, this weakened portion becomes thin and bulges out. There may be a break in the plasmagel at such a point, or the plasmagel tube may be open from the beginning (Mast, 1931). In these cases the plasmasol is in direct contact with the plasmalemma. If the advancing pseudopodium is attached to the substrate, the plasmalemma is drawn forward over the plasmagel at the upper surface, but remains stationary below; the result is a "rolling movement" of the plasmalemma. But when the pseudopodium remains free, the plasmalemma is stretched and moves forward equally on all sides, with no rolling movement.

Such a pseudopodium may become attached at the tip after it has been extended. Another pseudopodium may then be formed above it and, owing to the contraction at the posterior end of the body of the amoeba, moves over the first point of attachment. This results in "walking movement." In both cases the movement of the plasmagel and plasmasol is the same. The difference lies merely in whether the pseudopodium is attached to the substrate during its formation or becomes attached after it is formed.

As has been indicated, Mast agreed with Pantin that during locomotion plasmasol is changed into plasmagel at the anterior end and that a reverse change occurs at the opposite end. That is, the plasmagel tube at the anterior end is built up just as rapidly as it contracts and liquifies at the posterior end. The important point of difference between Mast's and Pantin's theory is in their concept of the nature of the locomotor force. For Pantin, the contraction of the plasmagel is the result of syneresis; Mast holds the plasmagel contraction to be a result of elastic recoil.

The movement of leukocytes in tissue cultures was studied by W. H. Lewis (1931, 1939, 1942), who found their manner of movement to be essentially like that of *Amoeba*, as described by Mast and Pantin. However, Lewis' concept of the locomotor force differs from that proposed by either Pantin or Mast. Lewis employed the time-lapse photomicrography technique for his studies. By projecting the film on paper and making tracings of the outlines of the cells at closely spaced intervals, he obtained accurate records of their changes in form during locomotion. As a leukocyte advances, a rigid mass of protoplasm drags behind the cell body so that the cell in motion resembles in outline the shape of a handmirror. A broad pseudopodium is present at the anterior end.

Lewis observed that when a leukocyte moves, a constriction develops at the point where the pseudopodium joins the cell body. This constriction remains stationary with regard to objects outside the cell while the leukocyte moves forward. During locomotion a succession of such "constriction rings" appears at regular intervals. Lewis assumed that the protoplasm composing the "constriction ring" remains there until the tail is reached. Thus all the protoplasm posterior to the ring, with the exception of the part composing the tail, must pass through the ring. As the "constriction ring" reaches the tail, the tail increases in length; between constriction rings, the tail shortens again. This explanation of the constriction rings is based on the assumption that white blood cells, like amoebae, have a superficial plasmagel layer, which has a contractile tension varying with its thickness or density. A local increase in thickness of the super-

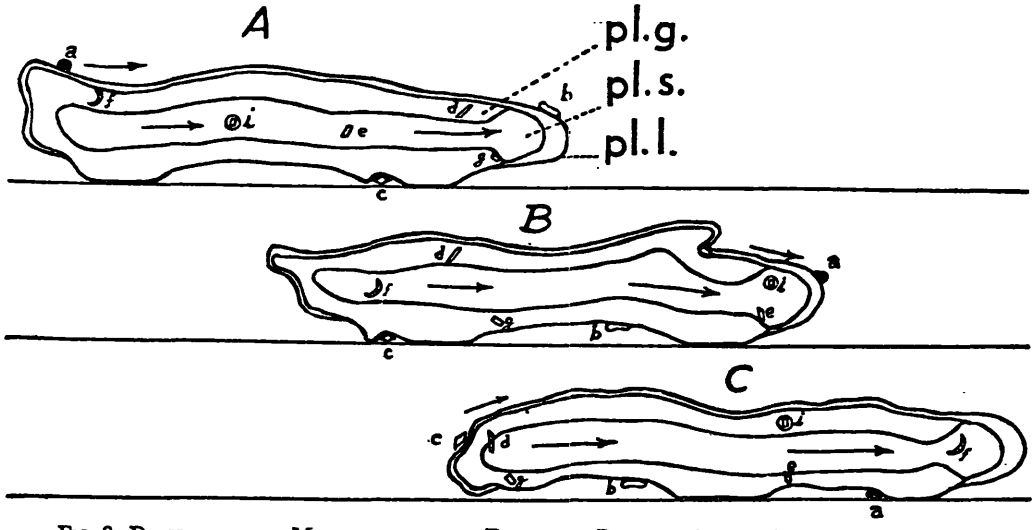

FIG. 8. DIAGRAM OF THE MOVEMENTS OF THE DIFFERENT PARTS OF AMOEBA DURING LOCOMOTION

a, b, c, particles attached to the plasmalemma (pl. l.). d, e, f, g, i, particles in plasmagel (pl. g.) and lasmasol (pl. s). (S. O. Mast, 1926).

ficial plasmagel would result in a greater contractile tension and so produce a local constriction.

Based on the above observations and assumptions, Lewis proposed an explanation for the mechanism of the locomotion of white blood cells (Fig. 9). "The contraction of the superficial plasmagel layer thrusts out a pseudopod at the weakened area and forces the endoplasm and nucleus forward through the constriction ring which does not move forward. As the plasmagel layer posterior to the constriction ring contracts, it becomes reduced in extent, gradually goes into solution until finally nothing remains but the constriction ring which then contracts down and

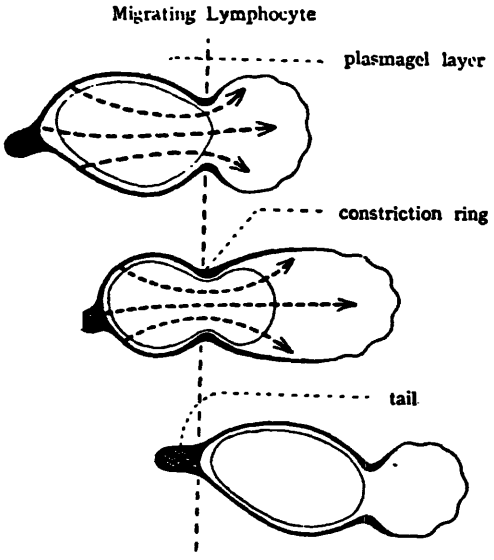

FIG. 9. LYMPHOCYTE MOVING WITH CONSTRICTION RING

For explanation see text (Lewis, 1939).

increases the length of the somewhat shortened gelated tail. The distortion of the nucleus indicates that the constriction ring exerts considerable tension. The decrease in the size of the tail in the second position indicates that some of it has gone into solution. Anterior to the constriction ring a new plasmagel layer is built up by progressive gelation at the surface from the ring inward. As the cell moves forward new constriction rings appear and the same process is repeated over and over."

As indicated above, the only difference between Lewis' hypothesis and the theories of Pantin and Mast is in Lewis' explanation of the locomotor force. Lewis regards the contractile tension exerted by the plasmagel as intimately associated with the process of gelation. "My idea is that protoplasm, like most colloids, automatically exerts contractile tension when it gels."

The investigations of De Bruyn (1946) on the movement of white blood cells in tissue cultures produced different results. Using essentially the same technique employed by Lewis, De Bruyn found that when two or more cells followed precisely the same path in the culture, they had constriction rings at precisely the same places. This indicates that constriction rings are caused by external factors and so cannot play an essential role in the locomotion of leukocytes; neither can they constitute the basis of any theories as to the mechanism of amoeboid movement.

De Bruyn observed further that when the movement of the leukocytes is speeded up by means of time-lapse photography, the sides of the cell seem to exhibit an undulating motion as if fine waves were passing over the surface from the anterior to the posterior part of the cell. Analyzing this phenomenon by means of tracings of the outlines of the cells at regular intervals, he found that actually the "waves" were stationary with respect to objects outside the cell (Fig. 10). A lateral part of the anterior pseudopodial area becomes immobilized, while the cell itself continues to advance. This particular part forms a protuberance on the lateral outline of the cell. As the tail end of the cell approaches this protuberance, the latter gradually diminishes in size, its surface becomes wrinkled and it finally shrinks away completely at the point where the tail joins the cell body. Observations on the behavior of the granules in heterophil leukocytes in locomotion indicated that the granules located in such a lateral protuberance, and frequently those located along the non-protuberant lateral outline of the cell, remain stationary with regard to points outside the cell. These granules remain stationary until they are reached by the posterior part of the cell, then they begin to participate in the streaming, which is generally in a forward direction.

De Bruyn agreed with Lewis that the movement of amoebae and leukocytes is of essentially the same nature, as indicated by (1) the similarity in the shape of moving leukocytes and moving amoebae, and (2) the behavior of the lateral protuberances as well as of the granules, which shows that in moving leukocytes, as in moving

amoebae, the lateral part of the protoplasm is stationary. Since in *Amoeba* it is the plasmagel which is stationary, it can be assumed that in leukocytes there is a similar superficial plasmagel layer, which is subjected to the same gelation process at the anterior end and solation process at the posterior end as described in *Amoeba* by Mast and Pantin. Making this assumption, De Bruyn interpreted his observations as follows. In leukocytes, the highly mobile anterior pseudopodial area consists of plasmasol, while the rigid posterior part is in the gel state. During locomotion the lateral part of the anterior plasmasol gelates and becomes immobilized. As the cell progresses this part of the plasmagel remains stationary with regard to objects outside the cell. A solation process must take place in the cell body, since plasmasol is continuously available at the anterior end. Besides giving evidence for the presence of a stationary superficial gel layer, the behavior of the lateral protuberances also indicates that a process of contraction takes place in the plasmagel. This contraction of the plasmagel has been observed only locally, i. e., in the lateral protuberances, and although this would be sufficient to account for a forward driving of the plasmasol, De Bruyn holds it probable that in analogy with the plasmagel in *Amoeba*, the whole plasmagel participates in the process of contraction.

That reversible sol ⇄ gel changes play an essential role in the mechanism of amoeboid movement seems at present well established by the observations of the above-mentioned investigators on moving amoebae and leukocytes. The question arises as to what are the causal factors of these reversible sol ⇄ gel changes. Various investigators have attempted to answer this question with very different results. Hyman (1917) believes that the sol ⇄ gel changes involve a reversal in phase in the sense that in the sol state the colloidal particles are dispersed in the fluid phase, while in the gel state the fluid phase is dispersed and the colloidal material forms the continuous

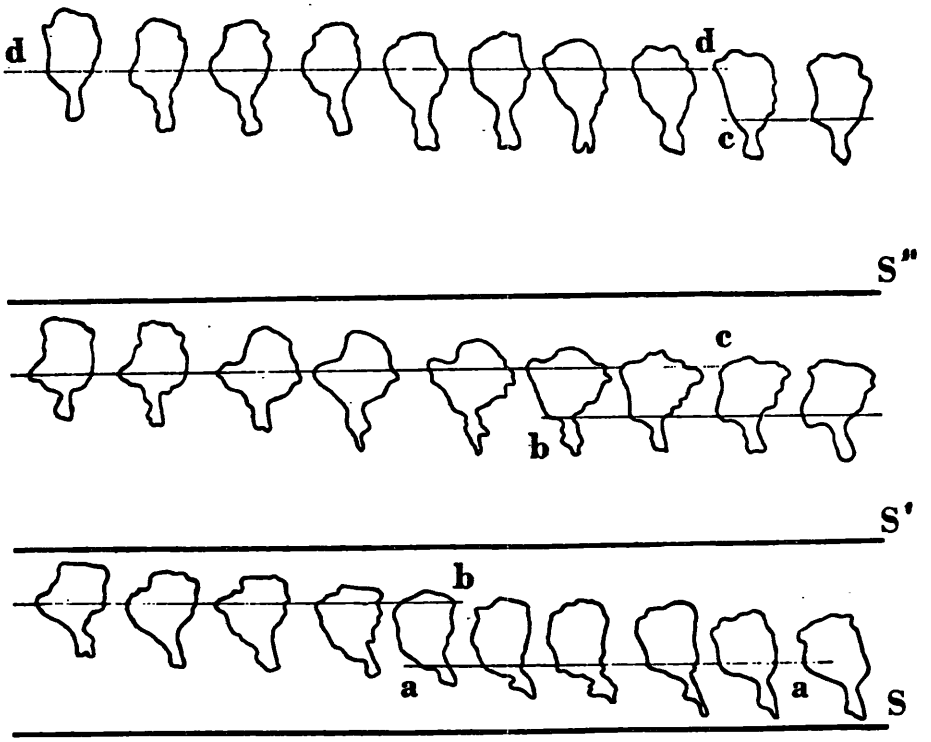

FIG. 10. OUTLINES OF A LYMPHOCYTE MOVING IN TISSUE CULTURES

The cell moves away from S(S', S''). a–a, b–b, c–c, d–d: stationary lateral protuberances. S, S', S'' are continuations of the same line. For further explanation see text. (De Bruyn, 1946.)

phase. These reversals in phase would be brought about by chemical changes originating within the amoeba. The external stimuli, which lead to the reversals in phase and thus to movement, would act via the protoplasm of the amoeba. While Hyman does not deny that an external stimulus can bring about liquefaction or gelation directly, she holds this to be improbable under the usual conditions.

Pantin (1923) related the gelation at the anterior end to the rise in hydrogen ion concentration which he observed, and which he said resulted in an increase in surface tension. Fürth thought that for the same reason the surface tension would be decreased. However, Pantin pointed out that while this is true for a protein-water interface, at a lipoid-water interface a rise in the hydrogen ion concentration results in an increase of surface tension. Since there is evidence that the cell surface is of a lipoid nature, he assumed that an increase in acidity raises the surface tension in this area so that forward movement results from work done against the increased surface tension (see also A. A. Schaeffer, 1920). However, Pantin believed that at the freshly formed surface at the sides of the advancing pseudopodium, proteins and other substances which tend to lower the surface tension will accumulate, in accordance with Gibbs' principle, so as to lower the free surface energy. This would account for the gelation of endoplasm. At the posterior end the hydrogen ion concentration is lower, the surface tension falls, and the concentration of substances at the surface diminishes.

Pantin's ingenious hypothesis of the mechanism of the sol $\rightleftarrows$ gel changes is based on the presence of an increased hydrogen ion concentration at the anterior end of a moving amoeba. But Mast (1926), using neutral red, was unable to find a difference in pH between the anterior and posterior parts of *Amoeba proteus*. From this he concluded that if the hydrogen ion concentration regulates solation and gelation, these processes must respond to smaller differences in pH than are detectable by neutral red; that is, any adjustment between sol $\rightleftarrows$ gel changes and changes in pH must be very delicate.

Chalkley (1929) believed that hydration and dehydration are involved in the transformation of plasmasol to plasmagel and the reverse. He found that changes in the gel/sol ratio and in the water content of *Amoeba* depend on the hydrogen ion concentration. The curves he obtained by plotting either the gel/sol ratio or the water content of *Amoeba* against the hydrogen ion concentration of the medium were similar to the curves obtained for the changes in hydration of an amphoteric hydrogel like gelatin under the influence of different hydrogen ion concentrations. On the basis of these observations, Chalkley postulated that there is in the protoplasm of *Amoeba* a colloid like gelatin which controls the water content as well as the gel/sol ratio. Because the water content changes directly with the gel/sol ratio, he believed that an increase of plasmagel, relative to plasmasol, was caused by the formation of plasmagel that resulted from hydration of this interprotoplasmic colloid. In the transformation of plasmagel to plasmasol a dehydration of this colloid would occur, resulting in flocculation.

The work of Chalkley indicates that the processes of solation and gelation in *Amoeba* are influenced by the medium. Even in 1905, Rhumbler assumed that ectoplasm was formed as a direct result of contact with the water. Since then it has been found that many factors influence solation and gelation in *Amoeba*. Temperature, salt content, and hydrogen ion concentration have a marked effect on the gel/sol ratio and the rate of locomotion. There is a complicated interaction between these factors in their effect on this ratio (Mast and Prosser, 1932; Pitts and Mast, 1933, 1934a, b). Heilbrunn and Daugherty (1934), studying the effect of similar factors on the viscosity of the plasmagel, found a correlation between plasmagel liquefaction and stoppage of movement. However, Mast and his coworkers did not detect a direct or specific correlation between the gel/sol ratio and the rate of locomotion.

These investigations on the influence of the above-mentioned physical and chemical factors on the gel/sol ratio do not indicate whether these factors play a role in the processes of gelation and solation as these occur in an amoeba, where they are an integral part of the mechanism of progressive movement. The relative thickness of the plasmagel and plasmasol may be only partially or not at all dependent on these processes. According to Pantin (1924), the rate of amoeboid activity is dependent on the rate at which the protoplasm can effect sol $\rightleftarrows$ gel changes. If this is true, then the lack of correlation between the rate of locomotion and the gel/sol ratio might indicate that the factors influencing the gelation and solation

processes at the anterior and posterior end certainly do not have a corresponding effect on the gel/sol ratio. On the other hand, Pantin's hypothesis also permits the interpretation that changes in the gel/sol ratio are caused by changes in the processes of gelation and solation at the anterior and posterior ends of the cell and thus are influenced by the same factors. The lack of correlation between the gel/sol ratio and rate of locomotion may then be explained on the basis that the factors increasing the rate of gelation do not affect equally the rate of solation, which would be necessary for an increase in the rate of locomotion. Finally, it is possible that the factors affecting the gel/sol ratio influence the rate of locomotion by another action, affecting, for example, the magnitude of the locomotive force, the absolute viscosity plasmasol, or "by their action on the surface, affecting adhesiveness and other properties of the surface, as well as by their action on permeability of the surface membrane" (Pitts and Mast, 1934b).

There is at present considerable evidence that protoplasm has thixotropic properties (Angerer, 1936; and others). The possibility that the sol ⇄ gel changes in amoeboid movement are based on this property has to be considered. The fact that amoebae in an electric current move toward the cathode, Heilbrunn and Daugherty (1939) ascribed to the thixotropic properties of the plasmagel. According to them, the protoplasmic particles in the amoeba have a positive charge and so move toward the cathode. The mechanical impact of the granules against the plasmagel produces a thixotropic solation by which a pseudopodium is formed. The fact that amoebae whose protoplasm is more alkaline move toward the anode they explain as resulting from a reversal of the charge of the protoplasmic particles.

A mechanism of the sol ⇄ gel changes during amoeboid movement based on the phenomenon of thixotropy has been proposed by Fenn (1945). He considers it possible that "the change from gel to sol during contraction is merely the result of movement which mechanically breaks up the gel, only to permit it to reform as soon as the movement ceases at the tip of the newly formed pseudopod." This hypothesis is particularly attractive, since it seems to explain the fact that there is quantitative correlation between the rate of gelation and the rate of solation in a moving amoeba. This becomes quite understandable if both processes are dependent on the same factor, i.e., on the mechanical agitation caused by the contraction of the plasmagel. In connection with this it is of interest to note that Freundlich and Soellner (1928) found that in thixotropic iron oxide sols the time necessary to set into a gel greatly depends on the pH. A similar phenomenon in the plasmasol of a moving amoeba would result in a dependence of the gel/sol ratio on the hydrogen ion concentration, without a correlation between this and the rate of locomotion. This is precisely what Mast and his coworkers found. As will be seen later, the protoplasmic changes in amoeboid movement are indeed very like the colloidal changes in a thixotropic gel.

THE NATURE OF THE LOCOMOTOR FORCE

Today most investigators agree that a contraction of the plasmagel is responsible for amoeboid movement, but there are varying opinions as to the physical nature of this contraction. W. H. Lewis (1931, 1939, 1942) says contraction is caused by the gelation process itself. Hyman (1917) also identified contraction with gelation. In connection with this hypothesis, Heyman's dilatometric investigations (1935, 1936) of the changes in volume during sol ⇄ gel transformations are of importance. He found that whether gelation is accompanied by an increase, a decrease, or by no change in volume depends on the type of sol. The thixotropic isothermal sol ⇄ gel change of an iron hydroxide sol is accompanied by no change in volume; during the setting of a methyl cellulose sol the volume increases; whereas a gelatin or agar sol decreases in volume during gelation. These changes in volume are extremely small; for example, during the setting of a 4.2 per cent gelatin sol the decrease is only 0.0026 per cent of the total volume. It seems doubtful whether such small changes could result in a contraction sufficient to produce the displacement of material seen in amoeboid movement. In addition, the work of Marsland and Brown (1936) indicates that the protoplasmic sol ⇄ gel transformation probably is not accompanied by a decrease in volume. Studying the effect of hydrostatic pressure on the protoplasm of *Amoeba dubia* and *A. proteus*, these investigators found that high hydrostatic pressures have a liquefying action on the plasmagel. From this, Marsland (1942) concluded that the protoplasmic sols are of the methyl cellulose type, which tend to increase in volume on gelation, a theory supported by the fact that gelatin sols, which

during gelation decrease in volume, gelate under pressure (Marsland and Brown, 1939). It seems, therefore, that the initial sol-gel transformation cannot of itself produce a contractile tension in the plasmagel.

An alternative interpretation of Hyman's or Lewis' hypothesis is that the tension in the plasmagel results from the decrease in volume which take place when a hydrophilic colloid dissolves or swells in a liquid. The volume reduction in such a system is considerably larger than the volume changes during sol $\rightleftarrows$ gel transformations (Neville and Theis, 1930). However, if such a process took place during the transformation of plasmasol into plasmagel, although there would be a contraction at the anterior end, the contraction at the posterior end, necessary to drive forward the plasmasol, could not be accounted for.

According to Mast (1926), the plasmagel is elastic and semipermeable, whereas the plasmasol is hypertonic. He said that as a consequence the plasmagel is in a state of elastic tension, and its contraction is an elastic recoil. This explanation can hold only if the plasmagel forms a closed tube system, but Mast reported later (1931) that the plasmagel tube frequently is open at the anterior end. In these cases the plasmagel can not be stretched by osmotic turgor. Since it is improbable that the mechanism of movement in amoebae with an open plasmagel tube is different from that in amoebae with a closed tube, it seems likely that an elastic recoil as a result of osmotic turgor cannot cause the contraction of the plasmagel.

Pantin (1923) believed that the contraction is caused by syneresis of the plasmagel, principally because he found evidence for an abstraction of water from the posterior part of the plasmagel. It was pointed out by De Bruyn (1946) that the sequence of protoplasmic changes in amoeboid movement shows a remarkable resemblance to the isothermal, reversible sol syneresis described by Heller(1942) in thixotropic iron oxide sols. The hypothesis that a similar process occurs in protoplasm during amoeboid movement would incorporate not only Pantin's theory but also Fenn's proposal, according to which the sol $\rightleftarrows$ gel changes are the result of the thixotropic properties of protoplasm. However, it is uncertain whether the physical basis of the colloidal changes in thixotropic hydroxide sols is actually the same as for the changes in protoplasm during amoeboid movement. In this connection it is of interest to note that the essential protoplasmic phenomena occurring during amoeboid movement are exhibited in vitro by plasmosin, the structural protein obtained by Bensley (1938) from the cytoplasm of liver cells. Bensley points out that the properties of plasmosin may well explain the reversible gelation of protoplasm. Plasmosin also shows a strong syneretic contraction.

Whatever the nature of the plasmagel contraction may be, the processes involved in locomotion indicate that contraction is greater at the posterior end than at the anterior end of the plasmagel and that there is a gradient in the rigidity of the plasmagel (Mast, 1926). Marsland and Brown (1936) found experimental evidence for the presence of such a gradient, and certainly a gradient would be expected if the contraction of the plasmagel is a syneretic process.

Seifriz' (1942, 1943) views are different from the ones discussed above. He believes that sol $\rightleftarrows$ gel changes are not an essential feature of the mechamism of amoeboid movement and says that the process of contraction is not limited to the plasmagel, but that the fluid protoplasm is equally capable of contraction. He does not elaborate his hypothesis, neither does he explain how his views account for the protoplasmic processes observed in amoeboid movement.

It is of interest to inquire whether the newer concepts of protoplasmic structure provide an adequate basis for the contraction as well as for the other processes observed in protoplasm during amoeboid movement. The prevailing concept is that the molecular structure of protoplasm is made up of a random arrangement of polypeptide chains, held together by cross-linkages of the side chains (Peters, 1937; Frey-Wyssling, 1938, 1940; Seifriz, 1942). Water, salt, carbohydrates, and fats are present in the meshes of this molecular network. According to Frey-Wyssling, side chains in varying numbers remain free. Because the side chains are never all loosened, the protoplasm is never in a true fluid state; however, as the number of loosened bonds increases, the protoplasm approaches the fluid state more closely. In streaming, the side chains would constantly be broken and reunited. Contraction can occur in such a system in two ways. First, the binding of additional side chains would cause the meshes of the three-dimensional reticulum to become narrower. Another possibility is that contraction

occurs owing to the folding of the polypeptide chains themselves (Astbury, 1939). In addition to shortening the individual polypeptide chains, this action would also narrow the meshes of the molecular reticulum, if the side linkages were maintained during the process.

In either case, contraction would be accompanied by a displacement of water, which would be taken up by previously contracted protoplasm by the reverse processes, i.e., by breaking up of side-chain linkages and/or by spring-like extension of the folded polypeptide chains. Both would produce a forward movement of the protoplasm. The process is essentially in agreement with Pantin's hypothesis, according to which water is displaced by the syneretic contraction of the plasmagel and water is absorbed at the anterior end. There is some experimental, but not conclusive, evidence that there is a differential imbibition of water in an amoeboid moving cell. When Pantin (1923) observed that a whole amoeba in hypertonic solution looks like the tail end normally looks, he concluded that during amoeboid movement water is abstracted from the tail end. De Bruyn (1946) noted that in moving leukocytes there was a wrinkling of the surface at the tail and at the lateral protuberances as they were approached by the posterior end. A similar wrinkling of the surface was observed by Mast and Doyle (1934) in amoebae, and they ascribed this appearance to a loss of water. If wrinkling of the surface in leukocytes also indicates a loss of water, it follows that the contraction of the cytoplasm during the amoeboid movement of leukocytes is accompanied by an abstraction of water.

SUMMARY

The various theories of amoeboid movement which have been proposed in the course of time have been closely related to prevailing concepts of protoplasmic structure. The early theories of amoeboid movement were based on the general protoplasmic property of contractility. To explain the physical basis of the contraction process, a contractile reticulum was postulated as the essential feature of protoplasmic structure. However, no conclusive microscopic evidence could be found for such a structure, so the early contractility theories of amoeboid movement gave way to surface tension theories. In these, amoeboid movement was seen as analogous to the behavior of a drop of fluid under the influence of a local lowering of the surface tension. The validity of these theories was questioned in turn when it was found that the currents in such drops were not entirely like the currents in an amoeboid moving cell. After it had been demonstrated that the protoplasm at the surface is not in a fluid state, making inapplicable considerations regarding the surface tension at liquid interfaces, the surface tension theories became untenable.

The introduction of colloid chemical concepts permitted for the first time an accurate description in colloidal terms of the protoplasmic processes in amoeboid movement, and the importance of reversible sol $\rightleftarrows$ gel changes was established.

The present theories of amoeboid movement resemble the early contractility theories in that protoplasmic contractility is again thought to be the locomotor force. The manner by which amoeboid moving cells progress has been established by observations on the movement of the different parts of the protoplasm, relative to each other and to points outside the cell. From these observations, it has been concluded that the contractility of the plasmagel is largely responsible for the movement.

To explain this process of contraction, protoplasm is conceived to be a three-dimensional network of protein chains, linked together by cross-linkages of the side chains. In such a structure contraction may occur either by a rearrangement of the side chain connections, resulting in a narrowing of the meshes of the molecular reticulum, or by a folding of the protein chain themselves.

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