THE INFLUENCE OF SALINE MEDIA ON THE TADPOLE. BY SYDNEY RINGER, M.D., F.R.S., AND ARTHUR G. PHEAR, M.B. *Cantab.*

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FOR some years we have been studying the action of various salts on living tissues, and we have found them to possess at least two distinct properties. Some salts are efficient in maintaining structural integrity of tissue; others are able to support functional activity. The two properties are not necessarily combined in a single salt: a salt which is competent to support function may be very inefficient or quite incapable of maintaining integrity.

We have shown[1] that the gill edges of water mussel if placed in distilled water quickly disintegrate, the cells becoming swollen and granular, and separating from one another. Many of the cells burst, and ciliary action ceases. The addition of a small quantity of calcium chloride or of sodium bicarbonate is sufficient to sustain both integrity and function for many days.

We have shown too that distilled water causes separation of the cuticle from fishes and newts[2].

In the present communication we shall put forward the results of further observations on the action of various salts on the intact organism. The organism employed has been the tadpole, those only being selected in which development had progressed beyond the gill stage.

The following is the method of experiment adopted. In each one of a series of beakers 200 c.c. of distilled water were placed. To the first of these nothing further was added. To the other beakers were added increasing quantities of a solution of the salt under examination, so that

[1] Ringer and Buxton. "Concerning the Action of small quantities of Calcium, Sodium, and Potassium salts upon the Vitality and Function of Contractile Tissues and the Cuticular Cells of Fishes." This *Journal*, VI. 154. 1885.

[2] Ringer. "The Influence of Saline Media on Fish, etc." This *Journal*, IV. VI. and V. 98.

a series was obtained of solutions of ascending strength. Into each of these vessels three or more tadpoles were introduced, and the action of the solutions on the tadpoles was observed and duly noted.

Tadpoles placed in distilled water rapidly become motionless, after a period varying as a rule between two and six hours. Paralysis is quickly followed by disintegration; indeed signs of desquamation may be observed before movement has entirely ceased; and we believe it to be by means of such disintegration that distilled water causes death. Certain salts when added to distilled water in proper proportion sustain motion in tadpoles, in some cases for many days. This fact of itself is no matter for surprise; since were it not for the sustaining properties of some salts tadpoles could not exist in the natural waters of rivers and ponds. Some of the salts which we have found to sustain are however powerful poisons, such as hydrocyanic acid, and cyanide of potassium, and the neutral oxalate of potassium. As illustrating this property of supporting life, experiments with potassium ferrocyanide and potassium oxalate will be described.

EXP. 1. *Potassium ferrocyanide.* In each beaker 200 c.c. of distilled water, and to all but beaker (1) potassium ferrocyanide added so as to make percentage of it in

(2) ·000125 p.c. (5) ·00075 p.c.
(3) ·00025 „ (6) ·001 „
(4) ·0005 „

April 23. 10 a.m. Tadpoles introduced.
4 p.m. (1) All tadpoles motionless.
(2) One moves languidly. Two motionless.
(3)—(6) Quite active.
7 p.m. (2) All motionless.
(3)—(6) Quite active.
April 28. (6th day.) 10 a.m. All tadpoles in beakers (3)—(6) quite active, with exception of one dead in beaker (6).
Experiment discontinued.

EXP. 2. *Potassium ferrocyanide.* 200 c.c. distilled water in each beaker; so much of the salt added as to make percentage in

(1) ·00125 p.c. (4) ·0075 p.c.
(2) ·0025 „ (5) ·01 „
(3) ·005 „

April 22. 11 a.m. Tadpoles introduced.
25. 10 a.m. Beakers (1)—(4). All active.
Beaker (5). One tadpole dead. Three active.

April 27. 10 a.m. (1)—(4) All active.
(5) All dead.
28. 10 a.m. (1)—(4) All active.

Experiment discontinued. Tadpoles in the first four beakers being as active after lapse of six days as at beginning of Exp.

EXP. 3. *Potassium oxalate* (neutral). In each beaker 200 c.c. distilled water; potassium oxalate added so as to make percentage of it in

(1) ·05 p.c. (3) ·1 p.c.
(2) ·075 ,, (4) ·15 ,,

April 8. 10 a.m. Tadpoles introduced.
9. 10 a.m. (1)—(3) All active.
(4) Two motionless. One quite active.
12. 10 a.m. (1)—(3) Active.
(4) One still moving, but sluggish.

Experiment discontinued, tadpoles being alive and active after lapse of four days.

Many other salts have been found to possess similar properties, particularly salts combined with weak acids, such as the carbonates, citrates, tartrates, and oxalates. The results of numerous experiments with these may be tabulated as follows:—

TABLE A. *Salts which sustain for an indefinite period.*

Salt	Strength found to sustain	Duration of experiment	Duration of life in control beaker containing distilled water only
Potassium Bicarbonate	1 in 1000 to 1 in 500	8 days	Under 7 hours
,, Citrate ($KH_2C_6H_5O_7$)	1 in 20,000 to 1 in 1000	3 ,,	
,, ,, ($K_3C_6H_5O_7$)	1 in 8000 to 1 in 700	3 ,,	
,, Tartrate ($KHC_4H_4O_6$)	1 in 10,000 to 1 in 1000†	6 ,,	
,, ,, ($K_2C_4H_4O_6$)	1 in 1000†	4 ,,	
,, Oxalate ($K_2C_2O_4$)	1 in 4000* to 1 in 800†	5 ,,	
,, Phosphate (K_2HPO_4)	1 in 2000 to 1 in 150†	5 ,,	2½ hours
,, ,, (K_3PO_4)	1 in 4000	5 ,,	2½ ,,
,, Cyanide	1 in 80,000	9 ,,	Under 3 hours
,, Ferrocyanide	1 in 400,000 to 1 in 15,000	6 ,,	
Sodium Chloride	1 in 300 to 1 in 200	7 ,,	
,, Sulphate (Na_2SO_4)	1 in 800*	5 ,,	
,, Bicarbonate	1 in 1000	8 ,,	Under 7 hours
,, Oxalate	1 in 4000*†	5 ,,	
,, Phosphate (NaH_2PO_4)	1 in 800†	5 ,,	3½ hours
,, ,, (Na_2HPO_4)	1 in 3000 to 1 in 800†	5 ,,	3½ ,,
Calcium Bicarbonate‡		6 ,,	
,, Phosphate §		6 ,,	
Iron Citrate	1 in 8000* to 1 in 1500	2 ,,	
Caffein Citrate	1 in 8000* to 1 in 2000	3 ,,	

In all these cases the tadpoles were as active, and with vitality apparently as unimpaired, at the close as at the commencement of the experiments.

* Solutions more dilute than these were not tested.
† Stronger solutions were not tested.
‡ Prepared by passing CO_2 through lime water.
§ Prepared by allowing distilled water to stand for 24 hours on tricalcic phosphate [$Ca_3(PO_4)_2$]. The solution thus obtained contains less lime than is present in tap water.

The limits of dose here given represent those ascertained by repeated experiment with each separate salt. In a number of experiments, although the main results are similar, yet there is some slight variation in the limits of the dose which sustains, doubtless owing to the varying susceptibilities of individual tadpoles. It will be noticed how varied is the quantity required of these several salts, and what a very minute dose of some is sufficient to support life. Potassium ferrocyanide is the most striking instance of this, a solution containing ·00025 p.c. being sufficient to maintain life and movement for a period exceeding six days. Since such minute quantities suffice, it might be supposed that the salts requisite for sustenance are derived from the fæces, which often are expelled in considerable quantity. Two considerations disprove this; first, the tadpoles do not survive in distilled water, although here as much fæcal matter is ejected as in the solutions which sustain; secondly, the tadpoles survive when transferred to a fresh solution, as soon as contamination with fæcal matter has occurred.

Other salts though not sustaining for these long periods, yet present a distinct superiority over distilled water in their powers of preserving life. These may be tabulated as follows:—

TABLE B. *Salts which sustain, but not indefinitely.*

Salt	Strength of solutions which support life	Duration of life in these solutions	Duration of life in distilled water
Sodium Sulphate (NaH_2SO_4)	1 in 7000	1 day	
,, Phosphate (Na_3PO_4)	1 in 4000 to 1 in 2000	3 days	3½ hours
,, Sulphite	1 in 700 to 1 in 400	3 ,,	
,, Arseniate	1 in 4000 to 1 in 900	1 day	Under 7½ hours
Ammonium Oxalate	1 in 4000 to 1 in 1000	4 days	

In some of these experiments it has happened that of four tadpoles some have become motionless within a short time, the remaining ones surviving for some days, even though transported into a fresh solution away from their dead companions.

In the case of a few salts we have been unable to discover any sustaining property, in spite of repeated trials and the employment of doses varying widely in range. Indeed, these salts seem to cause paralysis of movement and death sooner than distilled water. It will be noticed that many of these are potassium salts, and in association with the stronger acids.

TABLE C. *Salts which kill as soon as or sooner than distilled water.*

Salt	Solution in which tadpoles survive longest	Duration of life in these solutions	Duration of life in distilled water
Potassium Chloride	1 in 20,000	4 hours	4 hours
„ Sulphate ($KHSO_4$)	1 in 1500	3¾ „	
„ „ (K_2SO_4)	1 in 40,000	3 „	
„ Nitrate	1 in 4000 to 1 in 1500	20 mins.	
„ Chlorate	1 in 20,000 to 1 in 400	1¼ hours	Over 1¼ hours
„ Acetate	1 in 4000 to 1 in 700	1½ „	
„ Oxalate (KHC_2O_4)	1 in 4000	5 „	
„ Phosphate (KH_2PO_4)	1 in 800	2 „	2½ hours
Sodium Acetate	1 in 400	5¼ hours	
Calcium Chloride	1 in 3500 to 1 in 1500	6 „	
„ Sulphate	Sat. sol.	3 „	

It was with some surprise that we found that acids, inorganic as well as organic, support life even for days when employed in appropriate doses. With stronger doses of the inorganic acids paralysis supervenes very quickly, desquamation commencing even before death, and progressing at a very rapid rate. The caustic alkalies also were found efficient to sustain in doses which are large as compared with the extremely minute quantities of acid which suffice. The results of our experiments with acids and alkalies are summarised in the following table:—

TABLE D. *Acids and Alkalies.*

Acids	Strength of solutions found to sustain	Duration of life in these solutions	Duration of life in distilled water
Hydrochloric Acid	1 in 70,000 to 1 in 50,000	4 days	2 hours
Sulphuric Acid	1 in 200,000 to 1 in 80,000	2 „	5 „
Nitric Acid	1 in 100,000 to 1 in 50,000	9 hours	2½ „
Acetic Acid	1 in 20,000	1 day	
Citric Acid	1 in 80,000 to 1 in 15,000	2 days	
Tartaric Acid	1 in 20,000	2 „	
Oxalic Acid *	1 in 20,000	3½ hours	
Sulphurous Acid	1 in 1000	2 days	Under 4 hours
Hydrocyanic Acid	1 in 500,000	3 „	
„	1 in 1,000,000	1 day	
„	1 in 4,000,000	9 hours	Under 6 hours
Alkalies			
Potassium Hydrate	1 in 4500 to 1 in 2000	1 to 2 days	4 hours
Sodium Hydrate	1 in 5000 to 1 in 3000	1 to 2 „	4 „

* No supporting action discovered here; but minute doses were not tested.

The foregoing experiments and tables show clearly that certain substances when added to distilled water change this from a medium in which tadpoles live only for a few hours to a medium capable of maintaining life for a much longer time. Whatever be the precise value of the doses indicated,—and we believe that the dose must vary within wide limits according to the stage of development and individual well-being of the tadpoles employed,—the main results obtained in respect of maintenance or non-maintenance of life and motion remain unaffected.

With regard to the nature of this sustaining power, we are at present prepared to do no more than put forward an explanation, which so far as it goes we believe to be correct, and which is borne out by further experiments to be quoted. Distilled water causes death by disintegration, primarily of the cuticle. By the addition of certain salts in the right proportion, we believe that the integrity of the tissues is maintained, and thus the life of the whole organism is preserved. The prevention of disintegration of tissue by the presence of minute quantities of some salts has been well shown in experiments referred to above on the ciliary action of gills of fresh-water mussels. Portions of gill placed in distilled water rapidly changed; ciliary action ceased; the cells became granular and swollen, and separated from one another. In the presence of certain salts, however, ciliary action and structural integrity remained unimpaired for days.

Salts maintain life which to the heart of the frog are poisons,—for instance, the whole group of potassium salts, notably potassium oxalate and potassium cyanide. It cannot be supposed that a salt which paralyses the heart of the frog is inert when passing through the heart of the tadpole; we do not believe that these salts are absorbed when employed in such doses as we have found to sustain; we suppose them to be functional merely in maintaining the integrity of the skin and intestinal mucous membrane, thus protecting against themselves; since, with skin and mucous membrane intact both as regards structure and function, the physical laws of diffusion are held in abeyance, the cells exercising a selective power over what they absorb, or what they reject. Increase the dose, and the toxic action of the salt asserts itself; the selective function of the cells is paralysed, though in structure they remain intact; their controlling influence over the physical laws of diffusion is removed and the salt is absorbed, causing the death of the organism, although the structural integrity remains to all appearances unimpaired. This theory may be illustrated by the following experiment:—

EXP. 4.* *Potassium citrate* ($KH_2C_6H_5O_7$). In each beaker 200 c.c. distilled water; potassium citrate added so as to make percentage in

(1)	·00025 p.c.	(9)	·01 p.c.
(2)	·0005 „	(10)	·0125 „
(3)	·00075 „	(11)	·025 „
(4)	·001 „	(12)	·05 „
(5)	·00125 „	(13)	·1 „
(6)	·0025 „	(14)	·15 „
(7)	·005 „	(15)	·2 „
(8)	·0075 „	(16)	·25 „

April 25.	10 a.m.	Tadpoles introduced.
	2 p.m.	(1) Two motionless. Two sluggish.
		(2)—(16) All active (except a small one dead in 3).
26.	10 a.m.	(1)—(3) All motionless.
		(4)—(14) All active.
		(15) and (16) All motionless.
27.	10 a.m.	(4) Motionless.
		(5) Two motionless. One active.
		(6)—(12) Active (except one motionless in 10).
		(13) Three active. One motionless.
		(14) All motionless.
28.	10 a.m.	(5)—(7) All motionless.
		(8)—(12) All active (except one in 10).
		(13) Three motionless. One active.

* This experiment was performed in two parts on separate occasions; these have been arranged in a single series for the sake of clearness.

We believe that here the lowest doses of potassium citrate are not sufficient to obviate the distilled water action; the medium doses, at the same time as they suffice to sustain integrity, are not large enough to destroy the function of the cells, whereby diffusion is held in abeyance, and absorption of the salt prevented; the larger doses display the toxic action of the salt, paralysing the cells, allowing the physical laws of diffusion to act without check, so that the salt gains access to the tissues of the organism, and the tadpole dies.

Further evidence in favour of the non-absorption of salts in the doses in which they sustain is supplied by experiments with double solutions, containing potassium chloride and in addition one of various other salts. Of potassium chloride by itself, solutions have been tested varying in strength from 1 in 20,000 to 1 in 100; but only one (the weakest) has been found to maintain life so long as distilled water, none to maintain it longer (except in some cases of tadpoles in the gill stage, which present anomalous features). From experiments to be described, there is reason to believe that this lack of supporting power on the part of potassium chloride is due to its inability to entirely prevent the disintegrating action of distilled water; thus the salt gains access to the organism; and as would be expected the combined toxic action of the salt and disintegrating influence of distilled water cause death sooner than would distilled water alone. Disintegration must to some extent be obviated, since no gross changes occur comparable with those which are seen with distilled water alone; this however is no proof that minute breaches of integrity have not taken place, too small to be appreciated by the naked eye. If however a solution be employed, containing in addition to potassium chloride one or other of the salts which have been found to prevent disintegration, the result is very different; in such a double solution tadpoles survive for days, in many cases as well as in tap water, or in the sustaining solution itself free from potassium chloride.

We have tested in this respect solutions containing as well as potassium chloride one or other of the following salts,—potassium citrate, potassium oxalate, sodium oxalate, sodium phosphate, and calcium phosphate, and in every case the tadpoles have lived for days; in a solution containing potassium oxalate 1 in 4000 and potassium chloride 1 in 700 the tadpoles were alive and to all appearances quite healthy on the 8th day, when the experiment was closed. An experiment with potassium citrate + potassium chloride will be cited side by side with one in which potassium chloride was employed by itself.

Exp. 5. *Potassium chloride.* 200 c.c. distilled water in each beaker; to all but the first potassium chloride added to make percentage as follows:

(2) ·05 p.c. (5) ·2 p.c.
(3) ·1 „ (6) ·25 „
(4) ·15 „

April 16. 11.30 a.m. Tadpoles introduced.
1 p.m. (1) Tadpoles still moving.
(2)—(6) All motionless.
4.30 p.m. All motionless.

Exp. 6. *Potassium chloride + potassium citrate* ($KH_2C_6H_5O_7$). In each beaker 200 c.c. of a ·01 p.c. solution of potassium citrate in distilled water; potassium chloride added so as to make percentage in

(1) ·1 p.c. (3) ·2 p.c.
(2) ·15 „ (4) ·25 „

April 30.	10 a.m.	Tadpoles introduced.
May 3.	„	All perfectly active.
4.	„	All motionless.

It is seen that while the tadpoles in the potassium chloride solutions all died within $1\frac{1}{2}$ hours, those in solutions containing corresponding quantities of potassium chloride, and in addition a small quantity of potassium citrate, survived for more than three days.

With a view of determining the nature of this controlling action of one salt over the toxic properties of another, the following pair of experiments was devised.

EXP. 7. *Potassium oxalate* ($K_2C_2O_4$) + *potassium chloride.* In each beaker 200 c.c. of a ·025 p.c. solution of potassium oxalate in distilled water; potassium chloride added so as to make percentage in

(1) ·15 p.c.	(5) ·75 p.c.
(2) ·25 „	(6) 1 „
(3) ·35 „	(7) 1·25 „
(4) ·5 „	

April 29.	11 a.m.	Tadpoles introduced.
	2.30 p.m.	(1)—(6) All active.
		(7) All motionless.
30.	10 a.m.	(1)—(4) All active.
		(5) and (6) All motionless.
May 1.	„	No change.
2.	„	(1) and (2) Active.
		(3) and (4) One tadpole motionless in each.
3.	„	(1) and (2) Active.
		(3) and (4) All motionless.
4.	„	(1) All active.
		(2) Two active. One motionless.
5.	„	(1) All active.
		(2) One only active.
6.	„	No change.

Experiment discontinued.

By this experiment the dose was determined at which the toxic action of potassium chloride asserted itself despite the presence of a given quantity of potassium oxalate. A second experiment was then performed, in which this toxic dose of potassium chloride was employed as the constant, and to this solution increasing quantities of potassium oxalate were added.

EXP. 8. *Potassium oxalate + potassium chloride.* In each beaker 200 c.c. of a 1 p.c. solution of potassium chloride; potassium oxalate added so as to make percentage in

(1) ·025 p.c. (4) ·1 p.c.
(2) ·05 „ (5) ·125 „
(3) ·075 „

April 30.	10.30 a.m.	Tadpoles introduced.
	1.30 p.m.	(1) All active.
		(2) Two active. One motionless.
		(3) Two active. One motionless.
		(4) One moving sluggishly. Two motionless.
		(5) All motionless.
	7 p.m.	(1) All active.
		(2) One active. Two motionless.
		(3) and (4) All motionless.
May 1.	10 a.m.	All motionless.

These two experiments show that there is no direct antagonistic action between the two salts; for had there been such action, the dose of potassium chloride which in Exp. 7 was toxic, would have been antagonised by the higher doses of potassium oxalate in Exp. 8; whereas in fact the higher the dose of potassium oxalate in the last experiment, the sooner the tadpoles died. We believe the interpretation of these experiments to be as follows. Potassium oxalate sustains the integrity of the superficial tissues of the tadpole, and thereby permits the presence of a certain quantity of potassium chloride in the solution with impunity to the tadpole, absorption not taking place. On increasing the strength of potassium chloride beyond a certain point, its toxic properties become evident in the paralysis of the functional activity of the skin and mucous membrane; although the line of cells remains unbroken, their powers of selection are in abeyance, and the physical law of diffusion comes into action, absorption of both salts taking place, and the organism rapidly becoming motionless. When once this limit is reached, it matters not, as shown by the experiments, whether potassium oxalate or potassium chloride be added: in either case the result is the same. These two salts therefore only differ in the extent to which they preserve integrity; when once access to the organism has been gained, the one is as fatal in its effect as the other.