THE ACTION OF POTASSIUM, SODIUM AND CALCIUM SALTS ON TUBIFEX RIVULORUM.

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PREVIOUS experiments, for the most part recorded in the volumes of this Journal, have established the important part played by lime in the functions of living tissues. As an instance, we may mention the presence of lime as a factor essential to the contraction of cardiac muscle. The experiments demonstrating this shewed also the antagonism which existed between the influence of lime and of potassium salts. This was tested both physiologically[1] and toxically[2]. In a long series of experiments lime salts were shewn to be necessary to the act of clotting of blood and to the curdling (clotting) of milk, and again an active antagonism was found to obtain between the salts of lime and of potassium. True the clotting of blood can scarcely be classed as a vital process, still less the clotting of milk, yet it is difficult to dissociate vital from non-vital processes and a characteristic potency or function of a tissue may probably depend as much on a katabolic (disintegrating) as upon an anabolic (integrating) movement, we may therefore allow these experiments to stand here.

The influence of lime in purely non-vital processes, a physico-chemical action therefore, has also been tested in one direction, its power, viz., to affect the swelling of dried Laminaria when placed in watery solutions. Lime was found to control markedly the powers of imbibition, the Laminaria swelling out to a much greater degree when placed in distilled water than when a small percentage of a lime salt had been added. These experiments may have some bearing upon those about to be described.

[1] This *Journal*, Vol. IV. p. 29, 1883.
[2] This *Journal*, Vol. IV. p. 247, 1883.

The present set of experiments were undertaken in order to test the influence of salts of lime and potash and incidentally of soda also upon *whole* living organisms. The presence or absence of motor power was the chief test applied, but the organisms were also watched as to the presence of disintegration and its degree. The Tubifex Rivulorum (Lamarck) was chosen as suitable for the purpose. It is an actively moving minute worm tending, with its fellows, to form dense clusters or balls of filaments, each thread being in continuous wriggling movement. The structure, so far as it concerns us may be very briefly given[1]:—The integument, ringed and beset at intervals with minute bristles or hairs, consists of a cuticle, homogeneous in structure, and intimately united with an underlying corium. Internally this is bounded by a subcutaneous layer of muscular fibres in two layers, circular and longitudinal, of which the latter is the more developed. The contractions of the subdermal fibres cause the movements of the body. Traversing the body from end to end is the digestive tube and in close connection is the vascular system consisting of two longitudinal main trunks, dorsal and ventral, united by lateral branchings, a pair for each ring. Two so-called hearts are found near the head end on either side of the dorsal vessel and uniting it with the ventral vessel. In life the contractions of these and of the dorsal vessel are plainly visible under a low power through the transparent body wall. A perivisceral space intervenes between the body wall and the above tubes and transverse partitions one for each ring divide this space into compartments. Other structures, reproductive, secretory, we may omit. The digestive tube shews a very delicate mucous lining the surface of which is covered by a ciliated epithelium. There is also a delicate muscular coat surrounding the mucosa to which the peristaltic movements of the tube are due.

In tap water, renewed from time to time, the Tubifex will live for an indefinite period, exhibiting all the signs of an active vitality.

The following is the method of experimentation adopted: a series of beakers, each containing the same volume of fluid, received each a number of the worms, these were then watched and a record kept of their condition, in particular as to movement and disintegration. By varying the proportions of the salines added to the beakers it was possible to obtain an indefinite variety of separate series. In this way the effects of the salts, potassium and sodium chloride, calcium nitrate and phos-

[1] *Histoire naturelle du Tubifex des ruisseaux*, par Jules d'Udekem. Bruxelles, 1853; *Structure of Tubifex.* McIntosh, 1871.

phate, sodium oxalate were tested. Distilled water and tap water were the fluids used, and to which these salts were added—the tap water is that supplied by the New River Company.

We may now proceed to the actual experiments and first as to the effects of these two waters. In tap water, as has been already mentioned, the Tubifex will live for an indefinite period in full activity, this will be seen again and again in the experiments to be recorded and to which the tap water beaker served as the control. It will be noted however that the creatures, even in this medium, may not escape some degree of disintegration. This when present affects only a small percentage of the organisms and does not appear till late in the experiment, i.e. after some days, and it probably selects those only which have suffered mechanically in the act of transference, or in the act of separating the clustered colony. The separation was effected by allowing the tap to play upon them with moderate force, the transference to the test-medium by means of a pipette.

Distilled water acts very differently. In this medium the organisms continue for a time their active movements but gradually disintegration appears and ultimately they are all converted into a granular débris. The movements may continue for some hours becoming gradually more sluggish and the disintegration slowly advancing—within 24 hours the latter process is generally complete. The action of distilled water will be illustrated again and again in the experiments which follow.

If now we modify distilled water by the addition of salines we modify greatly the action of the medium. We will first consider potassium chloride.

On Sep. 29th, 1893 the following experiment was made:—five beakers were taken and received the following quantities of fluid:

I.	200 c.c. of distilled water					
II.	”	”	+ 0·5 c.c. of a 10 % sol. of potassium chloride			
III.	”	”	+ 1 c.c.	”	”	”
IV.	”	”	+ 1·5 c.c.	”	”	”
V.	”	”	+ 2 c.c.	”	”	”

Into each by means of a pipette several of the Tubifex organisms were introduced.

The experiment began at 2.15 P.M.

In 19 hours though disintegration was well marked in I., some of the organisms were active and even after 43 hrs. there were still some which were active though most were disintegrated. The action of distilled water was very exceptionally slow here.

In the beakers III., IV., V., the organisms were motionless within 19 hrs. (one Tubifex only was moving in beaker IV.).

In beaker II. after 43 hrs. all were motionless except one. A few only were disintegrated in this beaker.

When rendered motionless recovery experiments were made but these we omit for the present.

On October 3rd the following series was arranged:

I.	200 c.c. of distilled water		+ 2 c.c. of a 10 $^{0}/_{0}$ solution of KCl			
II.	,,	,,	+ 2·5 c.c.	,,	,,	,,
III.	,,	,,	+ 3 c.c.	,,	,,	,,
IV.	,,	,,	+ 3·5 c.c.	,,	,,	,,
V.	,,	,,	+ 4 c.c.	,,	,,	,,

The experiment was started at 9 A.M.

At 2 P.M., i.e. after 5 hours, a few organisms were moving slightly in beakers I., II., III. There was no movement in IV. and V.

At 5 P.M., i.e. after 8 hours, there was no movement in I., II., III.

All the motionless organisms were subsequently recovered.

On September 22nd a series was arranged to test the action of KCl and NaCl, separately, in modifying the action of distilled water.

I.	200 c.c. distilled water		
II.	,,	,,	+ 1 c.c. KCl solution, 10 $^{0}/_{0}$
III.	,,	,,	+ 2 c.c. ,, ,,
IV.	,,	,,	+ 1 c.c. NaCl ,, 10 $^{0}/_{0}$
V.	,,	,,	+ 2 c.c. ,, ,,
VI.	,,	,,	+ 1 c.c. KCl sol., 10 $^{0}/_{0}$ + 1 c.c. NaCl sol. 10 $^{0}/_{0}$.

I. After 24 hours most of the organisms were disintegrated, but there was still one moving after 48 hrs.

II. In 4 hrs. movements only just perceptible, in 8 hrs. motionless. No disintegration after 48 hrs.

III. In 4 hrs. slight movement only; in 8 hrs. one moving slightly; next morning, i.e. after 24 hrs., all motionless. No disintegration after 48 hrs.

IV. After 8 hrs. still active; after 24 hrs. motionless, some disintegration; after 48 hrs. all disintegrated or disintegrating.

V. After 8 hrs. active; after 24 hrs. very sluggish, but not disintegrated, after 28 hrs. same state; after 48 hrs. motionless but not disintegrated.

VI. After 4 hrs. sluggish; after 8 hrs. motionless; after 24 hrs. very slight movement was detected—no disintegration; after 48 hrs. no disintegration.

These experiments shew first the disintegrating power of distilled water and next the paralyzing power of potassium chloride with suspension or retardation of disintegration. Sodium chloride in the smaller dose of ·05 p.c. retards but does not suspend disintegration, it contrasts with potassium chloride in its relatively slight action upon movement—in the larger dose (·1 p.c.) disintegration is more effectually checked, but the effect on movement is even less marked, still the solution does slowly paralyze. In the effect of potassium and sodium chloride combined—the potassium action predominates in respect of paralysis, the fluid behaving quite like the solution containing 1 c.c. of KCl alone: disintegration, as we should expect is held in check.

On September 24th a longer series of KCl and NaCl solutions in distilled water gave results confirmatory of those just recorded. These results were as follows:—

Disintegration, complete, by the distilled water within 24 hrs.

In the KCl beakers nearly complete paralysis in 4½ hours, only one or two were moving sluggishly in each beaker; within 24 hrs. all were motionless. The beaker containing 1 c.c. each of KCl and of NaCl combined acted just like the 1 and 2 c.c. of KCl alone. In the NaCl beakers 1, 2 and 3 c.c. of NaCl 10 $^{0}/_{0}$ were contained. The influence upon movement was very slight, thus after 10 days there was some movement in two of the flasks containing respectively 1 c.c. and 2 c.c. In two other flasks containing these same quantities of NaCl movement was abolished in six days, for this discrepancy individual differences in vitality of the organisms might easily account, but either result, 6 or 10 days, contrasts sufficiently with the paralyzing action of potash made manifest in a few hours. The flask containing 3 c.c. NaCl gave practically the same results as those containing 1 and 2 c.c., so far as movement was concerned. As to disintegration this was held in check by the NaCl, thus after 6 days all the organisms in the NaCl flasks retained their shape. Under the microscope however the organisms in one flask, containing 2 c.c. NaCl, and which had lost their colour, shewed an absence of structure; in them some internal disintegration had evidently occurred. In the flask containing 3 c.c. NaCl preservation at the same stage was more complete, the organisms retained their red colour and though motionless to the naked eye the microscope shewed slow movements of the whole body and also contractions of the vascular canal.

We pass now to the influence of lime salts. We have seen that tap water will support life for an indefinite period. This fitness must be held to depend upon the presence of lime as one essential factor. This appeared in many experiments and first in the power of distilled water + nitrate of lime, and distilled water + phosphate of lime to support vitality. These two solutions seemed to replace tap water fairly satisfactorily. One or two cubic centimetres of a 10 % solution of nitrate of lime added to 200 c.c. of distilled water will be ample for the purpose, or a saturated solution of phosphate of lime in distilled water. The latter salt is so very insoluble that this saturated solution really contains less lime than is present in tap water, indeed the usual statement is that calcium phosphate is insoluble. We speak of the tricalcium salt. From the fact that tricalcic phosphate can act in such minute quantity, we must conclude that the amount of calcium nitrate employed by us was very excessive, in fact may be regarded as a luxus supply. This point we have not sought to determine quantitatively. Some of the experiments with a lime salt in distilled water seemed to suggest that the Tubifex suffered more disintegration than was observed with tap water, but when this process is slow, as it was in the lime experiments, it is difficult to make sure that the process is not rather due to some damage done to the organisms or to a weak vitality of the same. Even in tap water, in the course of an experiment extending over several days, disintegration was observed, it was generally slight but sometimes more marked.

The first proposition then, respecting lime salts, is that they will convert distilled water into a medium capable of supporting the vitality of the Tubifex for considerable periods, e.g. several days.

Next, it was found that lime, in the same proportions, was able to inhibit or antagonize the paralyzing action of potassium chloride. This was made quite definite by a number of experiments in which calcium nitrate or calcium phosphate was introduced along with potassium chloride. Tap water acted in the same way as a solution of a lime salt in distilled water and the inference was that it is the lime present in tap water which acts thus.

Thirdly lime was able to remove the effects of potash already produced, e.g. having paralyzed the organisms by potassium chloride it was possible to recover them quickly by the addition of a calcium salt.

Organisms which have been paralyzed by potassium chloride may be made to recover quickly by transferring them to a solution of a lime salt in distilled water or to tap water simply, but the recovery in these cases might be due solely to a diffusion out from the tissues of the paralyzing salt, KCl, and not to a direct antagonizing lime action, and it is probable that both these factors do come into play. To prove however that lime can antagonize without the aid of any diffusion the following experiment was made.

Oct. 1st. Four beakers were charged.

I.	200 c.c. distilled water	+ 1 c.c.	of a	10 % solution	of KCl
II.	,,	,,	,,	,,	,,
III.	,,	+ 2 c.c.	,,	,,	,,
IV.	,,	,,	,,	,,	,,

Thus II. and IV. were exact replicas of I. and III.

The organisms now introduced were found motionless in each case at the end of 7 hrs.

I. now received 1 c.c. of a 10 % solution of $Ca(NO_3)_2$ = I^a^.

II. the organisms were transferred to a flask containing tap water to which KCl had been added so as to make it of the same KCl strength as the original solution = II^a^.

III. received 2 c.c. of a 10 % solution of $Ca(NO_3)_2$ = III^a^.

IV. remained unchanged.

After 17 hours from the transferences the organisms in I^a^, II^a^, and III^a^, were all found active. In flask IV. the organisms were quite motionless.

Now to flask IV. 2 c.c. of $Ca(NO_3)_2$ solution 10 % were added, and within seven and a half hours from this there was some recovery and within 23 hours of the transference many organisms were active. This recovery was from a motionless state of 18 hours' duration.

Repeating these experiments on Oct. 2nd, completely confirmatory results were obtained, and it is thus clear that the lime directly and effectually antagonizes the potassium salt.

In an experiment made on Oct. 3rd, larger quantities of KCl were used, viz. 2 c.c., 2·5 c.c., 3 c.c., 3·5 c.c. and 4 c.c. of a 10 % solution. The organisms having been rendered motionless corresponding quantities of $Ca(NO_3)_2$ solution, 10 % were added and there was noted in each case recovery within a few minutes. The massive dose of the potassium salt was rapidly overcome by the massive dose of the lime salt. Recovery also took place in tap water which contained the same proportions of KCl as the above, but the recovery was much slower; the addition to the tap water of a massive dose of lime salt accelerated the recovery.

The lime effect was tested in yet another way by the addition viz. of sodium oxalate to distilled water. Of the oxalate varying quantities were used, viz. 0·5 c.c., 1 c.c., and 1·5 c.c. of 10 % solution—and three other flasks received the corresponding quantities of sodium oxalate + in each case 2 c.c. of 10 % solution of NaCl.—Each flask contained 200 c.c. of distilled water.

The organisms in the flasks containing 1·5 c.c. of sodium oxalate solution became almost motionless in 8½ hours, but after 24 hours there was still some slight movement present, however on transferring the organisms to tap water the movements greatly increased in a few minutes and thence onward they remained active. The 0·5 c.c. sodium oxalate flasks did not seem to influence the organisms, the 1 c.c. flasks caused sluggishness of movements in one case but not in the other.

The influence of sodium oxalate here is probably by withdrawing lime from the tissues or by rendering it useless within the tissues by precipitation.

These experiments were not made exhaustive.

Sodium oxalate seemed to completely prevent disintegration, during a period of three days (78 hrs. strictly).

To sum up, these experiments shew clearly :

1st. That a lime salt is essential to the vitality of the organism, Tubifex Rivulorum.

2nd. That whilst a very minute quantity of a lime salt seems to be adequate to the maintenance of this vitality, the quantity may be increased enormously without producing any very definite result—in other words, it is very indifferent to the tissues. Contrast here the quantity of lime present in a solution of tricalcic phosphate with that contained in 2 or 3 c.c. of a 10 % solution of calcium nitrate.

The minute dose of lime will represent the physiological one; the massive dose, the therapeutic or toxic one, only we did not reach the toxic limit.

3rd. That lime both in the minimal and in the massive dose antagonizes the paralyzing influence of a potassium salt, and that whilst a minimal dose of lime seems to have extraordinary powers of inhibiting or antagonizing the action of large quantities of potash, a massive dose of the potassium salt seems to be easiest overcome by a massive dose of the calcium salt.

4th. That sodium contrasts with potassium in the relative feebleness of its action—maintaining its character as an indifferent element.

We may add that all the salts potassic, sodic, calcic used in these experiments hold in check the disintegrating influence of distilled water. This action is probably physico-chemical. The experiments on this point shew :—

5th. The remarkable power which a minute dose of a lime salt, e.g. of lime phosphate, possesses in maintaining the integrity of the tissues. We may here again refer to the experiments already quoted in which it was found that the powers of imbibition of Laminaria were notably controlled by a lime salt. The quantities of sodium and potassium salts which also controlled the disintegrating process were maximal as compared with this quantity of the lime salts.

In what light we are to regard the physiologic and toxic antagonism existing between lime and potash it is hard to determine. That lime does not act by preventing the ingress of the potassium poison by imbibition or otherwise is clear from those experiments in which the potassic effect was first produced and then overcome *in situ* as it were, by the calcium salt. If we say that the molecular vibrations of the calcium salts do not harmonize with those of the potassium salts, and by mechanical interference annul each other's action in the same way that waves of light of different rates of vibration interfere with each other and may produce a negation of effect—viz. darkness—if we say this, we say nothing that really advances the understanding of the subject.