ON THE PHYSIOLOGY OF AMOEBOID MOVEMENT

VIII. A. THE ACTION OF CERTAIN NON-ELECTROLYTES.

B. A NOTE ON THE ISO-ELECTRIC POINT OF THE PROTEINS OF A MARINE AMOEBA.

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(With Four Text-figures.)

A. THE ACTION OF CERTAIN NON-ELECTROLYTES.

(1) *Introduction.*

In a previous series of experiments (Pantin, 1926 *a* and *b*) it was shown that calcium was essential for continued amoeboid movement in certain marine amoebae. Movement always took place in mixtures containing any of the major cations of sea water (Na, K, Mg, as chlorides), provided calcium was also present within a certain range of concentrations. But this range and the quality of the movement differed according to the other constituents of the mixture.

For continued movement, two conditions must obtain: (1) the general concentration of divalent cations must be sufficient to stabilise the cell, by preventing the cytolytic effects found in their absence (*e.g.* in pure NaCl); the divalent ion being either Ca^{++} or Mg^{++}: (2) a certain concentration of calcium itself must be present if *movement* is to occur, as opposed to mere inactive survival. Magnesium will not replace calcium in this respect. Therefore in a simple mixture such as $NaCl + CaCl_2$, the calcium has two distinct actions.

The question now arises: Are these effects due primarily to the presence of calcium in certain regions of concentration, or is it only when calcium is in the presence of some other antagonistic cation, such as sodium, that the effects are produced? This question is clearly of great importance if a comparison is to be made later between marine and fresh-water amoebae: the ionic composition of the medium is so different.

An attempt has therefore been made to study the relation of movement to calcium concentration independently of the concentration of other cations. In simple mixtures of salt solutions isotonic with sea water it is not possible to vary the calcium without concomitant changes in the concentration of other component

ions: otherwise the results would be overwhelmed by osmotic changes. The effect of simple differences in calcium concentration is therefore hard to disentangle.

In the following experiments, variations in calcium concentration were made independently of other cations by the addition of non-electrolytes to maintain the osmotic pressure equal to that of natural sea water. It is not to be supposed that a given non-electrolyte is necessarily physiologically inert apart from its osmotic action, but by comparing the results obtained when using non-electrolytes with those obtained when using salts, a much wider basis is provided for the ascription of physiological effect to calcium.

There are restrictions on the non-electrolytes which can be used. They must, as far as possible, be without special physiological effect; they must be very soluble, since a 1·05 *M* solution is isotonic with the sea water employed; they should not tend to break down into physiologically active substances; and they must be as diverse as possible in order to throw into relief any special effects they may possess.

(2) *Materials and methods.*

Marine limax amoebae originally obtained from the aquarium tanks at the Plymouth Laboratory were used for the experiments. They have already been described. When locomotion occurred, it was measured over the field of a Ghost-micrometer with a stop-watch. In any given solution about fifty amoebae were placed and the mean speed of five measured at each reading. The speed was compared with the mean normal speed in sea water. These methods have been fully described (Pantin, 1926 *a*).

Kahlbaum's purest salts were used. All solutions were freshly prepared and made isotonic with the sea water used. The strengths were 1·05 *M* for non-electrolytes, 0·6 *M* for NaCl, 0·4 *M* for $CaCl_2$ and $MgCl_2$. All mixtures were made from these. Where necessary, the *p*H was adjusted with traces of NaOH, $Ca(OH)_2$, or HCl. Experiments were conducted as far as possible at *p*H 7·0. In all cases the temperature was between 12 and 14° C.

Before being placed permanently in any solution the amoebae were washed twice with the experimental fluid to remove adherent sea water.

(3) *The action of sucrose and glucose.*

In natural sea water the amoebae are of the "limax" form with a single pseudopodium which is advancing continuously. In pure isotonic solutions of either of these sugars all activity rapidly ceases. The amoebae tend to become sphaeroidal with many irregular blunt pseudopodia. The amoebae fail to adhere to the Petri dishes in which they are placed. During the first hour of immersion the amoebae seem to shrink slightly, and the granules tend to clump in the endoplasm leaving the ectoplasm clear and transparent (Fig. 1). Ultimately the amoebae become rounded into irregular spheres.

In spite of these effects recovery can take place in sea water after one hour's immersion in the sugar solutions. Recovery takes about 2 hours, and while it is

in progress the amoebae pass into a "proteus" form with large irregular pseudopodia (Fig. 1).

No movement occurs in mixtures of isotonic sugars with isotonic $CaCl_2$ in any proportion. In all cases irregular proteus forms are found. Below 0·05 *M* Ca these are similar to those found in pure sugars: there is also a similar failure to adhere to the surface of the glass Petri dish. Adhesion always takes place in concentrations above 0·1 *M* Ca, and in such solutions the form of the amoeba is characteristic of calcium excess (cf. Pantin, 1926 *a*). All these effects are reversible for several hours.

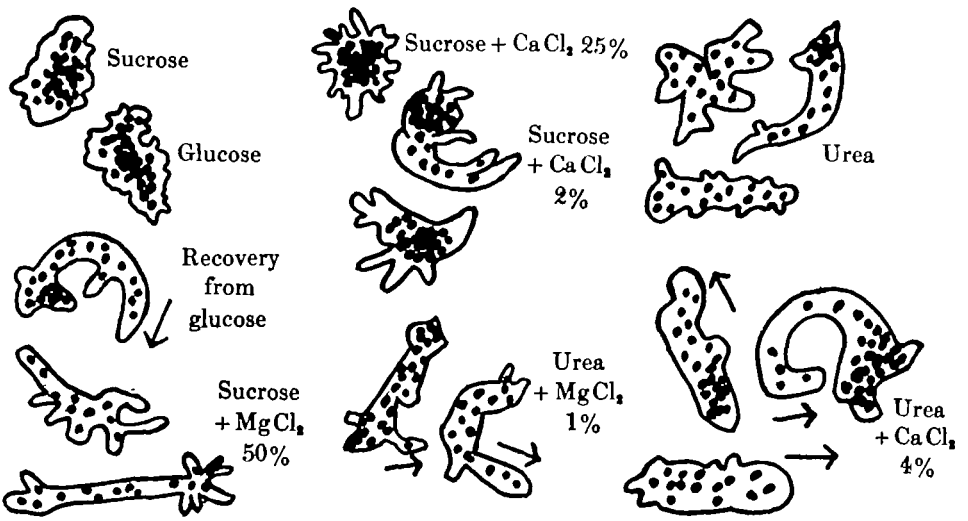

Fig. 1. Typical forms assumed by amoebae during first hour of immersion in various solutions. Arrows indicate direction of movement, if any. % expresses proportion of isotonic solution. Granules, diagrammatic.

Mixtures of these sugars with $MgCl_2$ illustrate the highly beneficial action of magnesium in stabilising the cell, particularly in fairly high concentrations (0·05–0·2 *M* Mg). In these, "proteus" forms are found with many finger-like pseudopodia typical of the action of excess magnesium (Fig. 1) (cf. Pantin, 1926 *b*). In a few individuals bizarre attempts at the limax form are made: these may even show slight activity for a short time especially in concentrations near 0·05 *M*. In low concentrations of magnesium (0·003–0·01 *M*) the effect is not sensibly different from that of pure sugars.

Since the mere addition of calcium to the sugar solution is not sufficient to maintain movement, experiments were performed in which both NaCl and $CaCl_2$ were added to sugar solutions in various proportions. Now in simple isotonic mixtures of NaCl and $CaCl_2$ good movement occurs for some time over a fairly wide range of relative proportions. But it was surprising to find that mixtures in any proportions between NaCl, $CaCl_2$ and sugars all inhibited movement, unless the sugar was below 0·004 *M* in concentration in the final mixture. Thus although

good movement takes place in a solution containing NaCl 0·56 *M* + $CaCl_2$ 0·025 *M* the addition of only 0·4 per cent. of an isotonic sugar solution prevents movement. Consequently it is evident that these sugars have a marked special physiological action on the amoebae. This is of great interest, though its significance is not obvious. The presence of the effect does, however, render experiments with sugars valueless for the present purpose—the determination of the effect of calcium in the absence of other ions.

This peculiar inhibition of activity by sugars even in the presence of salts is easily reversible and is accompanied by the assumption of a "proteus" form with well-formed pseudopodia arranged more or less radially (Fig. 1).

(4) *The action of glycerol.*

Pure isotonic glycerol inhibits all movement, but even after 2–3 hours this effect is reversible if the amoebae are returned to sea water.

Mixtures of isotonic glycerol with $CaCl_2$ do not allow movement to take place, though the amoebae are less "rounded" and have much better developed pseudopodia than in the sugar solutions. Indeed in low concentrations of calcium (0·05 *M*–0·003 *M*) some approach to the limax form was sometimes seen though no movement was actually observed. In low concentrations of calcium recovery is rapid and complete on return to sea water, even after 4 hours' exposure to the glycerol-calcium mixtures.

In the presence of glycerol, calcium by itself will not support movement; yet unlike the sugars, glycerol exerts no inhibitory effect in solutions which normally allow movement to occur, even when added in large quantities. When 75 per cent. of isotonic glycerol is added to a balanced solution of NaCl 0·56 *M* + $CaCl_2$ 0·025 *M*, good movement is still maintained. Fig. 2 A shows the relative velocity of amoeboid movement in mixtures of isotonic NaCl + $CaCl_2$ after 3½–4 hours. The abscissa represents the calcium concentration in the solutions, the remaining salt being NaCl. Fig. 2 B shows the effects of similar mixtures which have been diluted to four times the original volume by the addition of isotonic glycerol. This greatly diluted mixture permits movement, though over a lower absolute range of calcium concentrations. But the molecular ratio, Na/Ca, is about 20 in both cases for optimum movement.

From these experiments it would appear that in the absence of other cations, calcium in any concentration is unable to maintain movement, though the latter is maintained in balanced mixtures of NaCl + $CaCl_2$ even when greatly diluted with glycerol. This suggests that glycerol is without any specific inhibitory effect such as is found in the sugars.

These experiments indicate that movement itself is not conditioned solely by the presence of calcium in the external medium, but by the simultaneous presence of other cations. These, therefore, seem to play an active part in maintaining movement, so that within limits, the absolute concentration of calcium is less important than its ratio to other cations. This accords with the fact that the optimum calcium concentration for movement in a NaCl + $CaCl_2$ mixture depends on the degree

of dilution with isotonic glycerol: whereas the Na/Ca ratio remains approximately constant.

On this hypothesis we are supposing that the physiological effect of the glycerol is merely osmotic. But before accepting this it must be remembered that alternatively it is possible that glycerol actually inhibits activity to some extent; though successful in preventing movement only if calcium alone is present. Such an hypothesis would become very complex when modified to account for the inability of glycerol to inhibit movement in NaCl + $CaCl_2$ mixtures, and would offer no explanation for the constancy of the Na/Ca ratio over a wide range of dilution with glycerol. But the experiments to be described subsequently show that this hypothesis cannot be at once dismissed.

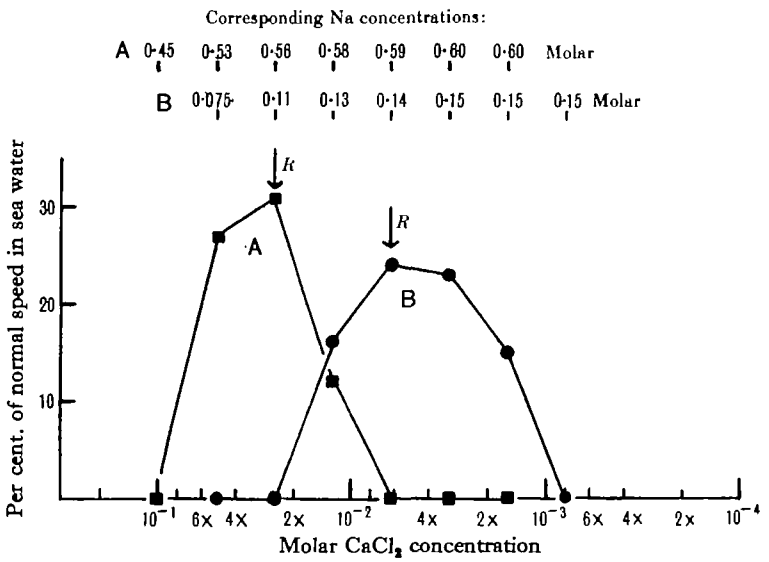

Fig. 2. Per cent. mean speed of amoebae after 3 hours. A, in mixtures of isotonic NaCl + $CaCl_2$. B, in mixtures of NaCl + $CaCl_2$ diluted 75 per cent. with isotonic glycerol, giving 0·79 *M* glycerol in each mixture. Points, *R*, correspond to molecular ratio Na/Ca = 22·5. All solutions at *p*H 6·8. The scale of $CaCl_2$ concentrations is logarithmic.

Whatever view we take with regard to movement, the mere presence of calcium or of magnesium in both glycerol and the sugars definitely enhances viability whether other cations are present or not.

(5) *The action of urea.*

Pure isotonic urea inhibits locomotion. But the condition of the amoebae is vastly superior to that in the other non-electrolytes studied. In urea a well-developed "proteus" form is found with large pseudopodia (Fig. 1) in which occasional streaming may be observed. Further, recovery is remarkably rapid; even after exposure to pure isotonic urea for 16 hours, recovery takes place in sea water within 2 hours.

If various amounts of isotonic $CaCl_2$ are added to the urea, the activity of the amoebae is greatly increased: indeed active locomotion can be measured over a wide range of Ca concentrations for several hours (Fig. 3), and is maintained for over 12 hours at the optimal concentration. Nevertheless the locomotion is considerably more irregular than the normal "limax" movement, and the majority of amoebae in such mixtures have irregular shapes with subsidiary pseudopodia, giving a "limaxoid" rather than a true "limax" type (Fig. 1). These pseudopodia are of characteristic form; they are large and curl round, often turning through an abrupt angle (Fig. 1).

At and above the somewhat low concentration of 0·05 *M* Ca, all activity is inhibited. But in concentrations below this, even down to 0·0008 *M*, movement takes place for some time. Urea not only allows movement to occur when calcium

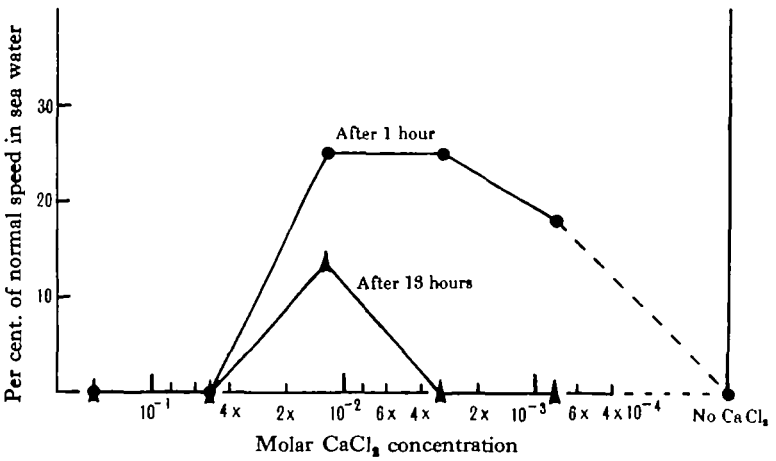

Fig. 3. Per cent. mean speed of amoebae after 1 hour and after 13 hours in mixtures of isotonic urea and isotonic $CaCl_2$. Except for the "no $CaCl_2$" point the scale of $CaCl_2$ concentrations is logarithmic. All solutions at *p*H 7.

alone is added, but the amoebae seem remarkably sensitive to the presence of calcium when urea alone is present; for inhibition occurs at a low concentration and only a trace is required for movement.

In mixtures of urea and magnesium the amoebae assume rather similar forms to those found with calcium, but the activity is irregular, very slight, and soon ceases. If the magnesium is below 0·05 *M* the form approximates more to the "limaxoid" than to the exaggerated "proteus" form typical of the action of pure isotonic magnesium (Pantin, 1926 *b*).

Though the proteus forms induced by urea and by magnesium differ, yet there is some resemblance between their actions. Both substances stabilise the cell even to some extent when in pure isotonic solutions. On the other hand, if urea and $MgCl_2$ themselves are mixed appropriately, the viability and also the activity are greater than in either of separate solutions: their relation to the conditions of viability and to the mechanism of movement is therefore not absolutely identical.

If isotonic urea is added to a mixture of NaCl + $CaCl_2$ it is found that in order to maintain the activity unchanged, calcium has to be added in proportion to the amount of urea added. Provided this is done, movement is as good as in simple mixtures of these salts. The molecular ratio, Na/Ca, for optimum movement thus decreases as the amount of added urea rises. Since a proportionate amount of calcium has to be added when an optimal NaCl + $CaCl_2$ solution is diluted with urea, the concentration of calcium remains almost constant: in effect the urea merely replaces sodium. Thus in Fig. 4 the velocity in different mixtures of NaCl + $CaCl_2$ is plotted against the calcium concentration: it will be seen that the curves are almost the same whether for the simple salt mixtures, or for those diluted with 50 per cent., or with 75 per cent. of urea.

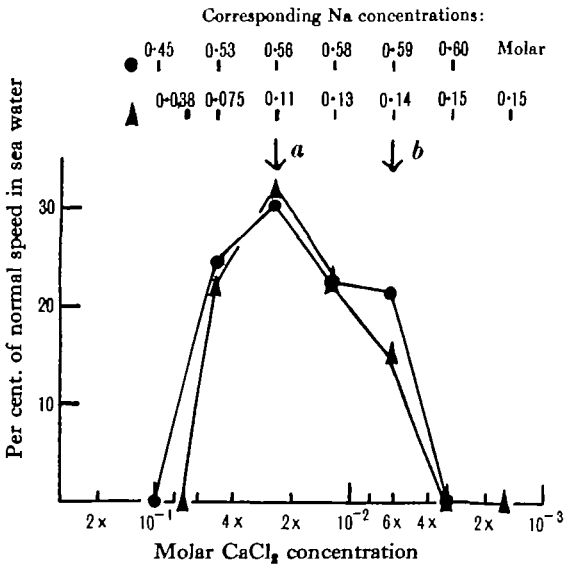

Fig. 4. Per cent. speed of amoebae after 2–3 hours. ● in mixtures of isotonic NaCl + $CaCl_2$. "*a*" marks molecular ratio Na/Ca = 22·5. ▲ in mixtures of NaCl + $CaCl_2$ diluted 75 per cent. with isotonic urea, giving 0·79 *M* urea. "*b*" marks Na/Ca = 2·25 for this series. All solutions at *p*H 6·9. The scale of $CaCl_2$ concentrations is logarithmic.

From the glycerol experiments it could be argued that amoeboid movement required the simultaneous presence of another cation as well as calcium in order to maintain movement, because the latter did not occur when calcium alone was added to the glycerol. But in the presence of urea, the addition of calcium alone permits movement to take place. On the basis of these experiments, it may therefore be argued with equal probability that of cations, calcium alone is required for movement; its absence in glycerol-calcium mixtures being supposedly due to some inhibitory effect of the glycerol. Similarly the effect of urea on activity in NaCl + $CaCl_2$ mixtures can be interpreted as illustrating the importance of the absolute calcium concentration as opposed to the Na/Ca ratio, though it is equally possible to consider the effect to be due to a synergic action of the urea with

sodium, so that added urea is merely replacing the latter in the balanced mixture. It is evident that the results need careful interpretation.

(6) *Discussion.*

The diverse effects of different non-electrolytes show at once that the mere absence of ionised groups in a molecule fails to allow us to suppose that it is necessarily physiologically inert. Any suggested relations must therefore be tentative.

It is noteworthy that the amoebae can recover from immersion for at least an hour in pure isotonic solutions of any of the non-electrolytes studied, and for very much longer periods in urea. In many cases, such as the amoebocytes of *Limulus*, isotonic solutions of non-electrolytes have a very injurious effect resembling that of hypotonic solutions, even when in the presence of considerable quantities of salts (Loeb and Genther, 1928). The swelling of cells, even to the point of cytolysis, in isotonic urea and to a less extent in glycerol, has been taken to indicate that many cells are freely permeable to these substances, and therefore swell owing to the unbalanced internal osmotic pressure. This is particularly the case with urea (Höber, 1926), and this substance has great powers of penetrating artificial membranes.

The marine amoebae are certainly permeable to water, for they swell rapidly in dilute sea water (Pantin, 1923). The long survival in isotonic non-electrolytes without obvious swelling therefore seems to indicate a high degree of impermeability to these substances.

The long survival of the amoebae in the non-electrolytes also seems to show that the cell does not suffer rapid loss of internal dissolved constituents, even when surrounded by such media quite devoid of the normal ions. The shorter survival in the sugars than in urea may indicate a relatively more rapid loss of dissolved constituents in these cases, especially since a slight shrinkage takes place—which would follow from the lowered internal osmotic pressure consequent on loss of dissolved substances.

The case of urea is truly remarkable. It penetrates artificial membranes and many cells with ease; its breakdown products, NH_3 and CO_2, appear to be able to penetrate all cells: yet the amoebae remain more normal in this than in any other non-electrolyte, or, for that matter, any pure isotonic salt solution. No irreversible changes are produced even after 12 hours, though the external urea is of molar concentration. A great impermeability to urea in high concentration is found in some organic membranes: Hill (1930) has pointed out that this obtains in the gills of fish, particularly of Elasmobranchs. To suppose a high impermeability to dissolved substances in these amoebae is in agreement with the results of earlier studies (Pantin, 1926 *b*). This stabilising action of urea and other non-electrolytes is in contrast to their apparent inability to prevent the dispersion of many protein systems, and of the intercellular matrix and the cells of certain tissues (Gray, 1926).

Survival in non-electrolytes is greatly enhanced if calcium or magnesium are added even in small amounts. Evidently the presence of a trace of a divalent cation stabilises the cell even though monovalent cations are absent.

Turning to the effects of non-electrolytes on movement, the inhibitory action of small amounts of a sugar is obscure. Edwards (1924) describes possibly related phenomena in fresh-water amoebae with sucrose. But Mast (1928) and Hopkins (1929) record no similar results with lactose. These marine amoebae are often found in forms resembling those seen in the sugars when in cultures rich in food. The limax form is strictly characteristic of clean surroundings. Perhaps the reaction to sugars is related to a reaction to food substances in the cultures.

The behaviour and movement of amoebae in glycerol and urea in the presence of salts evidently provides significant data for the interpretation of the rôle of calcium. The method of formulating the problem discussed earlier in the paper was shown to lead to conflicting hypotheses according as we make glycerol or urea the basis for comparison. Clearly therefore it is worth while reviewing the observations.

In order to clarify the succeeding argument, the main results of the experiments are represented in Table I.

Table I.

	Viability		Movement		
	Pure solution	Effect of Ca and Mg	Pure solution	Effect of added Ca	Effect of added Na + Ca
Sucrose / Glucose	About 1 hour	Ca and Mg enhance viability in all	o	o	o, down to 0·004 *M* sugar
Glycerol	About 2–3 hours		o	o	+, constant optimal Na/Ca ratio
Urea	About 16 hours		o	+	+, constant optimal Ca concentration

Since calcium added alone to a urea solution allows movement it is evident that at least the external presence of *metallic ions* other than calcium is unnecessary. It is not suggested that calcium alone supplies the necessary conditions for movement; but the remaining conditions are satisfied by the presence of any one of a variety of substances, which have so far been found to include the alkali metals, magnesium, and urea. But calcium stands by itself with respect to movement since nothing but the related element strontium can replace it: whereas the other conditions may be satisfied by very different substances.

Whatever these other conditions may be, they are not satisfied in the presence of glycerol and calcium alone.

Though one cannot say that movement requires an antagonistic balance between calcium and any specific substance, yet movement does depend on the antagonistic balance between some condition produced by calcium and one of a series of possible conditions set up by the presence of these other substances; and the mechanisms by which these antagonise the action of calcium may differ. Thus although movement will not occur in pure isotonic $CaCl_2$ or pure alkali metal chloride, yet it does take place in a balanced R^{+}/R^{++} mixture. The range of movement is not the same for each alkali metal, so that the antagonistic conditions differ (Pantin, 1926 *a*).

A similar argument holds for movement in mixtures of $MgCl_2$ and $CaCl_2$. The conditions set up by the calcium must be antagonised for movement to occur, but the conditions set up by magnesium differ from those set up by the alkali metals (Pantin, 1926 *b*).

In the same way, movement in urea + $CaCl_2$ results not from mere maintenance of certain conditions by calcium, but from a balance between these and conditions which are maintained by the presence of urea. This is confirmed by the fact that if urea is added to an optimum mixture of NaCl + $CaCl_2$, more calcium must be added to maintain optimum movement: the conditions antagonistic to the action of calcium which are set up by the sodium are reinforced by other conditions set up by the presence of urea. Similarly, in glycerol where the antagonistic conditions are not set up, dilution of an optimal mixture of NaCl + $CaCl_2$ does not affect the Na/Ca ratio required for movement.

We may now attempt to analyse these conditions. Previous work showed that isotonic mixtures of an alkali metal chloride with $CaCl_2$ permit movement for some time over rather narrow ranges of calcium concentrations. If the Ca concentration is small, movement rapidly ceases apparently because there is not sufficient divalent ion to stabilise the cell against the destructive effect which takes place in pure alkali metal chloride solutions. Increasing amounts of calcium allow longer and longer movement to be maintained, but beyond a certain concentration, calcium directly inhibits the movement mechanism. Moreover, since the concentrations at which these effects occur depend upon the alkali metal it seems that movement is conditioned by the antagonism itself between this and calcium.

Mixtures of $MgCl_2$ + $CaCl_2$ permit amoeboid movement over an enormous range of calcium concentrations. Now it is characteristic of magnesium that it stabilises the cell to a remarkable degree (cf. Gray, 1922). Amoebae are able to recover from longer immersion in isotonic magnesium salts than in those of any other cation.

When the amoeba is in sea water, the ionic conditions at the significant protoplasmic mechanisms are such as will maintain movement. Magnesium may stabilise the cell by maintaining a condition of impermeability of the cell surface so that even when the external calcium concentration is very low, the correct calcium and other ion concentrations at these mechanisms are only slowly lost. Conversely, movement continues in high calcium concentrations because the magnesium prevents the excess calcium reaching the mechanisms. Conditions set up by the magnesium in the presence of calcium thus permit movement, but maintain it by different means from those set up by alkali metals in the external medium.

It has been shown that urea stabilises the cell. In this, urea resembles magnesium, though its effect is more marked. It is not wholly similar since urea does not allow movement in very high calcium concentrations. In urea therefore as in magnesium we may suggest that movement primarily occurs owing to maintained impermeability of the cell, thereby preventing rapid loss of substances present from appropriate sites in the protoplasm when the amoeba is first transferred from sea water to the urea solution.

On this hypothesis, the failure of glycerol to allow movement even when

calcium is present follows from inability to maintain sufficient impermeability: witness the more rapid onset of irreversibility in glycerol than in urea.

The peculiar position of urea merits discussion. By what mechanism can it stabilise the cell surface in amoebae? This effect is in apparent contrast to its inability to prevent dispersion in many other cell systems. But the action of urea on proteins shows that it is certainly not an inert substance. It is probably significant that its structure is related to that of the peptide linkage and it is presumably these linkages which maintain proteins in the lyophil condition. Hopkins (1930) has recently drawn attention to the rapid denaturation of proteins by urea in concentrations which are approached by the solutions used in the above experiments. Now a denatured protein has passed into the lyophobe condition, and a lyophobe colloid is readily precipitated by divalent cations. We may perhaps suggest that the stabilising action of urea on the amoebae is due to a partial denaturing of the proteins at its surface. Divalent ions within the amoeba might thus react with these proteins to give a lyophobe, water insoluble surface layer, which well might be relatively impermeable to dissolved substances. The external presence of calcium or magnesium in the external medium would greatly assist this. It may be well to mention that there is some evidence that denaturation is not completely irreversible (Anson and Mirsky, 1929): such reversibility would be favoured in a reaction purely at the surface of a small cell freely exposed to the external medium.

A great concentration of urea not merely denatures proteins but tends to disperse the denatured product. This suggests that the dispersive action on tissues already referred to is actively brought about by the urea: indeed one wonders how far the alleged osmotic swelling of cells (Höber, 1926) may be in fact due to such an action.

A special relation of urea to the action of ions on contractility is well known in muscle (cf. Mines, 1912) and at times it appears that its action is related to the action of calcium (Hogben, 1925) in a manner which recalls the effects described here. In vertebrates there is a well-marked antagonism between the related substance guanidine and calcium (Minot, 1929; Stewart and Percival, 1928). Dreschel (1891) showed that calcium administered to mammals could be excreted as carbamate (or perhaps as cyanate, cf. Werner, 1923), which may indicate a more immediate metabolic relation of calcium to urea.

There is a further point: these experiments show that a particular unicellular marine organism is able to remain active for considerable periods in the presence of urea and low concentrations of calcium. Urea is a very common metabolic product. One is tempted to suggest that it is in this direction that one may seek for an explanation of the physiological differences underlying the relation between such marine organisms and the closely related types which inhabit those environments of low and varied salt content which constitute fresh water.

This work was largely carried out at the Marine Biological Laboratory, Plymouth, while I held the post of General Physiologist at the Laboratory. I wish to thank the Director and the staff of the Laboratory for their kind help and the facilities I enjoyed when working there.

B. A NOTE ON THE ISO-ELECTRIC POINT OF THE PROTEINS OF A MARINE AMOEBA.

For several years I have practised the cultivation of certain marine amoebae. In the course of various series of experiments the action of the hydrogen ion was always determined. Amoeboid activity was always inhibited by an increase in hydrogen ion concentration beyond certain values. The *p*H at which movement was inhibited varied considerably. In one species ("Type A," *Trichamoeba* sp.?) inhibition occurred at about *p*H 5·5. In another species ("Type B") upon which the majority of experimental work has been performed, the point of inhibition varied from *p*H 7·0 to 6·0 according to the stock from which the amoebae were raised (Pantin, 1923 and 1926). For any one stock the *p*H of inhibition was almost constant. Somewhat similar variations in the effect of the hydrogen ion on movement have been studied by Hopkins (1928) in fresh-water amoebae. He shows that considerable changes may be caused in the effect of this ion in a given species by previously cultivating it at different hydrogen ion concentrations.

Direct observation indicates that amoeboid movement is related to reversible changes of state, sol $\rightleftharpoons$ gel, in the protoplasm. Since the state of protein systems is strongly affected by the H-ion concentration in the neighbourhood of the iso-electric point, it becomes of interest to determine this for the chief constituents of the amoebae, and to compare it with the *p*H of inhibition of movement.

Amoebae from a culture in which the normal *p*H of inhibition was 6·0 were fixed in absolute alcohol, in order to disturb the chemical properties of the proteins as little as possible. They were then brought down through intermediate concentrations of alcohol to water. They were then stained with eosin or with methylene blue, or with a combination of both, in distilled water for 30 minutes. After this they were washed in buffer solutions at known *p*H's. The buffers used were mixtures of *M*/10 lactic acid + *M*/10 sodium acetate, and McIlvaine's citrate-phosphate buffers. The amoebae were examined after $\frac{1}{2}$, 1, and 18 hours. Similar experiments were also performed in which the dyes were added direct to the buffers.

In regions more acid than the iso-electric point the acid eosinate radical tends to be retained, owing to the basic nature of the protein. Conversely, on the alkaline side, the basic methylene blue radical tends to be retained. The principle is based on the work of J. Loeb on proteins and has been widely applied to plants and bacteria (Robinson, 1924).

The methylene blue was used in saturated solution. The eosin stained the protoplasm to some extent at all *p*H's and was hard to wash out if the staining had occurred in strong solutions. Good results were obtained after using trial dilutions of eosin. Typical examples of the results obtained are given in Table II.

There seems to be a fairly definite iso-electric point in the region *p*H 4·6–5·0. This is in the region of the iso-electric points of the majority of proteins.

It is thus evident that the *p*H of inhibition of movement is at a considerable and variable distance towards the alkaline side of the iso-electric point of the major proteins of which the amoeba is composed. It is therefore not possible to correlate

the influence of the external hydrogen concentration on the amoebae directly with its effect on the simple proteins, as exemplified by the Donnan effect on swelling etc. in the immediate neighbourhood of the iso-electric point. The effect of changes in the external hydrogen concentration near neutrality on the sol $\rightleftharpoons$ gel changes accompanying amoeboid movement must therefore depend either on a considerable modification of the properties of proteins in the presence of other substances in the protoplasm, or it may be related to some entirely indirect effect propagated from the surface into the cell.

Table II.

Buffer: Lactate-acetate Stain: Eosin		Buffer: Citrate-phosphate Stain: Methylene blue + eosin	
*p*H	Result after washing	*p*H	Result after washing
7·0	Faint red	5·8	Blue
6·0	Faint red	5·4	Blue
5·1	Slight red	5·0	Slight purple
4·1	Bright red	4·6	Purple
3·0	Bright red	4·2	Red
2·0	Bright red	3·8	Red
		3·4	Red

SUMMARY.

A.

1. The action of isotonic sucrose, glucose, glycerol and urea on a marine amoeba has been studied. Experiments were performed with these to elucidate the action of calcium on locomotion. No movement occurs in these pure non-electrolytes.

2. The amoebae have remarkable powers of survival in pure isotonic non-electrolytes (1·05 *M*). Survival ranges from 1 hour in the sugars to over 16 hours in the urea. Survival in the latter is far longer than in any other pure solution, including salts.

3. Even when the concentration is as low as 0·04 *M*, the sugars tested inhibit movement in solutions which normally support it.

4. Addition of calcium to glycerol does not support movement. But an optimum mixture of NaCl + $CaCl_2$ which supports movement will suffer at least 75 per cent. dilution with isotonic glycerol and yet maintain movement.

5. The addition of calcium to urea allows good movement over a wide range of low calcium concentrations: movement at the optimum is maintained for over 12 hours. The addition of urea to optimal mixtures of NaCl + $CaCl_2$ necessitates the further addition of calcium to balance the effect of the added urea.

6. The rôle of calcium in the external medium in relation to movement is reviewed.

7. The physiological action of urea in these amoebae is compared with its action on many cells. It is suggested that the long survival and continued move-

ment in the presence of urea is related to the maintenance of a condition of impermeability to dissolved substances at the cell surface. It is pointed out that this is in agreement with the denaturing action of urea in strong solutions on proteins. The possible significance of this in the adaptation of cell surfaces to a freshwater environment is mentioned.

B.

An attempt has been made to determine the iso-electric point of the proteins of a marine amoeba by staining with methylene blue and eosine after fixation in alcohol. There appears to be a fairly definite iso-electric point between *p*H 4·6 and 5·0. This is at a considerable distance on the acid side from the *p*H at which movement is inhibited in the amoebae. It is suggested that sol $\rightleftharpoons$ gel changes accompanying amoeboid movement cannot be related simply to the external H-ion concentration in a manner comparable to the relation of the latter to the properties of simple protein systems around the iso-electric point.

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