MICRURGICAL STUDIES IN CELL PHYSIOLOGY.

III. The Action of CO_2 and Some Salts of Na, Ca, and K on the Protoplasm of Amœba dubia.*

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The first paper (1) of this series described the action of the chlorides of Na, K, Ca, and Mg on the protoplasm of *Amœba dubia* as determined by micrurgical technique. A study of the effect of other salts of some of these cations forms the basis of this report.[1] Among those tested were a few which are often employed in preparing buffer solutions and some of general physiological interest, particularly solutions containing phosphate, borate, lactate, acetate, bicarbonate, carbonate, and CO_2. The details of the apparatus, its manipulation, and the terminology used have been fully described in a previous publication (1) to which the reader is referred.

Immersion Experiments.—When amebæ are immersed in toxic concentrations of any of the Na salts used in these experiments, the same effect is obtained as that found with NaCl (1), *viz.*, rounding, quiescence, and sinking of the heavier granules. In general, the toxicity of the phosphates depends upon the relative amount of Na in the salt (Table I). The only exception to this is the marked toxicity of NaH_2PO_4 in concentrated solutions due to the acidity of this salt (1). Alkalinity alone, as has been shown previously (1), is not a factor in the production of toxic effects. The phosphate ion may be a factor in contributing to the toxicity of these salts, since even in

*The ameba that has been used in the studies of this series previously identified as *Amœba proteus* is the form described by Schaeffer in his book on Amœboid movement (1920) as *Amœba dubia.*

[1] We wish to thank Mr. Kenneth Blanchard for his help in preparing and analyzing some of the chemicals used in this work.

the more dilute solutions amebæ do not appear as healthy as in control solutions containing an equivalent amount of Na.

The borate is more toxic than any other salt used in this series (Table I), a condition which is in keeping with the long established use of borates as an antiseptic.

$NaHCO_3$ and Na_2CO_3 are very toxic within the first 24 hours of immersion (Table I). Even in dilute solutions, ranging from M/1024 to M/8192, the amebæ become round and sluggish. In a few hours, however, those which have retained intact plasmalemmæ recover and remain living and well. Fresh amebæ placed in these solutions which have stood for several hours are not affected. This fact indicates

TABLE I.

Viability of Amebæ Immersed in Decreasing Concentrations of Salts.

Salts	Dead in						Living through 5 days
	1 hr.	1 day	2 days	3 days	4 days	5 days	
Monosodium phosphate	M/28	M/512	—	—	—	—	M/2048
Disodium phosphate	M/24	M/1024	—	—	—	—	M/2048
Trisodium phosphate	M/384	M/1536	—	—	—	—	M/2048
Sodium borate	M/768	M/1536	—	—	—	—	M/3072
Sodium bicarbonate	M/256	M/512	—	—	—	—	M/1024
Sodium carbonate	M/384	—	—	—	—	—	M/1024
Sodium lactate	M/4	M/32	—	—	M/384	—	M/1024
Sodium acetate	M/4	M/8	M/32	M/128	M/256	—	M/512
Calcium acetate	M/2	M/16	—	—	—	—	M/48
Calcium lactate	—	M/7.5	—	M/15	—	—	M/30

that a change takes place in solutions of Na_2CO_3 and $NaHCO_3$ on standing and the implication is that the loss of CO_2 is a factor in the change. To determine this, amebæ were immersed in water saturated with CO_2. Such a solution, tested immediately after preparation, had a pH of 4.8. The amebæ showed the same effects as in the carbonates. With the gradual increase in alkalinity due to the loss of CO_2, those amebæ recover whose plasmalemmæ have remained intact.

The lactate of Na, although not markedly toxic (Table I), can inhibit activity even in very dilute solutions.

The acetate is the least toxic of all the Na salts used (Table I).

Ca acetate and Ca lactate resemble $CaCl_2$ (1) in their non-toxicity

(Table I). It is interesting to note, however, that the lactate is the only salt of Ca which has a destructive action on the plasmalemma.

Injection Experiments.—In general the injection of the Na salts produces the same effect as that of the chloride (1). With NaH_2PO_4, however, this is neither marked nor sustained. The injection of this salt is characterized by an elevation of the plasmalemma with the appearance of a subjacent hyaline zone and the formation of a distinct membrane-like film or boundary around the granuloplasm within the hyaline zone. This boundary breaks down during recovery when flowing movements of the granuloplasm fill the hyaline zone with granules, Fig. 1. Na_2HPO_4 and Na_3PO_4 are more toxic than NaH_2PO_4 (Table II), as is to be expected because of their increased Na content. These salts do not form the granuloplasmic boundary peculiar to NaH_2PO_4.

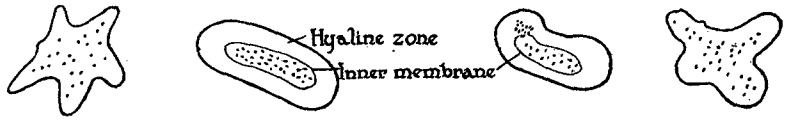

FIG. 1. The production of an inner membrane upon injection of NaH_2PO_4 into an ameba.

The borate is much more toxic than the phosphates to the interior of the ameba (Table II).

$NaHCO_3$ and Na_2CO_3 are more toxic than the other Na salts except borate because of their marked solvent action on the plasmalemma (Table II). To determine the rôle of the carbonate alone in producing this effect, bubbles of CO_2 gas were introduced into the ameba. In the cytoplasm they shrink, apparently by going into solution. If the injected bubble is larger in size than that of the nucleus, the plasmalemma fades and disappears over the entire ameba and the granuloplasm scatters. When a very small bubble of CO_2 is injected into the middle region, the plasmalemma disappears only at one end, usually the hind end of the ameba and the ameba recovers. The shrinking bubble tends to disappear before the surface breaks, Fig. 2. Control injections of bubbles of air do not affect the ameba unless the bubble is large enough to burst the ameba.

TABLE II.

Recovery of Amebæ from Injection of Decreasing Concentrations of Salts.

Salts	Recovery from			Water effect
	Small injection	Moderate injection	Large injection	
Monosodium phosphate	M/2	M/4	M/12	M/1024
Disodium phosphate	M/4	M/16	M/32	M/1280
Trisodium phosphate	M/4	M/16	M/64	M/768
Sodium borate	M/16	M/32	M/64	M/512
Sodium bicarbonate	M/16	M/32	M/64	M/256
Sodium carbonate	M/16	M/32	M/64	M/256
Sodium lactate	—	M	M/4	M/2048
Sodium acetate	M	M/3	M/8	M/128

Concentration	Pinching off effect	
	Calcium lactate	Calcium acetate
M/1	—	In 20 sec.
M/60	1 min.	—
M/120–M/130	Attempted only	In 1–2 min.
M/240–M/260	—	Delayed or attempted only
M/480	No attempt	—
M/520	—	No attempt

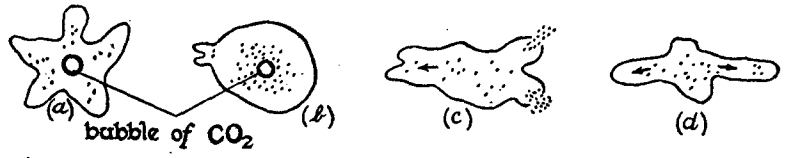

FIG. 2. The effect of injecting a small bubble of CO_2 into an ameba: (*a*) immediately after injection; (*b*) beginning rounding of ameba and sinking of granules, shrinking of bubble; (*c*) disappearance of bubble, beginning flow of ameba, breaks in plasmalemma; (*d*) recovery.

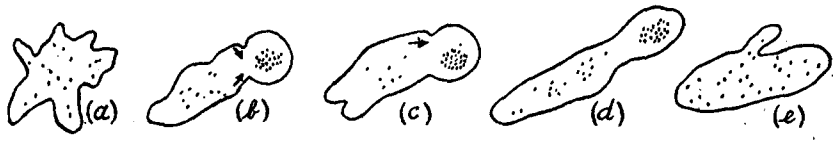

FIG. 3. The antagonistic effect of lactate on the pinching off reaction of Ca: (*a*) before injection; (*b*) beginning pinching off of solidified area; (*c*) inflow into injected area from healthy portion pushing back sluggish, constricting membrane of the stalk; (*d*) beginning incorporation; (*e*) recovery.

The lactate of Na, although relatively non-toxic (Table II), has a distinct quieting effect on the movement of the ameba and a solvent action on the plasmalemma.

The acetate is the least toxic of all the Na salts tested in this series.

Both Ca acetate and lactate have the same action as $CaCl_2$ (1), *viz.*, a solidification of the injected area which is pinched off by the living remnant. The rate of pinching off with the lactate, however, is slower than that with the acetate. This delay seems to be related to the visible sluggishness of the plasmalemma produced by the lactate. The pinching off process is frequently interrupted by an inrush of internal protoplasm. This frequently results in a failure to pinch off and, ultimately, in a consequent incorporation of the solidified material even with relatively concentrated solutions of Ca, Fig. 3.

In general, the effects of injecting the K salts were the same as those obtained with the Na salts except for the greater stability exhibited by the plasmalemma (1).

DISCUSSION.

It is evident that the predominant action of the salts is that of the cation. The anion may modify this effect without apparently changing its fundamental nature. Many of the salts tested are generally used as buffers. The usual strength of a buffer solution (M/20) is non-toxic when injected into the ameba. In immersion work, however, the buffer salts are too toxic to be used except in very dilute concentrations.

Some of the salts show individual peculiarities which are of interest. For equivalent concentrations of Na, the phosphates are more toxic in immersion experiments than the chloride. The probability that phosphates penetrate very slightly (2) suggests that their toxicity may be due to the extraction of substances, for example, Ca, from the ameba. A remarkable effect is the production of a membrane-like structure around the granuloplasm within the ameba when the acid phosphate is injected. This is perhaps due to a gelation or precipitation of some substance on the surface of the granuloplasm by virtue of an interaction with the injected phosphate. Whether the phosphate reacts with the Ca in the protoplasm it is not certain but in connection with this occurrence it is interesting to recall that an acid medium tends to accentuate the typical solidifying action of Ca (1).

The action of CO_2 and the carbonates in dissolving the plasmalemma is most significant because of the fact that CO_2 is more soluble in organic solvents than in aqueous solutions (3). This is evidence for the lipoid nature of the plasmalemma. Lillie (4) has shown in his work on the activation of starfish eggs by acids that CO_2 behaves like a fatty acid. However, the marked toxicity of CO_2 (5–7), has usually been attributed to its great penetrating power, thus implying that CO_2 exerts its toxic action on the interior. In fact, Jacobs (8) shows that CO_2 can enter and leave the cell with complete reversibility. This varies considerably with the cell used (9). The injection experiments indicate that in the ameba penetration into the interior is not the important factor in the production of lethal effects. In the ameba the ability to revert to normal depends upon the maintenance of an intact plasmalemma. The ameba is irreversibly injured only when CO_2 destroys the plasma membrane. This emphasizes again the importance of the surface in the maintenance of the life of the cell (10).

The lactates also act on the surface of the cell. Amebæ, immersed in these salts, are characterized by their sluggish plasmalemmæ. This is of interest in connection with Lillie's finding that lactic acid is relatively ineffective in activating the starfish egg, a condition which he attributes to difficulty of penetration. The dispersive action of the lactate on the plasmalemma is most evident when this salt is brought by injection into contact with the inside of the plasmalemma. This dispersing effect is further seen when amebæ are immersed in Ca lactate. This is the only Ca salt in which dead amebæ show a disrupted plasmalemma.

The relative non-toxicity of sodium acetate is rather surprising when one considers the frequent reports of its marked activity (4, 11). Loeb (12), however, pointed out that sodium acetate acts exactly like the chloride in its depressing effect on the viscosity of gelatin if the pH is kept constant. Furthermore, it is difficult to compare results from different materials or even different functions of the same material. Cohen and Clark, for example, point out that the effect of pH upon specific fermentative processes, upon reproduction in its several stages, and upon death, must be kept distinct. This may well be kept in mind in dealing with any factor.

CONCLUSIONS.

I. *Plasmalemma.*

1. Of the salts used in these experiments the anions have only a modifying effect on the cations. The dispersive action of Na and, to a lesser extent, of K, predominates. Borate increases the toxicity of Na and acetate decreases it.

2. CO_2 and carbonates dissolve the plasmalemma readily.

3. Na lactate tends to dissolve the surface especially when brought into contact with it from the interior by injection.

Lactate antagonizes the stimulating effect of Ca on the plasmalemma.

II. *The Internal Protoplasm.*

4. Acid phosphate of Na and K, when injected, causes a membrane to form around the granular endoplasm within the ameba.

5. Na borate increases the toxicity of Na inside the cell.

6. Bubbles of CO_2, injected into the cell, cause an increase of fluidity of the internal protoplasm. These bubbles shrink and disappear from the cell more readily than air bubbles.

7. The anions modify the typical cation effect. Carbonates accentuate the liquefying and solvent action of Na.

Phosphates prevent a complete rounding of the ameba caused by Na.

Lactate inhibits the solidification and pinching off effect caused by Ca.

III. *Physiological Significance of Salts.*

8. The buffer salts can be injected in high concentrations without toxic effects but amebæ can be immersed in them only in very dilute solutions without injury.

9. The inhibiting action of lactate and the dispersive effect of CO_2, carbonates, and lactate on the plasma membrane, must be of importance in a consideration of the functions of the organism and perhaps in the production of pathological changes.

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