On the Physiology of Amœboid Movement. I.

By

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With Figures 1–10.

Of the relation of amœboid movement to other forms of contractility, very little is known at present. Hypotheses have been advanced to explain the movement, but they differ widely among themselves, and are founded almost entirely on direct observations of the normal activities of amœba. More recently Loeb (**24**) (**25**) and others have tried to determine the *rôle* of various environmental factors, such as the presence of certain salts, in amœboid activity. It is on these lines that the present work is being conducted.

Material, Methods, etc.

Marine amœbæ were used in the experiments, since sea-water as a medium has advantages over fresh-water for the following reasons :—

(1) The osmotic pressure of sea-water can be increased or reduced with ease, but it is almost impossible to determine the effects of hypotonic solutions on fresh-water organisms (compare Greely, **14**).

(2) Solutions can be prepared which are isotonic with sea-water, but which have certain ions in excess or deficit.

(3) The hydrogen ion concentration can be kept more constant.

(4) Marine amœbæ usually, if not invariably, possess no contractile vacuole : their physiology is therefore probably simpler than that of fresh-water amœbæ.

The material was obtained from an open tank, 4 ft. by 2½ ft., which was fed by a slow stream of sea-water almost continuously. The supply of sea-water was pumped from the Laboratory storage tanks. This water, which will be referred to as " tank water," contains more phosphates and more organic matter than does water from the open sea. An account of this " tank water " has been given by Allen and Nelson (**1**).

The hydrogen ion concentration of the water in the open tank varied from pH7·8 to pH8·0; these values are higher than that of the "tank water" supplied, probably owing to the presence of an abundant growth of diatoms and filamentous algæ.

The water used in the following experiments for cultures, solutions, and so on, was open-sea-water, hereafter referred to as "outside sea-water."

The bottom of the tank was covered with a film of brown algæ, associated with diatoms and enormous numbers of a small brown flagellate (Chilomonas). A few ciliates of very diverse forms were present, and there were about half a dozen species of Amœba, readily distinguished by the character of their granules, the nature of their pseudopodia, and the average size.

The amœbæ used in the experiments were usually of the "limax" type. This type has the great advantage for experimental work that locomotion takes place by the formation of a simple pseudopodium, which, continuously pushing forward, is followed by the rest of the amœba. Three different species were used, but so little is known of marine amœbæ that as yet it has not been possible to identify them with certainty. They will, therefore, be referred to as "Type A," "Type B," and "Type C."

Type A (*Fig.* 1, *No.* 1 *and* 2).

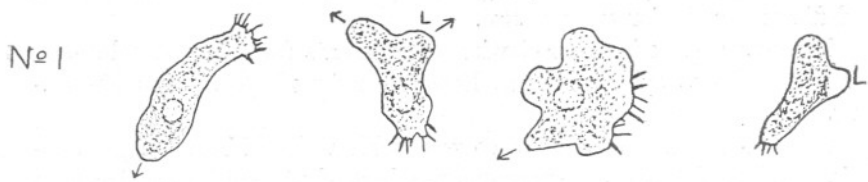
This amœba was usually of the limax form, varying in length from 50μ to 120μ, the average size being about 80μ long by 30μ wide. A large clear spherical nucleus was present. The advancing pseudopodium was large and rounded. At the hind end was a rugose tail-piece, which with careful observation could be seen to bear a number of fine clear processes, very different from the anterior advancing pseudopodium (Fig. 1, No. 2). These processes were capable of slow bending movements and of extension and retraction.

Although typically a limax form, this amœba often threw out a lateral pseudopodium (Fig. 1, No. 1). If this changed the direction of motion of the amœba, a fresh tail-piece developed at the new hinder end: the old tail-piece was either resorbed or else moved slowly towards the new hind end and ultimately fused with the new tail-piece (Fig. 1, No. 2). On stimulation the amœba often went into a temporary "proteus" condition with several pseudopodia, a tail-piece forming at the hinder end when locomotion commenced.

The entire mass of both endoplasm and ectoplasm usually consisted of translucent, but not transparent, protoplasm. A few fairly small granules were present in the endoplasm. The appearance suggested that the protoplasm was packed full of almost ultramicroscopic particles,

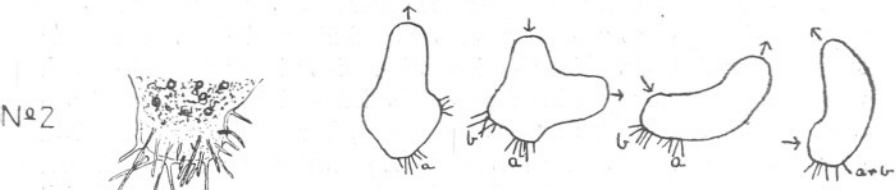
FIGURE 1.

TYPE A.



Limax. Limax-proteus. Note lateral pseudopodium (L). Proteus. Formation of a hyaline pseudopodium (L).

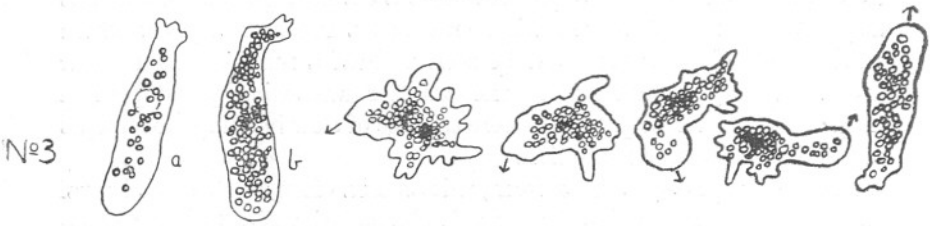
TYPE A.



Tail-piece enlarged.

Four successive positions of an individual amœba, to show formation of a new tail-piece (b) when the direction of motion is changed. Note migration of old tail-piece to new hind end.

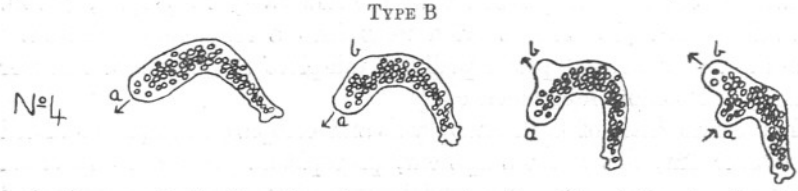
TYPE B.



(a) Specimen from tank.
(b) Specimen from culture.

Five successive stages showing the assumption of the limax from the proteus form.

TYPE B



Four successive stages in the formation of a new pseudopodium (b), and the retraction of the old one (a). Note that the granules seem to be pushed inwards by the swelling ectoplasm.

a condition also indicated by the brilliance with which the entire amœba shone under dark-ground illumination. The thin ectoplasm differed from the endoplasm in the relative absence of the small granules. If any of these were present they seemed to be temporarily fixed, and took no part in the irregular streaming movements of those in the endoplasm. However, occasionally a new pseudopodium had a cap of hyaline ectoplasm, like that of the fine pseudopodia of the tail-piece (Fig. 1, No. 1).

The protoplasm seemed to be very fluid. If an amœba were sucked up with a fine pipette it adhered to the substratum by the tail-piece. The body of the amœba formed a round droplet attached to the tail-piece by a neck as though the fluid protoplasm were contained within a weak surface membrane.

These amœbæ resembled, and may have been identical with, those described by Lebour (**22**) from the plankton of the Plymouth region (Form " C," p. 157). They also bore a resemblance to the amœbæ described by Orton (**27**) as occurring in the gastral cavity of Sycon and elsewhere.

Type B (*Fig.* 1, *No.* 3).

This amœba was a typical limax form, moving for relatively great distances without forming lateral pseudopodia. The animal was long and thin, varying in length from 70μ to 150μ, and in breadth from 20μ to 35μ. The tail-piece was rugose, but bore no fine pseudopodia. A " proteus " form was developed on stimulation, the pseudopodia of which were at first composed entirely of ectoplasm, while the endoplasmic granules were concentrated in a central mass (Fig. 1, No. 3). Later the granules flowed into one of the pseudopodia, which, increasing in size, became the advancing main pseudopodium of the amœba. The other pseudopodia now rapidly diminished to form the rugose tail-piece of the normal amœba.

The protoplasm was hyaline and highly refracting. There were large dark granules in the endoplasm. These were few in number in amœbæ taken from the tank, but in those obtained from cultures the number increased with the age of the culture till the protoplasm was densely packed (compare Fig. 1, No. 3, a and b).

During locomotion some granules became embedded in the ectoplasm : these, as in Type A, were relatively fixed in position, unlike the streaming granules in the endoplasm. The anterior end of the advancing pseudopodium was often free from granules, those of the endoplasmic stream being unable to penetrate it. A new pseudopodium formed at the side of the amœba at first consisted entirely of granule-free ectoplasm, and it was often observed that endoplasmic granules immediately below the pseudopodium were actually pushed inwards as the pseudopodium swelled (Fig. 1, No. 4).

The amœbæ were of a stiffer consistency than Type A. When sucked into a pipette they retained their shape and could be set down elsewhere without great change of form, though ultimately the mechanical stimulation caused them to take on the proteus form.

So far this amœba has not been identified with a described species.

Type C.

This amœba resembled Type A, except that there was a much greater tendency to assume the proteus form. The size ranged from 70μ to 150μ in length, by 40μ to 80μ in breadth. The protoplasm had a faint yellow tinge, and was more granular than in Type A: vacuoles were sometimes present. The protoplasm was rather less fluid than Type A. Fine processes were present on the tail-piece as in Type A.

This amœba also has not yet been identified.

Cultivation.

All three types of amœbæ can be grown in culture, though Type A cultures tend to become infested with ciliates (Euplotes and others). The cultures were prepared by a modification of Taylor's method for fresh-water amœbæ (**34**). A litre of "outside sea-water" and some Petri dishes were heated to 80° C. for half an hour, in order to kill the ciliates; once ciliates obtained a footing in a culture they multiplied rapidly, and the amœbæ fell off in numbers and disappeared. Wheat grains were now crushed and boiled in water till they were swollen. About twelve wheat grains were put in a Petri dish with 50 c.c. of sterile sea-water. A few drops of tank water were now added to the dish to infect it with bacteria, the water being drawn up slowly with a clean capillary pipette while under the microscope, to ensure that no ciliates were taken up.

In about a week's time a thin bacterial scum had formed over the bottom of the dish, and the remaining wheat grains were removed. Amœbæ from the outside tank or from previous cultures were now removed individually into clean sea-water by means of a fine pipette. This was done under the microscope. Three or four of these amœbæ were then retransferred to each culture dish. At the end of ten days most of the cultures contained large numbers of amœbæ. The cultures lasted for about two months, though one lasted over four (Type B). Sooner or later Type C amœbæ appeared in large quantities in these cultures, though no special precautions were taken to ensure their presence. In this way an abundant supply of Type C amœbæ could always be obtained from old cultures.

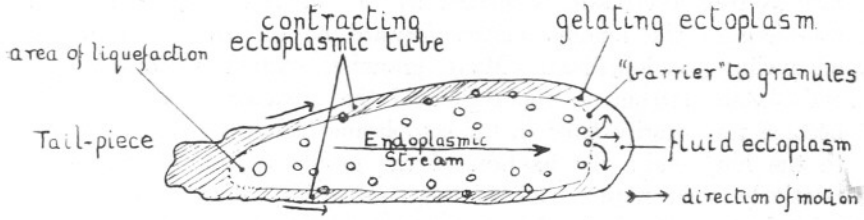
(1) THE LIMAX FORM OF LOCOMOTION.

This form of locomotion can be readily studied in Type B amœbæ, with their clear ectoplasm and large granules.

It has already been pointed out that we may look upon limax forms as amœbæ which obtrude a single persistent pseudopodium which is

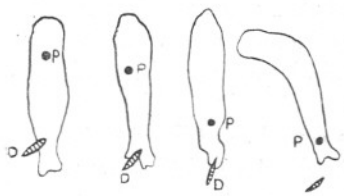
FIGURE 2.

No. 1.

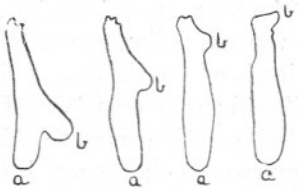


Diagrammatic illustration of the movement of a Type B amœba.

No. 2.



Four successive drawings of a Type B amœba to show migration of particles (P and D) to the tail-piece.



Four successive drawings of a Type B amœba to show the migration of a retracting pseudopodium (b) to the tail-piece.

No. 3.

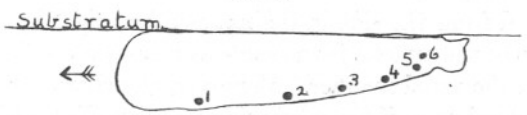


Diagram founded on observations of successive positions of a single large granule embedded in the ectoplasm of a Type B amœba (seen from side).

advancing continuously. In Type B amœbæ the endoplasm and its included granules stream towards the advancing pseudopodium (Fig. 2, No. 1). The head of the pseudopodium consists of a greater or less amount of clear fluid ectoplasm ; and it is important to note that endoplasmic granules are usually checked in their forward flow just behind this clear area, as though there were a weak barrier to the entrance of the granules into the anterior region of the ectoplasm (Fig. 2, No. 1).

The ectoplasm at the sides of the clear anterior region seems to become more solid. Since the ectoplasm in the middle and that which is anterior is advancing continuously, this solidification at the sides results in the formation of a tube of gelated ectoplasm. As the advancing pseudopodium continuously adds fresh solid ectoplasm, each portion of this tube, once formed, moves further and further back towards the hind end of the amœba.

The tube of gelated ectoplasm contracts continuously, the contraction increasing as the hind end is approached ; the hind end is contracting as fast as fresh ectoplasm is formed at the anterior end, so that the tail-piece of the amœba moves forward at the same rate as the advancing pseudopodium. Many granules, carried forward by the endoplasmic stream to the region of the advancing pseudopodium, become temporarily fixed in the ectoplasmic tube as this passes back to the hind end : the fixation of the granules attests both to the relatively solid nature and to the contractility of the ectoplasmic tube, because the increasing contraction of the tube as the hind end is approached is shown by the successive positions of individual granules (Fig. 2, No. 3).

If a lateral pseudopodium is formed and then retracted, it passes back to the tail region just as do the granules in the ectoplasm. The same thing is seen in the case of diatoms, lamp-black particles, etc., which have adhered to the ectoplasm (Fig. 2, No. 2).

The greatly contracted hind end of the ectoplasm tube, together with remnants of old retracted pseudopodia, forms the highly gelated rugose tail-piece (Fig. 1, No. 2).

Within the hind end of the amœba, in front of the gelated tail-piece, is a place of liquefaction : here, as Schæffer (**32**) has pointed out, the endoplasmic stream begins.

When viewed from the side in the manner described by Dellinger (**9**), the movement of the amœba is the same as it appears to be when viewed from above in the usual manner. Moreover, the character of the movement is unchanged even if the advancing pseudopodium is lifted clear of the substratum, or if the amœba is in contact both with the substratum and with a surface at right angles to it.

A forward rolling movement of the upper anterior surface of the amœba, as described by Jennings (**20**), was in no case observed ; nor did my observations on these limax amœbæ agree with the contractile network theory of Dellinger (**9**). The character of the movement is essentially the same as described by Rhumbler (**30**) : there is an "ento-ectoplasmic process," and the streaming does resemble a "fountain current," but there is no backward *current* at the surface, because the ectoplasm is not fluid ; it is the continuous contraction of the gelated

ectoplasm and its continuous formation at the anterior end which causes the outer surface to pass towards the tail-piece.

We can, therefore, conceive of the amœba as a contracting tube of gelated ectoplasm closed at its hind end. The endoplasm streams forward through this tube from a place of liquefaction within the hind end, and apparently forms ectoplasm at the anterior end. This anterior ectoplasm adds to the contracting tube by becoming gelated at the sides of the advancing pseudopodium.

(2) THE EFFECT OF OSMOTIC PRESSURE: THE WATER-CONTENT OF THE CELL.

The experiments were performed mainly on Type A amœbæ, though observations on Type B were used to check the results.

Hypotonic solutions of sea-water were made by mixing distilled water and "outside sea-water" in known proportions. For the hypertonic solutions a stock solution of 100·5 gms. of Tidman's sea salt in a litre of distilled water was made, the pH being adjusted by the addition of sodium carbonate to pH8·1, the usual pH of "outside sea-water." This solution has approximately three times the salt content of the "outside sea-water." Hypertonic solutions of various strengths were made by adding this solution to outside sea-water.

The effect of the "three-times-strength sea-water" solution was checked by dilution to three times its volume, when amœbæ were found to behave in it as they did in normal sea-water.

Hypotonic Solutions: Type A.

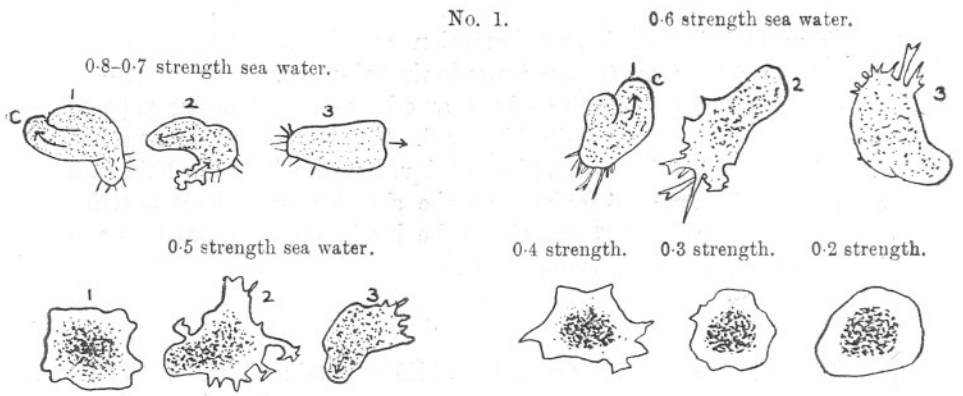
In hypotonic solutions the amœba absorbs water. Sometimes the activity of the amœba is slightly increased at first. The protoplasm becomes rather more fluid in hypotonic than in "outside sea-water," and this fluidity is often accompanied by the eruption of pseudopodia, which flow round the body in the manner described as a "circus movement" by Loeb (**25**) in the amœbocytes of Limulus (Fig. 3, No. 1).

If the osmotic pressure of the medium is lowered by stages the amœbæ are capable of considerable adjustment to the conditions.

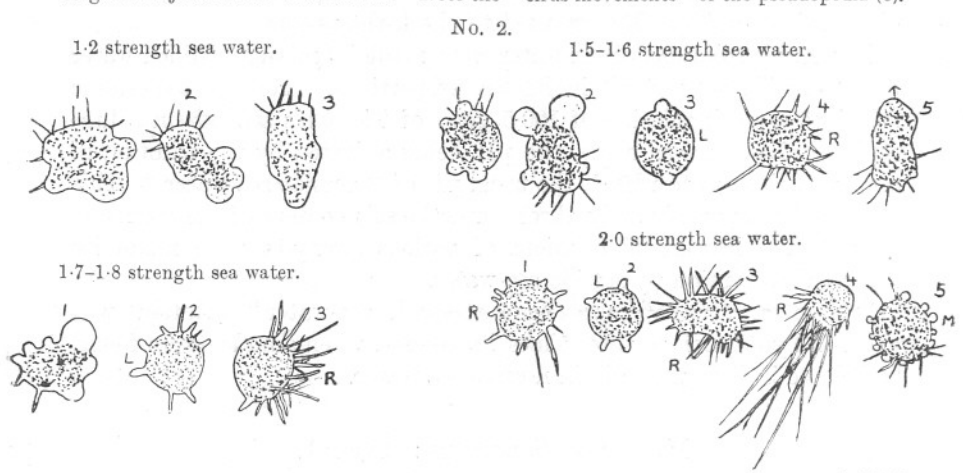
Figure 3, No. 1, shows drawings of changes in shape of individual amœbæ when the osmotic pressure was lowered in successive stages.

In "outside sea-water" the amœbæ were either in the limax, or in the "limax-proteus" form with a tendency to throw out new pseudopodia (Fig. 1, No. 1). When the medium was changed for 0·8–0·7 strength sea-water the amœbæ swelled slightly, and at first approached more

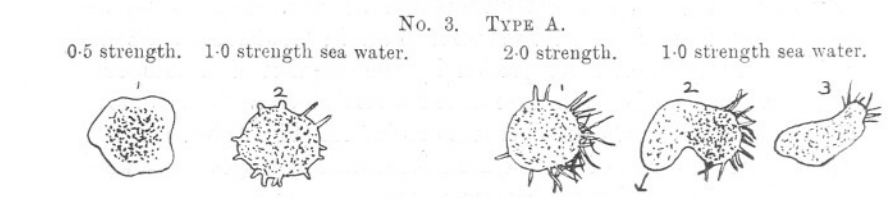
FIGURE 3.



Changes of form in Type A amœbæ in hypotonic solutions. The numbers 1, 2, 3 indicate successive stages in adjustment to the medium. Note the "cirus movements" of the pseudopodia (c).

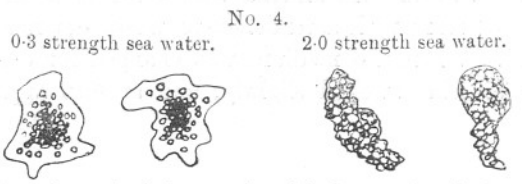


Changes of form in Type A amœbæ in hypertonic solutions. Note the "lemon" and allied forms (L), the "radiosa" forms (R), and the "morulate" form (M).



To show assumption of the radiosa form on transference from hypotonic to sea water of natural strength.

To show direct passage of radiosa pseudopodia into "fine processes" of the tail-piece on transference from hypertonic to sea water of natural strength.



Type B amœbæ in hypotonic and in hypertonic solutions.

nearly to the proteus form. The tail-piece increased in extent, and its fine processes became slightly larger. Pseudopodia, once put out, sometimes retracted with some difficulty, irregular projections appearing over them. These projections were finally absorbed into the tail-piece, and appeared to change into fine processes like the others covering this region. After a short time recovery took place and reversion to the normal type occurred.

If the solution was now changed to 0·6 strength sea-water the amœba again swelled. At first its form might be normal, but soon broad, rounded pseudopodia appeared, composed largely of clear ectoplasm: at the same time the fine processes of the hind end became broader and began to take on an appearance similar to the pseudopodia. At this stage the "fine processes" and the pseudopodia were somewhat flattened and adhered to the substratum. Recovery began after five or ten minutes, and ultimately a fairly typical limax form might be attained, but the movement was slower than in "outside sea-water."

On changing the solution to 0·5–0·4 strength sea-water the amœba again swelled. The ectoplasm formed a clear border round a central granular mass. Both the "fine processes" and the pseudopodia became similar broad sheet-like extensions of the ectoplasm, firmly attached to the substratum. These sheet pseudopodia underwent changes of shape, but the animal seemed incapable of locomotion. Later, as the amœba adjusted itself to the medium, the granular endoplasm began to penetrate the clear ectoplasmic region. The sheet pseudopodia thickened and became differentiated into broader rounded pseudopodia and finer ones. Ultimately the broader pseudopodia became fairly typical, while the finer ones congregated at the hind end as the "fine processes" of the tail-piece. However, the "fine processes" were still abnormally large and might even be represented by blunt pseudopodia.

When the osmotic pressure was lowered to 0·3–0·2 strength sea-water these effects were increased: the ectoplasmic border was more marked, though the activity of the sheet pseudopodia lessened progressively till in the end the amœba was almost circular with a clear sheet of ectoplasm surrounding a granular mass. This form resembled the amœbocytes described by Loeb (**25**) as possessing, under similar conditions, a structure resembling ova with a fertilisation membrane. In the amœbæ this resemblance was superficial, because in reality they were not spherical when in this condition, but flattened against the substratum.

Amœbæ could still recover if returned to "outside sea-water" from 0·2 strength sea-water. If, however, the 0·2 strength sea-water was replaced by distilled water they were incapable of recovery. They might not undergo immediate cytolysis, but they swelled till almost spherical.

Sometimes while swollen a number of violent contractions occurred, at each of which water was discharged from the cell; these contractions ended in cytolysis.

Hypertonic Solutions: Type A.

In hypertonic solutions water is abstracted from the amœba. The animals are capable of considerable adjustment to the medium if the osmotic pressure is changed slowly. Figure 3, No. 2, illustrates changes in individual amœbæ accompanying a progressive increase of osmotic pressure.

On raising the solution from 1·0 to 1·2 strength sea-water only slight changes occurred in the amœbæ. The animals became more sluggish, and tended to throw out lateral pseudopodia. The tail-piece sometimes increased in area, and there were more "fine processes" on it.

In 1·2–1·4 strength sea-water the limax form was at first lost; there were several pseudopodia, and these of a characteristic rounded appearance. The fine processes elongated slightly, and they sometimes arose over half the surface of the animal. Later adjustment took place, and the "limax-proteus," or even the limax form, was attained, though often with an enlarged tail-piece.

When the osmotic pressure was raised to 1·5–1·6 strength sea-water the amœba tended to become spherical. Small pseudopodia appeared all over the body (Fig. 3, No. 2); they were more numerous than those formed in 1·4 strength sea-water and consisted largely of clear ectoplasm. The fine processes might appear at any point on the body. A few amœbæ even went into a peculiar "radiosa" or a "lemon" form (Fig. 3, No. 2).

In the "lemon" and similar forms the amœba consisted of a rounded granular mass bearing a few nipple-shaped pseudopodia of clear ectoplasm. In the "radiosa" form the fine processes of the tail-piece increased in size, and often developed at points all over the surface of the rounded granular body of the amœba. The originally limax amœba came more to resemble a naked Foraminiferan than an amœba. There is a strong similarity between these "radiosa" forms and those described by Loeb (**25**) in the amœbocytes of Limulus under similar conditions.

In the 1·5–1·6 strength solution the amœbæ might effect partial adjustment to the medium after some time, but adjustment rarely exceeded the mere limitation of the fine processes to a definite area of the amœba.

In 1·7–1·8 strength sea-water many amœbæ assumed the "lemon" or "radiosa" forms, but the majority consisted of a compact granular mass bearing small clear pseudopodia. It was then impossible to distinguish between the pseudopodia and the enlarged fine processes.

On raising the osmotic pressure to twice the strength of sea-water, the clear ectoplasmic pseudopodia reached the condition seen in the nipples of the " lemon " form. Finally, these pseudopodia lengthened, and in the majority of amœbæ the " radiosa " form was attained. The radiosa pseudopodia might even extend to several times the body length. Some " radiosa " forms contracted the pseudopodia into small droplets, so that they took on a " morulate " appearance (Fig. 3, No. 2). This occurred more readily if the rise in osmotic pressure had been sudden.

Further increase in the osmotic pressure caused great shrinkage without further change in the type of pseudopodia. In and above 1·8 strength sea-water the amœbæ were incapable of reverting to the normal form by adjustment to the medium. Cytolysis did not take place for some hours even in 3·0 strength sea-water, and recovery could take place if this solution was slowly brought back to the strength of ordinary sea-water. Great swelling, usually followed by cytolysis, occurred if amœbæ were suddenly transferred from 3·0 to 1·0 strength sea-water.

It is interesting to note the strong resemblance of the effects of hypotonic and hypertonic solutions on such widely different amœboid individuals, as " Type A " amœbæ and the amœbocytes of Limulus.

Loeb (25) considers pseudopodium-formation is due to liquefaction at the advancing tip of the pseudopodium, followed by gelation at the sides. Liquefaction will necessarily be more limited when the protoplasm is more gelated, and also gelation will occur more readily at the sides of the pseudopodium. For these reasons, when the protoplasm of an amœba is more gelated, we should expect to find long thin " radiosa " pseudopodia, and when more fluid we should expect wide liquid pseudopodia.

Loeb points out that some of the effects of hypotonic and hypertonic solutions can thus be accounted for by assuming that the imbibition of water by the amœbocyte (or the amœba) from a hypotonic solution causes an increased fluidity of the protoplasm resulting in broad liquid pseudopodia. Conversely the loss of water which occurs in a hypertonic solution causes an increase in consistency, so that fine " radiosa " pseudopodia develop. Additional evidence that the effects are due simply to imbibition of water by, or its abstraction from, the protoplasm, and are not due to variation of the concentration of particular ions, is provided by the behaviour of amœbæ which are transferred to normal sea-water after previous adjustment to a hypotonic medium. Under these circumstances the amœbæ often go into a typical " radiosa " form at first, though ultimately recovering the normal limax form (Fig. 3, No. 3). Similarly, radiosa amœbæ which have been kept for some time in 2·0 strength sea-water approach the typical limax form when first put in 1·5–1·6

strength sea-water. The effects of osmotic pressure are due to the altered water-content within the cell.

However, it is difficult to see how mere increased liquefaction of the protoplasm could cause the flattened sheet-like character of the amœbæ in solutions of very low osmotic pressure : a condition very similar to that of leucocytes exhibiting the stereotropic reaction to the substratum. Again, increase in consistency alone can scarcely account for the " morulate " forms sometimes seen in hypertonic solutions, nor can it explain the great length and number often attained by " radiosa " pseudopodia.

We have seen that both the fine processes characteristic of the tail-piece and the true pseudopodia tend to become similar sheet-like extensions of the ectoplasm in a hypotonic medium. We have also seen that both the pseudopodia and the fine processes can become transformed, with but little change in the latter, into the " radiosa " pseudopodia in hypertonic solutions. This suggests that even in normal amœbæ the fine processes of the tail-piece are true pseudopodia, but formed under different conditions.

Taking into account the fluid character of the advancing pseudopodium of the limax amœba on the one hand, and the strong resemblance of the " fine processes " and the " radiosa " pseudopodia on the other, one may be justified in concluding that in normal locomotion water is being imbibed by the protoplasm at the advancing pseudopodium while it is being abstracted from the region of the tail-piece, which therefore tends to form " radiosa " pseudopodia.

The Effect of Osmotic Pressure : Type B.

The effects of changes in osmotic pressure on Type B amœbæ (Fig. 3, No. 4) are essentially the same as on Type A. Activity is only possible within certain limits of osmotic pressure.

The ultimate effect of hypertonic solutions is the assumption of a rugose form by the entire amœba. A " radiosa " form is not developed, and this is possibly correlated with the absence of fine processes on the tail-piece of the normal Type B amœba.

In very hypotonic solutions the endoplasmic granules become condensed into a central mass, the whole amœba swells, and the ectoplasm forms flat sheet-like pseudopodia. The effect is similar to that occurring in Type A under similar circumstances, but the ectoplasmic sheet is not so flattened and the pseudopodial extensions are more rounded.

As in Type A, a certain amount of adjustment is possible to alterations in the osmotic pressure.

Like muscle (**5**) and cilia (**12**) amœboid activity is altered and inhibited

by an abnormal osmotic pressure : for efficient activity there must be a certain water-content in the cell.

The observations suggest that water is imbibed during pseudopodium formation. From the character of the tail-piece this water appears to be abstracted from the hind end. The streaming of the endoplasm would follow as a consequence of this movement of water within the amœba.

Water imbibed during pseudopodium formation probably is not extracted directly from the external medium. On the one hand, a limax form amœba can move for long periods by the continuous extension of a single pseudopodium. If water were imbibed from the outside medium the amœba must continuously increase in volume, whereas the volume seems to remain constant (taking the length and breadth of the amœba as an index of the volume). On the other hand, when an amœba thrusts out a main pseudopodium from the resting condition the size of the resting mass can be seen to undergo progressive reduction as the size of the pseudopodium increases (Fig. 1, No. 3). It might be argued that the volume of the amœba would remain constant though water were imbibed by the pseudopodium from the external medium if a corresponding extrusion of water took place at the hind end. But this would entail a current within the amœba from the pseudopodium to the hind end : a condition the reverse of that observed.

Imbibition of water from the external medium would result in currents in the medium : these currents have not been observed. Currents in the medium have been looked for, since they should be present on the Bütschli-Rhumbler hypothesis of pseudopodium formation by means of a local lowering of surface tension at the surface of the amœba.

(3) THE RELATION OF AMŒBOID ACTIVITY TO HYDROGEN ION CONCENTRATION.

A. Variations of pH associated with Pseudopodium Formation.

The development of an acid reaction in muscle during contraction is well known. The work of Gray (**12**) indicates that the contraction of cilia is probably associated with the production of acid. It seemed possible, therefore, that a change of hydrogen ion concentration might accompany amœboid activity.

In the following experiments the amœbæ were stained with neutral red. This indicator has many advantages :—

(1) It is readily absorbed by living cells.

(2) Homer (17) has shown that the salt and protein errors of this indicator are negligible unless it is employed for very accurate work. Bayliss supports this (4).

(3) The indicator is much less toxic than other indicators (17 and 4).

(4) The range of the indicator is about the neutral point.

(5) Owing to the colour change being from yellow to red in the presence of acids, a local increase in acidity is much more marked than would be the case if the acid reduced the depth of colour (as in phenol red).

Staining was accomplished by the addition of two or three drops of a 0·05% solution of neutral red in distilled water to about 5 c.c. of sea-water containing the amœbæ. As soon as the amœbæ were stained sufficiently for the true tint to be appreciated with the condenser diaphragm well open, the water was changed for clean sea-water.

The pH corresponding to a particular tint was determined by a method described elsewhere (28), the principle of which is as follows. Test tubes are filled with buffer solutions ranging from pH6·6 to pH8·0, and neutral red is added as indicator. A strip of wood carrying the test tubes is hung in the window in front of the microscope. The image of the series of buffer solutions is now focussed in the plane of the object by means of an achromatic condenser. On looking down the microscope the object stained with neutral red is seen in juxtaposition with the image of the series of tubes. By tilting the mirror the images of successive tubes can be brought opposite the object until a tube is found with a corresponding tint.

It is obvious that the accuracy of the method relies entirely upon the change of tint of the indicator with a change of pH and not upon a mere change in the intensity of the colour. It must be admitted that the change of tint in the case of neutral red does not render it an ideal indicator for the method.

Various amœbæ were tested, but only those of Type A and Type C were found to be satisfactory. In all other amœbæ tried, the presence of large granules in great numbers or the presence of large deeply staining bodies in the endoplasm rendered them unsuitable. Moreover, in amœbæ such as Type B the clear cytoplasm took the stain very feebly or not at all.

In Types A and C the minutely granular translucent ectoplasm took the stain fairly evenly. Type A was more suitable than Type C, because the latter normally has a faint yellow colour and sometimes has a few moderate-sized granules in the endoplasm. In both these amœbæ the tint corresponded to a pH within the range of neutral red.

The observations described below refer to Type A, though results obtained with Type C were almost identical.

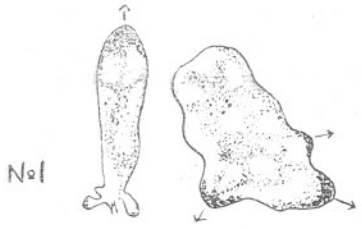
Observations on normal active amœbæ.

The Type A amœbæ were stained with neutral red, though not too deeply. The colour changes described here are obscured by overstaining. The endoplasm stained a dirty yellow, and the ectoplasm stained red.

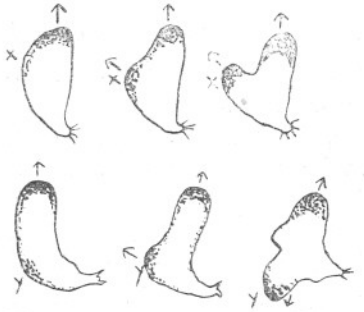
The colouring of the ectoplasm was not even or constant. The colour

FIGURE 4.

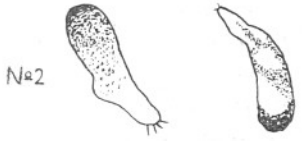
The brightness of the red colour in the protoplasm is represented by the depth of shading.



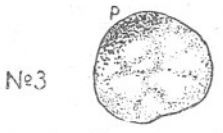
A Type A and a Type C amœba, to show the network.



Three successive stages in two different amœbæ (Type A), cases of apparent local increase in redness before pseudopodium formation (at X and Y).



A sluggish and an active Type A amœba. Note the sharp definition of the red colour in the latter.



Resting Type A amœba, to show the network and greater redness at the pole (P).

pattern took the form of an ill-defined network of dirty red, though brighter in some parts of the network than in others (Fig. 4, No. 1). The network was difficult to draw owing to its constantly changing aspect. In order to be certain that this was not due to local condensations of staining granules the amœbæ were stained with methylene blue. This stained the amœbæ fairly evenly throughout their entire mass, and there was no evidence of a network.

In active amœbæ the anterior and sides of the pseudopodia were bright red. The more active a pseudopodium was, the brighter was its colour.

This was especially the case with eruptive pseudopodia which were sometimes formed. An important feature of eruptive pseudopodia was that they appeared suddenly to gelate at the surface after eruption. The red colour became much more intense on gelation; though afterwards the colour faded back to the tint of the ectoplasmic network.

Dr. E. J. Allen, Director of the Laboratory, and Dr. W. R. G. Atkins kindly allowed me to demonstrate to them the colour change accompanying pseudopodium formation. They both agreed with me as to the definite character of the change.

By the method already described the endoplasm was found to correspond to about pH7·6–7·8, the network of ectoplasm to about pH7·2, and the active pseudopodia to about pH6·8.

The extension of pseudopodia.

Pseudopodium formation was sometimes actually preceded by a slight local increase in the red colour of the ectoplasmic network (Fig. 4, No. 1). But this did not occur invariably, the increase in redness often taking place at the same time or even lagging behind pseudopodium formation.

The bright red of the ectoplasm was sometimes sharply marked off from the underlying endoplasm, though the colour transition from ectoplasm to endoplasm was often gradual; this was especially the case in sluggish limax forms, in which the redness at the anterior end was only of moderate intensity (Fig. 4, No. 2).

The retraction of pseudopodia.

This was accompanied by a change from bright to dull redness. Soon after a pseudopodium ceased activity the apparent pH rose fairly rapidly from about pH6·8 to pH7·0 and thereafter more slowly to pH7·2 (the pH of the network). At times the apparent pH fell very slowly indeed; this was often associated with resumed activity in the pseudopodium.

The resting amœba.

The amœba was spherical in the resting condition. The difference between the colours of the ectoplasm and endoplasm was less marked. The pH of the endoplasm was about pH7·6, while the greater part of the ectoplasm formed a stationary network at about pH7·2. There was sometimes a local concentration in the ectoplasm at about pH7·0 (Fig. 4, No. 3).

The effect of the pH *of the medium: cytolysis.*

The pH of the medium was varied by the addition of N/100 HCl or N/100 NaOH in sea-water. Between about pH9·0 and pH6·0 the internal

hydrogen ion concentration did not seem to fall appreciably. The endoplasm remained at about pH7·6–7·8, whilst the active pseudopodia varied from about pH6·8 to pH7·0.

At a pH of the medium between pH6·0 and pH5·5 the activity was progressively reduced, the amœba usually becoming spherical; at the same time the entire amœba rapidly became very bright red. These effects culminated in cytolysis in a medium between pH5·5 and pH5·0: the ectoplasm of the spherical amœba suddenly became very active, and large fluid spherical pseudopodia were thrown out, accompanied by great endoplasmic streaming. Some of the protoplasm was discharged into the medium, where it coagulated. The pseudopodia often detached themselves from the amœba, so that it became disintegrated into three or four spherical masses of protoplasm.

During this process the colour of the neutral red faded entirely away, leaving the spheres of protoplasm colourless. After a short period of quiescence the separate masses of protoplasm sometimes resumed activity and cytolysed completely, but this did not always occur, the spherical masses sometimes appearing to be coagulated. Once cytolysis had started the amœbæ were incapable of recovery by transference to normal sea-water.

The colour of the stain sometimes redeveloped in the spherical masses for a short time after fading, especially if activity was resumed. Ultimately the colour faded completely away. This fading or bleaching of the neutral red was quite different from the change of the indicator from red to yellow in alkalis: the observations suggested that the stain was chemically altered, possibly by reduction or oxidation.

Cytolysis did not always occur in the manner described above. Sometimes a Type A amœba became spherical at about pH5·5, the surface ectoplasm broke down and the central protoplasm remained as a coagulated mass.

The effect of osmotic pressure.

The internal pH of the amœba was unaffected by osmotic pressure except that reduced activity in hypotonic and hypertonic solutions was accompanied by an appearance resembling that of the normal resting amœba.

General considerations.

Some Type A amœbæ showed the effects very much better than others. This was due partly to differences in staining. After a few hours the stain sometimes collected in small masses in the protoplasm; when this occurred the changes of tint were very difficult to make out, and even seemed to be absent in some cases.

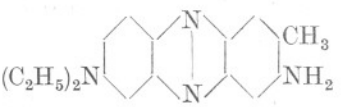
The lighting conditions are very important. A white light is the best, and the diaphragm of the condenser must be opened till the angle of the cone of light from it is equal to the angle of the cone of light entering the objective. Less light than this obscures the colour tint, whilst more light fogs the image.

Apart from differences in staining and lighting conditions there was still considerable variation in the degree to which various amœbæ showed the colour change.

Experiments with other intra-vitam stains.

Atkins (3) has shown that brom-thymol blue can be used as an intra-vitam indicator. This substance only stains amœbæ with difficulty, and is toxic in relatively strong solutions.

By subjecting Type A amœbæ to solutions of relatively moderate strength, the body of the amœba could be stained a pale blue, while the pseudopodia were almost colourless. The effect was very poor, but it does corroborate the neutral red experiments to some extent, because this indicator turns from blue to yellow as the acidity increases.

A di-ethyl homologue of neutral red, 

specially prepared for the author by the Cooper Laboratory, Watford, was also tried.

This substance is a fairly good intra-vitam stain, and is also an indicator. The range is much extended, from pH8 to pH5, the colour changing from yellow to reddish brown. The colour change is not so marked as that of neutral red. Unfortunately, though the stain is readily soluble in fresh-water, sea-water precipitates it almost completely.

Neutral violet (also prepared by the Cooper Laboratory) was also tried. But this substance is a poor indicator and precipitates in sea-water.

The experiments point to the following conclusions: that in these Type A amœbæ the ectoplasm is more acid than the endoplasm, and that pseudopodium formation is accompanied by an acid reaction of the protoplasm. Moreover, the internal pH of the amœbæ varies only slightly, if at all, as the acidity of the medium is raised until a critical pH is reached at which amœboid movement ceases. This cessation of movement is usually followed by cytolysis in Type A.

When drawing conclusions from these experiments it must be borne in mind that, in spite of the small protein error of neutral red, the concentration of protein in the cell may be so great that estimations of the pH by intra-vitam indicators may be very wide of the mark.

It has already been shown that in amœbæ with clear cytoplasm, such as Type B, the stain is not taken up by the cytoplasm but by the included granules. It is almost certain that the stain is taken up by minute granules in Type A amœbæ also, and not by the cytoplasm itself, because unstained transparent pseudopodia can at times be formed even in this amœba.

The pH measured is therefore the pH of the granules and not that of the cytoplasm itself. An assumption that the change in tint is due to the production of acid in the cytoplasm is not necessarily justified ; chemical changes might occur in the granules during the formation of ectoplasm in the pseudopodium. Again, Loeb (**23**) points out that the pH within gelatine particles is quite different from the pH of the surrounding medium owing to the Donnan equilibrium ; the same may hold true for granules in protoplasm.

Were the acid change restricted to granules alone it might be merely incidental to the mechanism of pseudopodium formation, since granule-free pseudopodia can at times be formed.

Chambers (**6**) has found it possible to inject solutions of dyes into the living cell. He finds that under certain circumstances the dyes diffuse through the cytoplasm. It is hoped that in this way indicators such as brom-thymol blue and phenol red may be injected into the cytoplasm, so that changes in the reaction of the cytoplasm itself could be determined.

(B) Amœboid Activity and the pH of the Medium.

In these experiments the velocity of locomotion has been used as a measure of amœboid activity. The energy of amœboid activity is only a function of the velocity if all the energy of pseudopodium formation is directed to locomotion. But more accurate methods of estimating the activity do not seem to be forthcoming.

Most of the experiments were performed on Type B amœbæ. These are peculiarly suited to the method. If placed in a clean dish of sea-water they move for long periods in a typical limax manner by the continuous advance of the single anterior pseudopodium. There is very little tendency to form lateral pseudopodia. Considerable distances, 10 mm. or more, can be covered in a straight line. (It should be mentioned that no evidence was obtained of the wavy path described by Schæffer (**32**) as characteristic of many amœbæ.)

If the conditions of the medium were kept constant the velocity varied but little. The following table (Table 1) gives the velocity at long inter-

vals of two Type B amœbæ in sea-water pH8·1. The only variable factor was the room temperature :—

TABLE 1.

	Time.	Temperature.	Velocity: μ per sec.
	11.45 a.m.	14·0°C	2·29
Type B	0.15 p.m.	14·6	2·40
Amœba (1)	1.00 p.m.	14·8	2·43
	2.00 p.m.	14·4	2·38
	April 13th.		
	(a) 10.45 a.m.	13·4	2·09
Type B.	(b) 3.05 p.m.	15·1	2·18
Amœba (2)	3.15 p.m.	16·4	2·48
	April 14th.		
	(c) 10.30 a.m.	13·4	2·06

In the intervals between the values (a) and (b), amœba (2) had been used for experiments during which it was paralysed with acid. On removal to sea-water pH8·1 complete recovery took place, and the values (b) and later (c) were obtained. The variation in velocity is not great, and always appears to be correlated with the one variable factor, temperature.

Methods.

To determine the effect of various solutions a clean Petri dish on the microscope stage was filled with 50 c.c. of "outside sea-water." A single amœba was then transferred to the dish by means of a fine pipette. After one to five minutes the amœba adjusted itself to the sea-water and moved in a normal limax manner.

A ghost-micrometer (**10**) was placed at such a distance from the microscope that the lines appeared to be 25μ apart when the image was focussed in the plane of the amœba observed.

The velocity was measured with a stop-watch by finding the time taken for the hind end of the amœba to cross one or more divisions of the micrometer. Observations were discarded unless the motion of the amœba was free from irregularities due to sudden changes of direction, and so on.

By turning the Petri dish, the direction of movement of the amœba was kept at right angles, or at 45°, to the divisions of the micrometer. When first transferred to the dish, or when the solution was changed, the amœba moved irregularly ; movement sometimes ceased for a short

time or was abnormally fast, probably owing to direct stimulation. For this reason observations of the velocity were taken after a lapse of about ten minutes, so that adjustment to the new medium might have been completed.

The velocity in a solution was calculated from the mean of ten to forty observations of the time taken to traverse a single interval of the micrometer. Where possible the times taken to traverse blocks of five or ten intervals at a time were taken. In solutions which strongly inhibit amœboid movement, sometimes only a few readings could be obtained.

The velocity remained constant for long periods in various solutions once the initial adjustment had taken place.

Effects of various acids and salts were determined by the addition of successive amounts of 0·01N solutions to the sea-water in the Petri dish. The 0·01N solutions were made up by the addition of 0·1N solutions of the acids or salts in distilled water, to known amounts of " outside sea-water," sufficient 3·0 strength sea-water solution being added to render the solution isotonic with " outside sea-water."

Owing to decomposition of carbonates these 0·01N acid solutions were much weaker than 0·01N acid in distilled water. But the normality of the acid radicle was 0·01N (over and above the concentration of the acid radicle normally present in sea-water).

The pH was determined with the aid of Sœrensen's buffer solutions and the indicators suggested by Clark (**8**). Due allowance was made for salt error. One cubic centimetre only of the solution was taken for pH determination, so that the total volume of the solution should not be greatly changed.

General effects of acids and alkalis.

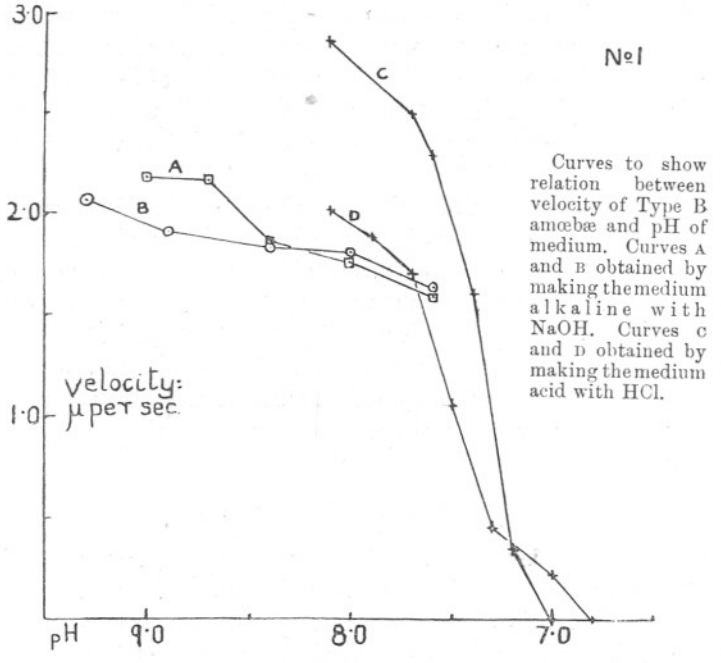
As the hydrogen ion concentration of the medium rises the velocity of the amœba falls, becoming zero at a fairly definite pH. For most acids, a Type B amœba becomes completely paralysed below a critical pH6·8–7·0 ; but no cytolysis occurs till about pH4·0 is reached. In Type A amœbæ paralysis occurs at pH5·0–6·0 and is often accompanied by cytolysis, since this takes place at, or but little below, the pH of paralysis.

This inhibition in acid solutions is completely reversible. A type B amœba paralysed with acid will recover completely on transference to sea-water at pH8·1. The shorter the time of exposure to acid, the more rapid is recovery, though even after twelve hours' exposure at about pH6·8–7·0 recovery commences in about an hour and ultimately becomes complete. The same amœba may be paralysed many times in succession with different acids, and each time the initial velocity is approximately recovered on transference to sea-water at pH8·1 (see Table 1, amœba (2),

also Fig. 6, No. 2). So far as it was possible several experiments were performed on a single amœba, that variations of individual behaviour might be detected ; such variations are found to be small if the conditions are constant.

If normal sea-water is made more alkaline the velocity of an amœba rises slowly up to pH9·6 (Fig. 5, No. 1). Type B amœbæ behave normally in solutions more alkaline than this. But precipitation of the magnesium in the sea-water commences about pH10, and the nature of the solution is thereby greatly modified. For this reason the study of amœboid

FIGURE 5.



movement in solutions more alkaline than this is deferred until the completion of experiments (now in progress) on the effects of the constituent ions of sea-water.

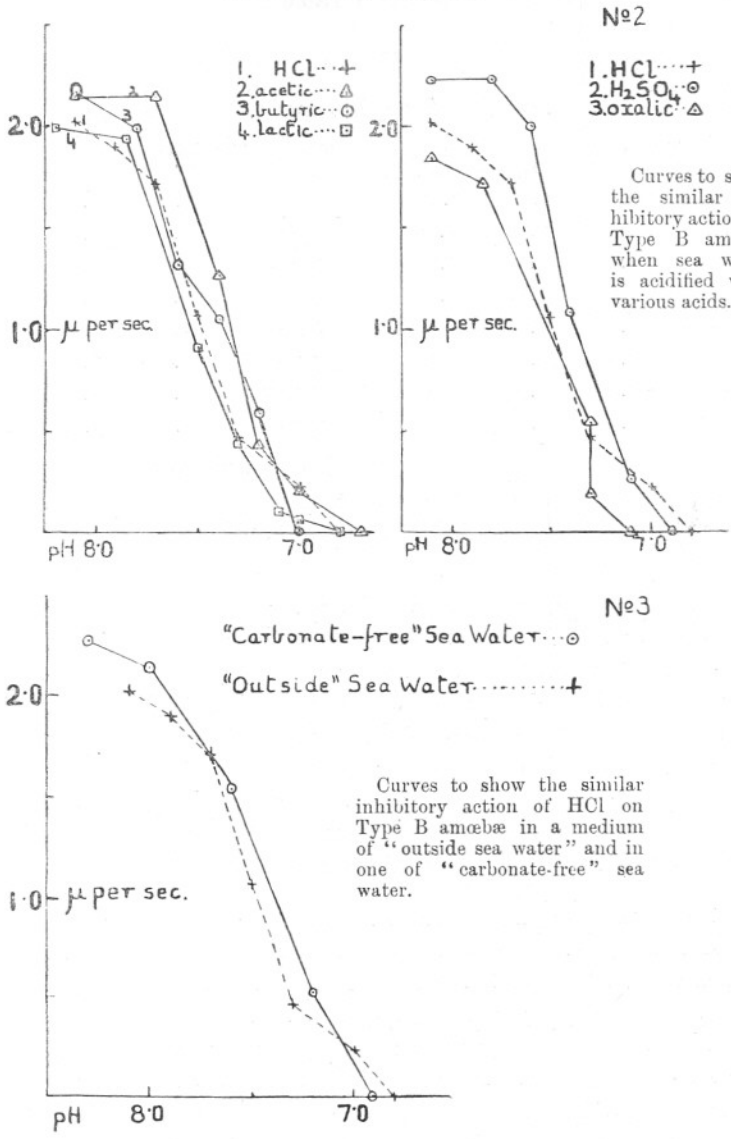
Effect of HCl *and other acids : Type B.*

When a solution of "0·01N HCl in sea-water" is added to sea-water pH8·1 the velocity of the amœba falls slowly as pH7·6–7·5 is approached. The velocity now falls rapidly as the pH is lowered from pH7·5 to pH7·0. Just below pH7·0 the velocity reaches zero (Fig. 5, No. 2).

There is little change in the form of the amœba while the velocity is falling slowly, but when the fall becomes rapid the amœba becomes

shorter and thicker, ultimately losing entirely its elongated shape at pH7·0. The limax form of locomotion continues right up to the paralysis

FIGURE 5 (*continued*).



point, though it becomes more and more difficult. This seems to be due to increasing gelation of the protoplasm, fewer and fewer granules being free to move in the endoplasm. Finally, at the paralysis point itself the

amœba is contracted into an irregular mass the shape of which alters slowly, though even this slow change ceases later.

This contracted phase can be retained for long periods, provided the solution is kept at about pH6·8 (see Table 2).

TABLE 2.

Type B Amœba.

Time. p.m.	Temperature. °C.	pH.	Velocity: in μ per sec.	Time. p.m.	Temperature. °C.	pH.	Velocity: in μ per sec.
3.10	14·0	8·1	2·45	4.45	14·1	7·0	0
3.30	14·0	7·7	2·09	5.00	14·1	7·0	0
3.45	14·0	7·3	0·58	5.30	13·8	7·1	0
4.00	14·5	7·2	0·09	6.15	13·0	7·2	0
4.10	14·5	7·0	0	6.50	13·0	7·3	0
4.15	14·5	6·9	0	7.10	13·0	7·4	0
4.20	14·5	6·8	0	7.20*	13·0	8·1	0
4.35	14·5	6·9	0	7.30	13·0	8·1	0·1
					Lost before complete recovery		

The pH was lowered by adding 0·01N HCl. Variations in pH after the seventh value are due to the evolution of CO_2 by the acid sea-water, the pH rising in consequence. This rise was corrected by further additions of acid sea-water to keep the solution at about pH7·0.

In another experiment an amœba was paralysed at 1.10 p.m. in a solution at pH7·0. Later the pH began to rise, attaining the value pH7·5 at 7.15 p.m. The amœba at the same time partially recovered, the velocity being 0·5μ per second. The solution was now acidified, and paralysis again occurred and continued till 10.20 a.m. next day.

If the hydrogen ion concentration of the sea-water is increased by the addition of 0·01N solutions of the acids, acetic, butyric, lactic, oxalic, or sulphuric, in sea-water, the velocity : pH curves resemble those obtained with hydrochloric acid within limits normally met with in individual experiments (Fig. 5, No. 2).

It might at first be thought that the weak acids, such as butyric, which penetrate the cell rapidly, would alter the form of the velocity : pH curve. But it has already been pointed out that the velocity was measured after the amœba had become adjusted to the medium. Adjustment may take place more quickly with weak acids, but so far this point is undetermined, owing to the irregular movement of the amœba after the solution is first changed.

None the less, it might be assumed that the inhibitory action of acids is partly an immediate surface effect, and that a considerable time must be allowed for an amœba to attain complete equilibrium in an acid

* Changed back to "outside sea water."

solution. But each velocity : pH curve takes from one to five hours to determine, and during the greater part of this time the amœba is in contact with divers strengths of the acid solution. Thus, even were equilibrium attained only slowly there is ample time for acids with various rates of penetration to affect differentially so small a cell, and consequently to alter the form of the velocity : pH curve. However, since the curves are the same for acids such as hydrochloric and butyric, equilibrium must be attained within the time allowed for an amœba to adjust itself to a changed medium.

The similarity of the curves obtained from very different acids suggests that inhibition of amœboid movement in acid solutions is due to hydrogen ions, and not to the acid radicle.

Now 4 c.c. of a 0·01N solution of " acid in sea-water " are required to bring 50 c.c. of sea-water from pH8·1 to pH7·0, the paralysis point for Type B amœbæ. In such an acid solution the concentration of the acid radicle, over and above its natural concentration in sea-water, is therefore

$$0{\cdot}01\mathrm{N} \times \frac{4}{50+4}, \text{ or about N/1300.}$$

If the effects of both strong and weak acids are due entirely to the hydrogen ions and not to the acid radicle, the velocity of the amœba should be unaffected by a concentration of the acid radicle of N/1300, provided the hydrogen ion concentration is kept constant. Table 3 shows that this is the case for the butyrate radicle. In this experiment the concentration of the butyrate radicle was raised by the addition of a solution made up by bringing 0·01N " butyric acid in sea-water " up to pH8·1 with sodium hydroxide.

TABLE 3.

Solution maintained at pH8·1.

Concentration of Butyrate radicle.	Temperature. °C.	Time. p.m.	Velocity. μ per sec.
Nil	14·2	4.25	1·87
N/5100	14·3	4.40	1·87
N/1700	14·3	4.50	1·89
N/1100	14·4	5.00	1·92
N/600	14·3	5.10	1·94
N/350	14·3	5.30	1·90
N/350	13·0	6.20	1·95
N/200	13·3	6.30	1·90
N/100	13·3	6.45	1·71
N/100	13·3	7.15	0·83
N/100	13·0	10.30	0 (cytolysed)

Before the inhibitory effect of an acid solution can be certainly attributed to the hydrogen ions, one other possibility must be investigated. When sea-water at pH 8·1 is acidified to pH7·0 the carbonates are partially decomposed and CO_2 is evolved. Possibly it is the rise in the concentration of carbon dioxide in the sea-water which inhibits amœboid movement. Jacobs (**19**) has shown that owing to the manner and rapidity of its penetration into living cells, carbon dioxide can exert a powerful specific inhibitory effect not shared by other acids.

To test this point, velocity : pH curves were obtained from amœbæ placed in a solution of artificial sea-water from which carbonates had been excluded. The solution was not absolutely free of carbon dioxide, since some of the gas was dissolved from outside air during preparation, but the solution was never in equilibrium with the carbon dioxide in the laboratory air. Carbon dioxide was absorbed from this air even when the solution was acidified to pH7·0, for on standing it became more acid at the surface exposed to the air, as indicated by the colour change of brom thymol blue. However, the amount of CO_2 in the acidified artificial sea-water is very much less than the amount in acidified natural sea-water.

Were the inhibition of amœboid movement due to the production of CO_2 in an acid solution, the very great difference in CO_2 content of acidified artificial and acidified natural sea-waters must cause a great change in the form of the velocity : pH curves.

It is hoped to repeat these experiments later in a closed chamber, where the partial pressure of CO_2 can be controlled absolutely.

The artificial sea-water was made up in the manner described by Allen (**2**), except that no carbonate was added. The solution contained :—

NaCl	28·13 gms.
KCl	0·77 ,,
$CaCl_2$	1·20 ,,
$MgCl_2$	2·55 ,,
$MgSO_4$	3·50 ,,

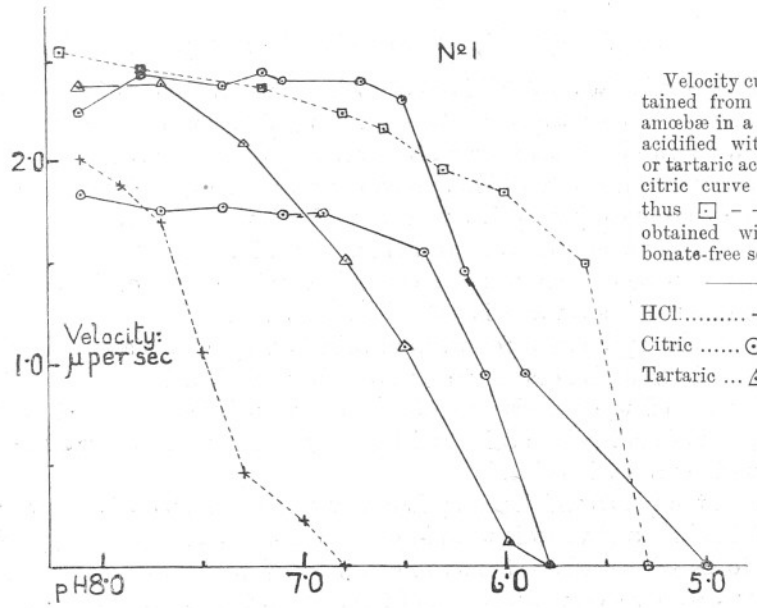
Boiled distilled water, 1000 c.c.

This made a solution of about pH6·8. The solution was oxygenated by shaking with air outside the Laboratory so that a minimal quantity of CO_2 was absorbed. Just before use this solution was brought to pH8·0–8·5 by the addition of pure NaOH solution.

0·005N solutions of acids in this artificial " carbonate free " sea-water were prepared by adding 1·0 c.c. of 1·0N pure acid in distilled water to 199 c.c. of the artificial sea-water. Because there were no carbonates to neutralise, these solutions were more acid than those prepared as already described from " outside sea-water."

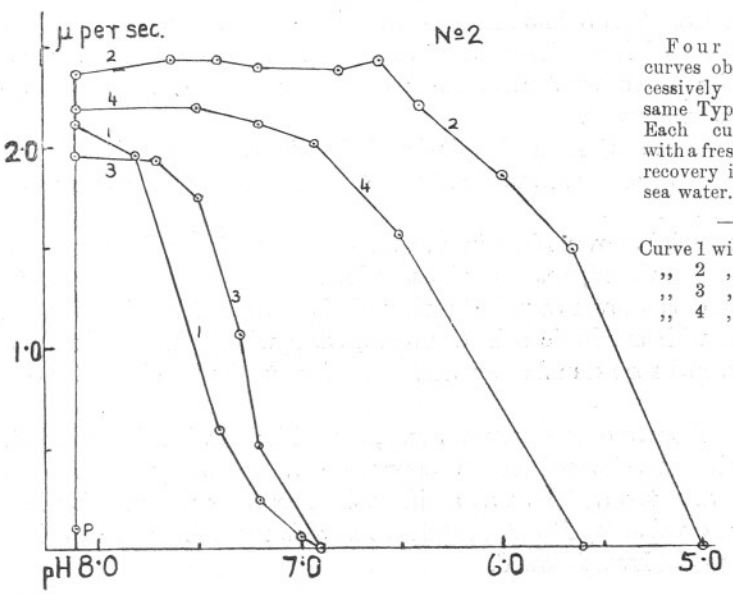
The velocity : pH curves obtained from amœbæ in "carbonate free" sea-water are of exactly the same type as those obtained in normal sea-water (Fig. 5, No. 3).

FIGURE 6.



Velocity curves obtained from Type B amœbæ in a medium acidified with citric or tartaric acids. The citric curve marked thus ⊡ – – –, was obtained with carbonate-free sea water.

HCl..... +
Citric ..... ⊙ and ⊡
Tartaric ... △



Four velocity curves obtained successively from the same Type B amœba. Each curve taken with a fresh acid, after recovery in "outside sea water."

Curve 1 with HCl.
,, 2 ,, Citric.
,, 3 ,, H_2SO_4
,, 4 ,, Tartaric.

The inhibition of amœboid movement in acid sea-water is therefore not due to the increase of the carbon dioxide in the sea-water. Since the inhibition has already been shown not to be due to the acid radicle, it seems that it must be directly related to the hydrogen ion concentration itself.

The effect of citric and tartaric acids: Type B.

When "outside sea-water" is made acid by the addition of a 0·01N solution of citric or tartaric "acid in sea-water," the velocity: pH curves are quite different from those obtained with other acids. With citric acid the velocity remains almost constant from pH8·1 to pH6·5. At pH6·5 the velocity begins to fall rapidly, the amœba becoming paralysed at between pH5 and pH6 (Fig. 6, No. 1).

The same type of velocity: pH curve is obtained from citric acid if "carbonate free" sea-water is used (Fig. 6, No. 1).

The velocity: pH curve for tartaric acid is very like that of citric. As in citric, paralysis does not take place with tartaric acid until pH5–6, But instead of the velocity remaining constant between pH8·1 and pH6·5, as it does in citric acid, it falls fairly slowly from about pH7·5 down to the paralysis point.

Though the pH at which paralysis occurs corresponds to a far greater acidity in citric and tartaric than in other acids, yet the paralysis itself seems to be of essentially the same character, since complete recovery readily takes place on transference of the amœba to sea-water pH8·1.

Figure 6, No. 2, shows four velocity: pH curves obtained from the same amœba. The animal, in sea-water pH8·1, was first paralysed by the addition of hydrochloric acid (curve 1). The solution was then changed for fresh sea-water at pH8·1: complete recovery had taken place two hours afterwards.

The amœba was then paralysed with citric acid (curve 2). The solution was again changed for sea-water at pH8·1, and recovery had taken place one hour later.

After remaining over night in a small vessel containing "outside sea-water," under the microscope, the amœba was next day transferred to a dish full of sea-water at pH8·1; the velocity was normal. It was then paralysed with sulphuric acid (curve 3), after which the solution was again changed for normal sea-water. Recovery took place in under one hour.

The amœba was once more paralysed, but with tartaric acid (curve 4). The solution was changed for sea-water pH8·1 and recovery commenced. Unfortunately the amœba was accidentally lost after the velocity had reached the value "p," but doubtless had this not occurred, complete recovery would have followed.

The curves are typical of their respective classes of acids—and all obtained from the one individual amœba. The slight differences in initial velocity are more or less correlated with temperature (Table 4).

TABLE 4.

Initial solution temperatures for curves in Fig. 6, No. 2.

Type B amœba.		pH.	Velocity. μ per sec.	Temperature. °C.
1st Day	curve (1)	8·1	2·12	12·2
	,, (2)	8·1	2·37	14·5
2nd Day	,, (3)	8·1	1·96	13·0
	,, (4)	8·1	2·20	13·7

From these experiments it seems that the presence of citric or tartaric acid radicles protects Type B amœbæ from the onset of hydrogen ion paralysis which occurs normally at pH7·0. This protection is not broken down till the hydrogen ion concentration is raised about twenty-fold. If this is really a protective effect, the addition of the citrate or tartrate radicle should cause recovery in an amœba paralysed at pH7·0 by means of HCl. Figure 8, Nos. (1) and (2) show that this recovery does take place.

Solutions of 0·01N citric or tartaric acids in sea-water were neutralised with NaOH to pH7·0. An amœba was now paralysed at about pH7·0 by the addition of 0·01N HCl in sea-water. It requires 4 c.c. of a 0·01N acid solution in sea-water to bring 50 c.c. normal sea-water from pH8·1 to pH7·0 ; 4 c.c. of the neutralised citrate (or tartrate) solutions were therefore added to the sea-water at pH7·0, containing the paralysed amœbæ. The amount of citrate (or tartrate) now present in the sea-water is approximately the same as the amount which would have been present had the original sea-water been acidified from pH8·1 to pH7·0 by means of 0·01N citric (or tartaric) acid solution in sea-water.

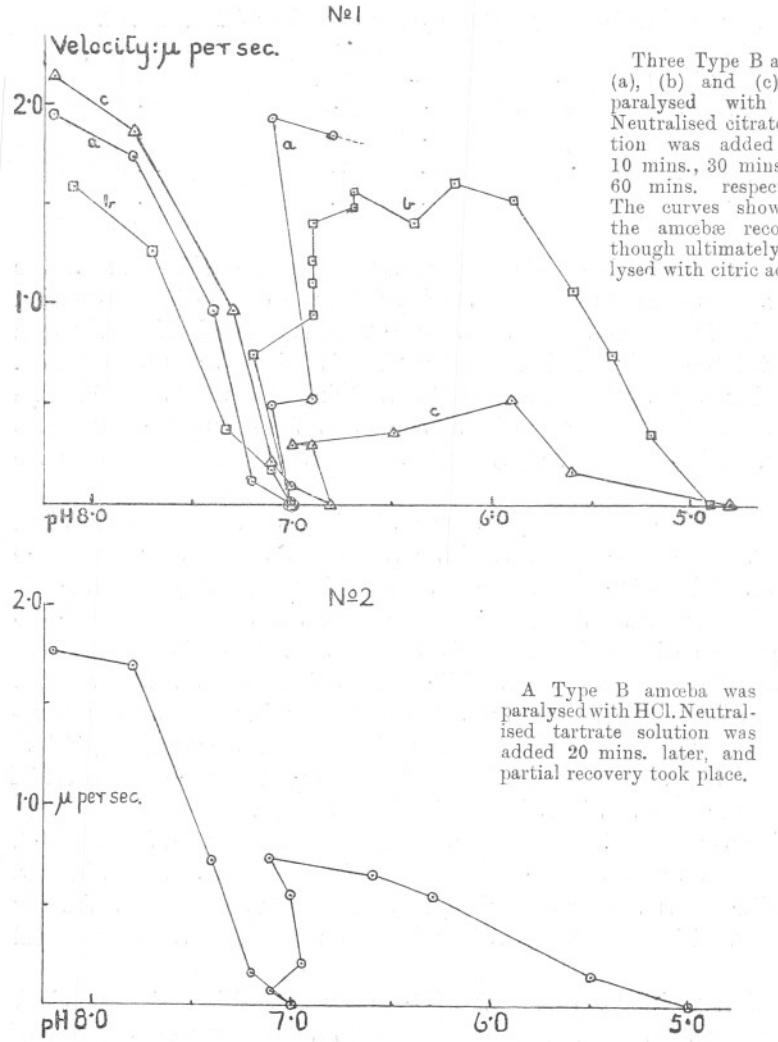
Owing to the evolution of CO_2 the pH now tends to rise above pH7·0. To counteract this some 0·01N solution of citric (or tartaric) acid (not neutralised) was added to the dish, thus bringing the pH down again. By further additions of these acid solutions the pH was lowered still more.

Amœbæ, which recovered at pH7·0 by the addition of neutralised citrate or tartrate solutions, were not paralysed by the addition of citric or tartaric acids till pH5–6, the normal paralysis point for these solutions.

In curve (a), Fig. 7, No. 1, the neutralised citrate was added ten minutes after paralysis with HCl. Recovery was complete one hour later. In curve (b) the citrate was added thirty minutes after paralysis

with HCl, and recovery did not commence for nearly one hour. Recovery was not complete till a certain amount of 0·01N citric acid solution in sea-water had been added—sufficient to raise the hydrogen ion concentra-

FIGURE 7.



Three Type B amœbæ (a), (b) and (c) were paralysed with HCl. Neutralised citrate solution was added after 10 mins., 30 mins., and 60 mins. respectively. The curves show that the amœbæ recovered, though ultimately paralysed with citric acid.

A Type B amœba was paralysed with HCl. Neutralised tartrate solution was added 20 mins. later, and partial recovery took place.

tion to pH6·7 : this was two hours after the commencement of recovery. In curve (c) the citrate was added one hour after paralysis. Recovery commenced in about one hour, but was never complete. The highest velocity after recovery was reached two hours after its commencement,

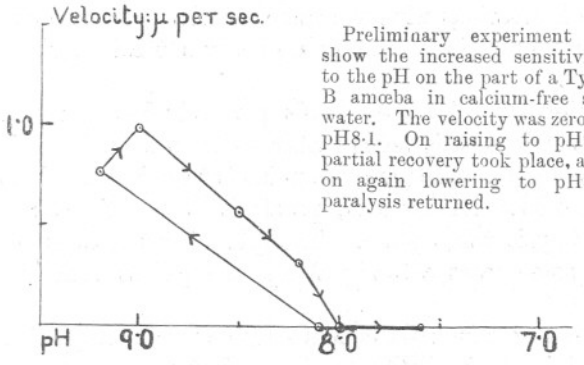
when sufficient 0·01N citric acid solution had been added to raise the hydrogen ion concentration to pH5·9.

The addition of neutralised tartrate solution also causes recovery in Type B amœbæ paralysed at pH7·0 with HCl (Fig. 7, No. 2).

There seems to be no doubt that these results are due to a protective effect of the citrate and tartrate radicles. This protective effect does not seem to be related to the basicity of the acid, since behaviour is normal in sulphuric and oxalic as well as in the monobasic acids. Moreover, it does not seem to be related directly to the presence of the >CHOH group as such, because normal behaviour occurs in lactic acid.

Gray (13) has shown that citrates and tartrates have a unique effect

FIGURE 8.



Preliminary experiment to show the increased sensitivity to the pH on the part of a Type B amœba in calcium-free sea water. The velocity was zero at pH8·1. On raising to pH9·2 partial recovery took place, and on again lowering to pH8·0 paralysis returned.

upon the cilia of Mytilus gills, but the effect is not protective, it is inhibitory (at pH7·8). In the present experiments the citrates and tartrates have never exceeded a moderate concentration, and have always been in the presence of the normal anions of sea-water at their usual concentration: in Gray's experiments the normal anions were entirely replaced by citrate or tartrate. It is probable that the action of these acid radicles is quite different under these two sets of conditions.

However, Gray points out that in the presence of citrates and tartrates the calcium in sea-water is probably not present in its ionic form, and he brings evidence to show that the effects of these acid radicles on ciliary activity are due to this absence of the calcium ion. In view of this it should be mentioned that from experiments still in progress on the effects of ions on amœboid activity, it has been found that the behaviour of Type B amœbæ in calcium-free sea-water bears no resemblance to their behaviour in the experiments with citrates and tartrates. The behaviour of the amœbæ in calcium-free sea-water seems to be analogous to the behaviour of cilia under like conditions, as described by Gray (13). The amœbæ are very sluggish in calcium-free sea-water at pH8·1,

but become active when the solution is made more alkaline (Fig. 8, p. 55), though the velocity is below normal. Absence of calcium, in fact, seems to increase the sensitivity to hydrogen ions, the critical pH being raised from pH7·0 to pH7·6–8·0. This is the very opposite of the effects observed in the citrate and tartrate solutions. For the present the peculiar effects of citrates and tartrates must go without an explanation.

The effects of acids: Type A.

The movement of Type A amœbæ is much less regular than that of Type B. Velocity measurements had to be taken by averaging a large number of readings obtained across single divisions of the micrometer; the movement rarely proceeded uninterrupted for five successive divisions. Also, velocity observations with the same amœba under the same conditions differed more widely among themselves than in the case of Type B amœbæ.

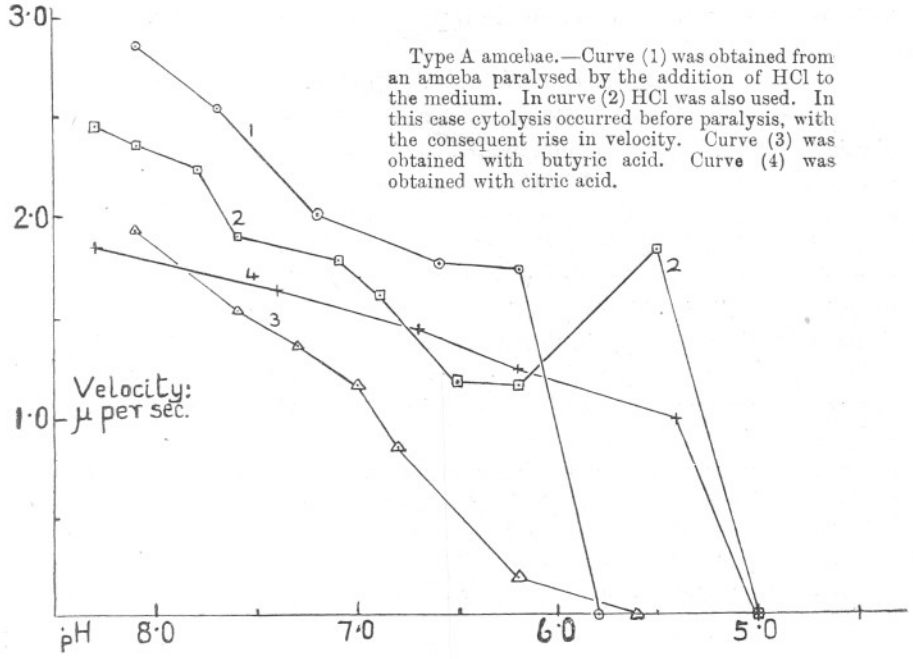
The velocity of Type A amœbæ falls gradually between pH8·1 and about pH6·5, and from this point rather more rapidly, becoming zero between pH6 and pH5. The paralysis point is therefore well below that of Type B amœbæ. In Type A, paralysis is sometimes reversible, but usually cytolysis takes place. The pH of cytolysis is only just below, and in some cases actually above, the pH at which paralysis occurs.

Figure 9 shows velocity : pH curves obtained from Type A amœbæ in sea-water acidified with hydrochloric and butyric acids respectively. Butyric acid seems to be rather more effective than hydrochloric, the critical pH being a little higher. The difference is, however, within the limits of variation for Type A.

In some amœbæ cytolysis began before the paralysis point had been reached. When this occurred there was a sudden large rise in the velocity accompanied by marked changes in the amœba (Fig. 9). The surface of the protoplasm became more rounded. The tail-piece, instead of having the usual rugose appearance, began to cytolyse, so that a mass of coagulated protoplasm was carried at the hind end of the amœba. The "fine processes" of the normal tail-piece were absent, but in their place large droplet pseudopodia were formed. These were rapidly extended and retracted and even became detached from the amœba (Fig. 10, No. 1).

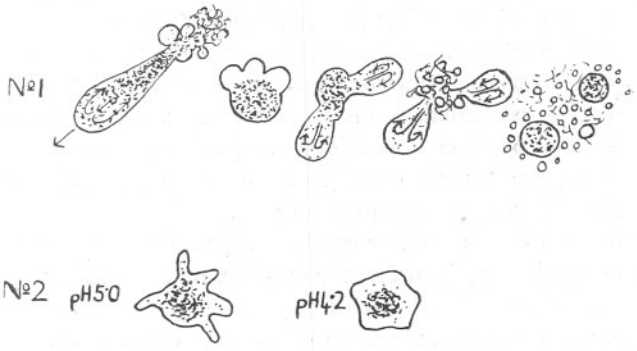
The endoplasmic streaming was very violent, but the velocity though much increased did not rise in proportion. The ectoplasm of the under surface continually slipped from its hold on the substratum, so that although activities normally associated with movement proceeded at a great speed yet locomotion was very inefficient.

FIGURE 9.



Type A amœbae.—Curve (1) was obtained from an amœba paralysed by the addition of HCl to the medium. In curve (2) HCl was also used. In this case cytolysis occurred before paralysis, with the consequent rise in velocity. Curve (3) was obtained with butyric acid. Curve (4) was obtained with citric acid.

FIGURE 10.



No. 1.—Changes in a Type A amœba when cytolysis in an acid medium precedes paralysis. The first figure illustrates the form during the period of increased activity.

No. 2.—Type A: to show changes in form below the point at which the velocity becomes zero when citric acid solution is added.

Ultimately locomotion ceased. The endoplasmic streaming still continued, and large droplet pseudopodia formed. Complete cytolysis followed in the manner described in a previous section of the paper.

Effect of citric acid: Type A.

There was no marked difference between the velocity : pH curve obtained from a Type A amœba in citric and in other acids. Locomotion ceases at a rather lower pH in the presence of citric acid, but this pH is close to the limit of variation for the paralysis pH in HCl (Fig. 9).

But in citric acid the cessation of locomotion does not mean a complete paralysis of amœboid movement. Pseudopodial activity, though progressively diminishing, sometimes continues down to pH4·2. The character of the pseudopodia changes, however. Instead of the formation of the single limax pseudopodium, many pseudopodia are formed. These become blunter as pH4 is approached (Fig. 10, No. 2). The translucent endoplasm with its minute granules concentrates into a central mass, the ectoplasm at the same time becoming relatively clear. Ultimately pseudopodial activity ceases at about pH4·0, when the amœba is almost spherical. Cytolysis follows, and the ectoplasm is destroyed, leaving a central coagulated mass of protoplasm.

The protective effect of citrates is far less marked in Type A than in Type B amœbæ.

DISCUSSION AND CONCLUSION.

In many respects amœboid activity resembles that of cilia and muscle. The protoplasm of the ectoplasmic tube of a limax amœba is contractile. Contractility is more marked in the pseudopodia of the Foramenifera and Difflugia ; so much so that Dellinger (**9**) has suggested that all amœboid activity is due to the contraction of a semi-permanent reticulum pervading the entire individual. This suggestion fails when the movement of obviously fluid amœbæ is considered. Nevertheless some degree of contractility is characteristic of all forms of amœboid activity, just as it is of ciliary (**12**) and of muscular activity.

The osmotic pressure experiments show that the water-content of the cell must be within certain limits for efficient amœboid activity. Gray (**12**) has shown that this also holds for ciliary activity, and the same thing applies to muscle (e.g. the work of Carlson on the heart of Limulus (**5**).)

All three forms of contractility are inhibited by raising the hydrogen ion concentration (**12**) (**4**), and this inhibition is reversible.

The production of acid in muscular activity is well known, and Gray (**12**)

has inferred that the same thing occurs in ciliary activity. The foregoing experiments with neutral red indicate that an increase of hydrogen ion concentration also occurs during pseudopodium formation, though it has been pointed out that this conclusion must be accepted with caution. However, the parallel between the inhibition by acids of amœboid and of other forms of contractility does support this.

Whereas contractility is well marked in muscle, cilia, and certain specialised amœboid individuals, it is less marked in amœbæ with fluid protoplasm. The earlier hypotheses to account for amœboid movement concentrated on this fluid character of the protoplasm; they assumed that an amœba could be considered as a fluid drop in a medium with which it was immiscible. It was natural, therefore, to assume that the energy of amœboid movement was derived from surface tension changes.

According to Berthold the amœba flowed passively under the influence of local variations of surface tension between the fluid protoplasm and the substratum. Such a passive reaction of the amœba cannot explain all the phenomena observed, and, as Berthold himself realised, the hypothesis breaks down for free pseudopodia projected into the medium.

Rhumbler (**30**) suggested that a pseudopodium was formed by a local lowering of surface tension at the surface of the amœba. This was brought about by hypothetical minute droplets of "enchylema," which burst on the surface of the ectoplasm. The endoplasm then flowed towards the region of lowered surface tension, and, turning outwards at the tip of the pseudopodium, flowed backwards along the surface, having been changed into ectoplasm by the "ento-ectoplasmic process." Rhumbler called this streaming of the protoplasm a "fountain current." The advantage of this hypothesis over that of Berthold was that it assumed pseudopodium formation to be an active process on the part of the amœba.

Great difficulties are in the way of surface tension hypotheses of amœboid movement. In the first place, the surface of protoplasm must be supposed to be fluid. The microdissection studies of Seifriz (**33**), Chambers (**6**), and Kite (**21**) all go to show that whereas the endoplasm is fluid, the ectoplasm is gelated at the surface, though the thickness of the gelated layer seems to vary in different cases. It is difficult or impossible for a body with a solid surface to alter its form under the influence of surface tension. Moreover, the existence of the filose pseudopodia of the Foramenifera shows that in this case, at least, surface tension is negligible. Were it not so, the pseudopodia would be unstable; they would become moniliform, or even break up into droplets—a change which actually occurs in the pseudopodia of Polystomella on cytolysis with sodium hydrate.

Apart from these considerations Mast and Root (**26**) have shown that surface tension cannot supply the energy required to account for the relatively great force exerted by the pseudopodia of amœba. The pseudopodia can actually pinch a *Paramœcium* in two.

Though the surface tension hypothesis offers an explanation of the movement of fluid amœbæ, it breaks down in cases where contractility is highly developed. It fails in the case of Difflugia which Dellinger (**9**) has observed to move by the extension and retraction of pseudopodia. Cases such as these are the very ones that serve to link amœboid movement with other forms of contractility, and must be taken into account in any complete hypothesis of amœboid movement.

Rhumbler realised that the surface tension hypothesis could not account for the movement of amœbæ with a firm ectoplasm, such as *A. verrucosa*. He suggested that in these cases movement was the result of a local liquefaction of the ectoplasm. In a later paper (**31**) he works out this hypothesis in greater detail on *A. terricola* and allied forms.

Hyman (**18**) extends this new hypothesis to cover all forms of amœboid movement. She points out that microdissection evidence shows that in all amœbæ the ectoplasm is gelated, though local liquefaction is present during pseudopodium formation. She therefore suggests that in the formation of a pseudopodium there is a change from the gel to the sol state in the ectoplasm. Conversely in the retraction of a pseudopodium there is a change from the sol to the gel state (cf. Chambers (**7**)).

So far as concerns the fluid nature of the protoplasm in the advancing pseudopodium, my own observations are in agreement with those of Hyman. But in limax amœbæ continuous movement also demands the "ento-ectoplasmic process." The essential feature of the limax movement is the formation of fluid ectoplasm at the anterior end of the advancing pseudopodium by the "ento-ectoplasmic process." This ectoplasm, becoming gelated at the sides of the pseudopodium, forms the contracting ectoplasmic tube, the gelated ectoplasm being absorbed again into the endoplasmic stream within the hinder end of the amœba. The streaming of the protoplasm agrees with the "fountain-currents" of Rhumbler (**30**), except that there is no backward *current* at the sides of the amœba, but a gelated ectoplasmic tube, which passes back to the hind end as it contracts. The description of the limax movement given in this paper agrees with that of Schæffer (**32**), except that he does not consider that the endoplasmic stream flows passively under the pressure of the contracting ectoplasmic tube.

The experiments of Loeb (**25**) on the effect of osmotic pressure on amœbocytes, as well as my own experiments on its effect on marine amœbæ, support the conclusion that pseudopodium formation is accompanied by local fluidity of the ectoplasm. This conclusion offers an ex-

planation of the behaviour of pseudopodia in solutions of different osmotic pressures. But the experiments go further : the similarity of the condition of the tail-piece of an amœba to that of the entire amœba when water has been abstracted from it, together with the fluid character of the advancing pseudopodium, suggest that water is actually abstracted from the hind end of the amœba, and imbibed by the protoplasm at the anterior end. A water current is therefore set up towards the anterior end. This current, aided by the contraction of the ectoplasmic tube, would give rise to the endoplasmic stream.

Schæffer (32) considers the energy of amœboid movement to be derived from the streaming endoplasm itself. But, as he points out, where pseudopodia are highly contractile this streaming does not seem to accompany amœboid activity. Again, a pseudopodium when first formed may consist entirely of swelling ectoplasm, the endoplasmic granules actually being pushed inwards (Fig. 1, No. 4). The endoplasm does not burst into the pseudopodium till later. Jennings (20) also describes the bursting of the endoplasm into an ectoplasmic pseudopodium.

If it is assumed that amœboid activity is brought about by the imbibition and abstraction of water with consequent swelling and contraction of the protoplasm, both the movement of amœbæ with fluid protoplasm, and the contractile movements of Difflugia and other forms, become special cases of the same general phenomenon.

Fürth (11), in a theoretical consideration of the subject, has attempted to unite both the imbibition and the surface tension theories. He calls attention to the effects of the addition of acid to a protein suspension. A myosin suspension in the presence of acid increases in dispersion, and there is a tendency to imbibe water : at the same time the surface tension falls.

Fürth supposes a substance, "lactacidogen," to be present in the protoplasm. This substance is broken down locally, forming lactic acid. The increase in acidity causes imbibition of water from the surrounding protoplasm, with the result that a pseudopodial projection tends to be formed. This projection would be surrounded by a ring-shaped depression, owing to the abstraction of water in the neighbourhood of the projecting pseudopodium. The acid is supposed to cause local lowering of surface tension at the surface of the pseudopodium ; the surface tension over the rest of the amœba therefore tends to push out the swelling protoplasm of the pseudopodium, at the same time effacing the depression round its base.

Fürth considers that all forms of amœboid activity could be explained by this hypothesis, including the filose pseudopodia of the Foramenifera.

But the essential feature of his hypothesis is that the pressure of the swelling pseudopodium is acting against the pressure due to the surface tension over the surface of the amœba. These two pressures must therefore be of the same order of magnitude, and as we have seen from the observations of Mast and Root (**26**), sufficient energy cannot be derived from pressures of this order. Moreover, the same objection holds here that held against Rhumbler's surface tension theory ; the presence of gelated ectoplasm over the body of the amœba must render the mechanical effects of surface tension negligible. As in Rhumbler's theory, it is impossible to see how the long filose pseudopodia of the Foramenifera can remain stable unless the forces due to surface tension are too small to produce an effect.

With regard to an increase in acidity during pseudopodium formation Fürth's hypothesis is very suggestive. The writer, independently, had come to the same conclusion from the results of the experiments described in this paper. A rise in acidity does seem to occur during pseudopodium formation. Moreover, the importance of the hydrogen ion concentration in relation to amœboid activity is probably general, because the activity not only of these amœbæ but also, according to de Haan (**15**), of leucocytes varies with the pH.

Hyman's observations (**18**) also may indicate that a chemical change is occurring in an advancing pseudopodium. She finds that the tips of the youngest pseudopodia are the first parts of the amœba to cytolyse in dilute KCN. She argues on Child's hypothesis that the rate of metabolism is higher at these places than elsewhere.

We have seen that the inhibitory action of acids on amœboid movement is reversible, and that it is directly related to the hydrogen ion concentration. Gray (**12**), in considering the inhibition of ciliary activity by an acid medium, points out that according to Kondo the rate at which lactic acid is produced from its precursor depends upon the hydrogen ion concentration. If the production of acid in amœboid activity be due to a chemical mechanism similar to that which produces lactic acid in muscle, the inhibitory effect of an acid medium may be due to this cause.

There is possibly another way in which increased acidity of the medium may inhibit amœboid movement. The rise in hydrogen ion concentration may increase the gelation of the ectoplasm. Gelation may proceed too rapidly in the advancing pseudopodium where ectoplasm is being formed from endoplasm. The resistance to amœboid movement would thus increase in proportion to the penetration of the acidity of the medium.

Before investigating this second possibility further, the effects of an acid medium on the surface tension must be considered. Fürth, as we have seen, suggests that the surface tension at the surface of the amœba

will fall in the presence of acid, on the analogy of the effect of acid on a suspension of myosin. But living protoplasm is very different from a simple protein suspension. There is evidence that the cell surface is not of a protein, but of a lipoid nature (**4**).

The effect of a rise in hydrogen ion concentration on a lipoid-water interface will be very different from the effect on a protein-water interface. Hartridge and Peters (**16**) have shown that for an olive oil-water interface the surface tension increases, and increases with great rapidity, as the hydrogen ion concentration rises ; near neutrality there is a rise of 35% for a fall of 1·0 of pH. They point out that this is in keeping with Langmuir's observations on the effects of acids and alkalis on films of oil and of fatty acids spreading on water.

The lipoid surface of the cell would probably behave in a similar manner ; a rise in hydrogen ion concentration, instead of diminishing the surface tension at the surface of an amœba, would cause it to increase considerably.

Schæffer (**32**) brings evidence to show that an increase in surface tension does occur at an advancing pseudopodium. He shows that a thin surface tension layer moves over the amœba towards the advancing anterior end. It is probable, therefore, that contrary to Fürth's assumption, work is actually done against the surface tension when a pseudopodium is formed.

Assuming from experimental evidence that there is an increase of acidity in pseudopodium formation, and assuming that a lipoid film covers the amœba, the following hypothesis to account for amœboid movement suggests itself.

The production of acid at some point in the protoplasm causes water to be imbibed : this assumption has also been made by Fürth. Proctor (**29**) has shown that acid causes imbibition of water by gelatine ; and he points out that this occurs both in gelatine gel and in the gelatine particles of a gelatine sol.

The above assumption implies that the protein constituents responsible for the imbibition are in the neighbourhood of the iso-electric point (cf. Loeb (**23**)).

The swelling resulting from the imbibition of water will cause a pseudopodium to be protruded, so that the surface is increased by stretching the surface layer. As swelling continues the protoplasm pushes beyond the old surface layer, and a fresh surface of fluid protoplasm is continuously exposed to the medium at the advancing anterior pseudopodium.

Since the acidity has raised the surface tension at the lipoid interface between the protoplasm and the medium, proteins and possibly other substances will become condensed at the freshly formed surface in order

to lower the free surface energy. This follows from Gibbs' principle, which shows that substances which lower the surface tension tend to concentrate at the surface (**4**). A familiar instance of the same principle is the surface coagulation of a solution of albumen.

Thus as the fresh surface of protoplasm is formed at the sides of the advancing pseudopodium, the protoplasm immediately beneath the surface will tend to gelate, forming ectoplasm. This is perhaps the explanation of the " ento-ectoplasmic " process.

As locomotion of the amœba proceeds and the pseudopodium continues to advance the already formed ectoplasm approaches the hind end of the amœba. The acidity at the same time diminishes, owing either to neutralisation and diffusion, or possibly, as in muscle, to partial reconversion into a precursor substance. The reduction of acidity will cause the reversal of imbibition, that is, syneresis and loss of water by the swollen particles of protein ; evidence for this loss of water has already been advanced. Moreover, the surface tension will fall and the concentration of substances at the surface will diminish. The contraction of the " ectoplasmic tube " and the absorption of protoplasm into the endoplasmic stream might be explained in this way. These effects, combined with the imbibition of water taking place at the anterior end of the amœba, would give rise to the endoplasmic stream.

The inhibitory action of acids in the external medium follows on this hypothesis because the surface tension will be raised ; there will be an increased condensation of substances at the surface. At the pH of paralysis this condensation will be so great and take place so rapidly where fresh surface is exposed on the advancing pseudopodium, that the swelling protoplasm is unable to overcome the resistance of the now gelated surface.

It is interesting to note that the imbibition of water by a protein solution in acid is accompanied by an increase in viscosity (**23**). Possibly it is owing to this increase that the granules of the streaming endoplasm so often seem to be checked behind the clear protoplasm of the advancing pseudopodium.

The advantage of this hypothesis is that the same explanation is offered to account for the activity of amœbæ with a fluid protoplasm and for the activity of those with highly contractile pseudopodia of high consistency. The swelling and syneresis of a protein according to the hydrogen ion concentration occurs whether the protein is in the sol or gel state (**29**).

Perhaps, as Fürth suggests, lactic acid is produced during pseudopodium formation. But this is a bold assumption in view of the fact that the carbohydrate-lactic acid mechanism of contraction has only been studied in highly specialised muscular tissues. It is possible that

there is an unspecialised chemistry of contraction in the unspecialised activity of amœba.

These suggestions rather overstep the basis of the existing knowledge concerning amœboid movement. They are only intended to form a working hypothesis to guide further research.

SUMMARY.

(1) Marine limax amœbæ were used for these experiments. A limax amœba may be looked upon as a contracting tube of gelated ectoplasm, closed at the posterior end; the anterior end is occupied by the fluid ectoplasm of the advancing pseudopodium. The fluid endoplasm streams forward through this tube from a place of liquefaction within the posterior end of the amœba. On reaching the anterior end, the streaming endoplasm apparently forms the fluid ectoplasm of the advancing pseudopodium. This fluid ectoplasm continuously adds to the contracting tube by gelation at the sides of the pseudopodium.

(2) As in the activity of muscle and cilia, amœboid activity can only take place provided the water content of the cell is within certain limits. Solutions with an abnormal osmotic pressure produce marked changes in the character of the pseudopodia: these changes can in part be accounted for, by the increased gelation of the protoplasm owing to the abstraction of water in hypertonic solutions on the one hand, and on the other by the increased fluidity owing to imbibition of water by the cell in a hypotonic solution.

(3) Comparison of the normal amœba with those in hypertonic and hypotonic solutions suggest that water is abstracted from the hind end of an active amœba, while the fluid protoplasm of the anterior pseudopodium imbibes water.

(4) Experiments with certain amœbæ stained with neutral red indicate a rise in hydrogen ion concentration in an active pseudopodium. The ectoplasm seems to be more acid than the endoplasm. For reasons given in the text it is not certain that the effects indicate the production of acid in the cytoplasm itself; changes in the minute stained granules might alone be involved.

(5) Experiments were performed to determine the relation between the velocity of an amœba and the pH of the medium. An acid

medium inhibits amœboid movement, the velocity reaching zero at a fairly definite pH for each kind of amœba.

As in muscle and cilia, this inhibition in acids is reversible, provided the acidity be not too great. Amœbæ paralysed with acid recover completely on transference to sea-water at pH8·1.

(6) The velocity of a Type B amœba, which has become adjusted to the medium, falls slowly as pH7·6 is approached and afterwards more rapidly, complete paralysis occurring at pH6·8–7·0. In Type A amœbæ the velocity falls gradually to about pH6·5, and then more rapidly to pH5–6, where paralysis occurs.

In Type A, cytolysis usually occurs near the paralysis point. Sometimes cytolysis occurs before this point is reached: this is accompanied by a sharp rise in the velocity, together with marked changes in the amœba.

(7) The same velocity : pH curve is obtained whether the medium be acidified with hydrochloric, acetic, butyric, lactic, sulphuric, or oxalic acids. The inhibition of amœboid movement in an acid medium depends neither on the acid radicle added nor on the carbon dioxide evolved by decomposition of the carbonates of the sea-water: it depends upon the hydrogen ion concentration.

(8) Citrate and tartrate, however, exert a protective action on Type B amœbæ, and possibly also on Type A, though to a much smaller extent. In the presence of these acid radicles the paralysis point of Type B amœbæ is shifted from pH7 to pH5–6. In spite of this, inhibition with citric and tartaric acids is reversible, just as it is in other acids.

(9) The protective action of citrate and tartrate seems to be unrelated to the basicity of the acids, because sulphuric and oxalic acids produce the same kind of velocity : pH curve as the monobasic acids. Nor does the action seem to be directly related to the presence of a =CHOH group, because lactic acid gives a normal velocity : pH curve.

(10) The protective action is not due to the absence of calcium ions through the formation of complex molecules between calcium and the citrate or tartrate radicle. Preliminary experiments show that absence of calcium raises the paralysis point of Type B amœbæ to pH7·6–8·0, the opposite effect to that of citrate or tartrate. The action of these radicles cannot yet be explained.

(11) A working hypothesis is suggested to account for amœboid movement. The local production of acid causes imbibition of water by the protoplasm. This causes swelling, and a pseudopodium is protruded. For reasons given in the text it is suggested that the acid raises the surface tension over the advancing pseudopodium. Substances in the protoplasm will now concentrate on the freshly formed surface at the sides of the advancing pseudopodium, in order to lower the surface energy. This would account for the formation of the gelated ectoplasmic tube by the "ento-ectoplasmic" process.

As the gelated tube passes back towards the hind end of the amœba the acidity disappears; the imbibed water is lost by syneresis with a resulting contraction of the protoplasm. At the same time the surface tension falls and the surface concentration of substances diminishes. This would account for the contraction of the ectoplasmic tube and its internal absorption into the endoplasmic stream.

The imbibition of water at the anterior pseudopodium and the syneresis at the posterior end, together with the force of the contracting tube of ectoplasm, would cause the endoplasmic stream to be driven forwards.

I wish to thank Mr. J. Gray, of King's College, Cambridge, with whose kind help and advice this research was commenced.

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