THE ACTION OF ACIDS AND ALKALIES AND OF ACID, ALKALINE AND NEUTRAL SALTS UPON THE TADPOLE OF 'RANA TEMPORARIA'

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Many observations have been made upon the action of inorganic salts and variations in their concentration upon living cells. We shall not attempt a complete review of the literature of the subject, but will confine ourselves to the consideration of those observations which are more nearly related to our own experiments.

Ringer and Phear¹ stated that distilled water was toxic for tadpoles, and tested the effects of various chemicals in lessening its supposed harmful action. Locke², however, showed later that distilled water is not necessarily fatal, but that if the least trace of copper be present the solution acquires toxic properties. He further showed that this action of copper can be destroyed in various ways, and suggested that the results of Ringer and Phear might be due to the presence of traces of copper, and that the actions of the agents employed consisted in rendering the copper inert. Ringer³, in a later communication, confirmed Locke's work, showing that distilled water, free from traces of copper and other metals, had no toxic effect on tadpoles.

Loeb and Gies⁴, using the eggs of *Fundulus*, found that a single salt added to distilled water stopped the development, whilst controls in distilled water grew perfectly, but that a second salt of different valency, when used in a suitable ratio, counteracted this effect.

1. *Journal of Physiology*, Vol. XVII, 1895, p. 423.

2. *Journal of Physiology*, Vol. XVIII, 1895, p. 319.

3. *Journal of Physiology*, Vol. XXII, 1897, Proceedings, p. 14.

4. *Arch. f. d. ges. Physiol.*, Vol. XCIII, 1903, p. 246.

Loeb¹ also studied the effect of altering the reaction of the medium upon the growth of *Tubularia*. He found that regeneration was hastened on adding a trace of alkali to an artificial sea water in which the stems were placed. Loeb² also found that the addition of minute traces of alkali to sea water hastened the growth of *Echinus* eggs.

The observations of Moore, Roaf, and Whitley³ upon altering the reaction of natural sea water, in which the fertilized eggs of *Echinus esculentus* were growing, show that alkalies and alkaline salts favour cell division when added in sufficiently small amounts, but that on increasing the strength the growth becomes arrested, whilst acids and acid salts possess no favouring action but inhibit the development of the eggs throughout.

In the present experiments we have studied the effect of adding various chemicals to a solution in which the tadpole can live for some length of time. For this purpose we used the tapwater supplied to this laboratory, after allowing it to run for some time in order to get rid of the water which had been standing in contact with the brass taps and other connections.

The effect of varying the reaction has been studied as well as the action of neutral salts containing similar ions to those used for altering the reaction. The organisms used were the larvae of *Rana temporaria*, which were all of the same age and probably came from a single spawning.

EXPERIMENTAL METHODS.

The experiments were conducted in flat earthenware dishes to expose a large surface for the exchange of gases necessary for the respiration of the animal. In each of these dishes was placed 300 c.c. of a mixture consisting of tap water and the requisite amount of standard solution to give the desired concentration. These solutions were changed every few days to prevent alteration of the strength by evaporation and by the excreta of the animals.

1. *Arch. f. d. ges. Physiol.*, Vol. CI, 1904, p. 340; also, *University of California Publications Physiology*, Vol. I, 1904, p. 137.
2. *Arch. f. Entwicklungsmechanik d. Organismen*, Vol. VII, 1898, p. 631.
3. *Proc. Roy. Soc. B.*, Vol. LXXVII, 1905, p. 102.

In nearly all cases six tadpoles were used, but preliminary experiments were often performed in which a single tadpole was used to determine the rough limits of concentration within which the tadpoles could live. By these means we obtained the concentrations necessary to kill the organisms. The rate of growth was studied in weaker solutions by observing the weight at the end of the same period of growth in the different solutions, and also by noting the time at which the four-legged stage was reached.

The weights were obtained by removing the excess of moisture from all the tadpoles grown in one dish and then weighing them. Each lot was treated in exactly the same manner and the weight of the tadpoles was divided by the number of tadpoles remaining alive, by which means the average weight of the tadpoles in each solution was obtained. In this series the experiments were all started on the same day and were kept going for the same length of time, thus the results are strictly comparable [Table VII (a)].

The solutions of the chemicals to be tested were made up in distilled water, and by adding a definite amount to tap water the requisite strength was obtained. The acids and alkalies were standardized by titration in the presence of an appropriate indicator, the carbonates were weighed and then titrated, whilst the phosphates were weighed, titrated, and finally controlled by estimating the phosphoric acid by means of uranium acetate. The neutral salt solutions were made by weighing the desired quantity and then dissolving in distilled water to make up the necessary volume. Carbon dioxide was bubbled through tap water and the resulting fluid titrated against decinormal sodium hydrate in presence of phenolphthalëin. As the neutral point to this indicator is reached when all the carbon dioxide is in the form of sodium bicarbonate, the number of c.c. used provides data from which to calculate the concentration of carbon dioxide in solution. Dilutions of this solution in tap water were used for testing the effect of carbon dioxide on the tadpoles. In the tables the strengths are expressed as molecular concentrations.

The Composition of Liverpool Tap Water, expressed as parts per 100,000, was as follows:—Total solids, 8 parts, half of which is

organic ; chlorine, 1.1 parts ; hardness, 3.5 (calcium = 1.4 parts) ; phosphates absent.

The reaction of the tap water was compared with distilled water by titrating in the presence of phenol-phthalëin and di-methyl-amido-azo-benzole. The quantity of water was large, and, therefore in each case it was necessary to add a certain amount of the standard solution before the ionic concentration of hydrogen or hydroxyl was sufficiently raised to affect the indicator.

1	litre of distilled water to 'phenol-phthalein' needed	1.6 c.c. decinormal alkali
1	" tap " " " "	0.3 c.c. " "
1	" distilled " 'di-methyl' "	0.9 c.c. " acid
1	" tap " " " "	3.0 c.c. " "

From these figures one sees that to both indicators tap water lies to the alkaline side of distilled water and possesses some constituent which very slightly tends to check change of reaction, as shown by adding the number of c.c. necessary to affect the different indicators. Thus for distilled water the limits between which neither indicator is affected are 2.5 c.c. (0.00025 N), whilst for tap water they are 3.3 c.c. (0.00033 N) apart. These values are so small that gas batteries would not show any difference between distilled and tap water, but they are of interest, as showing that with the small quantity of reagent added in the acid and alkali experiments the change of concentration of hydrogen and hydroxyl ions would be slightly less than if the same quantity of solution were added to distilled water.

SECTION A. EFFECTS OF ACIDS, ALKALIES, AND SALTS UPON LIFE OF ORGANISM

TABLE I. ALKALIES

Molecular concentration of added chemical in the solution	OBSERVATIONS
	(1) <i>Controls</i>
Tap water ...	First tadpole died at the end of 46 days, three were dead at the end of 54 days, and at 99 days the remaining three died.
Distilled water	First tadpole died at the end of 10 days, two more died 2 days later, and all were dead by the 14th day.
	(2) <i>Sodium Hydrate</i>
0.0003 M } 0.0006 M } ... 0.0010 M }	None of these died within 28 days, when the experiment was discontinued.

- 0'0013 M ... First died in 48 hours, the remainder lived for 20 days when two died, and the three which were left died on the 30th day.
 0'0017 M ... One died in 22 hours, the remaining five only lived 48 hours.
 0'0023 M ... First died in $1\frac{1}{2}$ hours, the rest lived only $3\frac{1}{2}$ hours.

(3) *Potassium Hydrate*

- 0'0003 M ... None of these died within the time of duration of the experiment.
 0'0006 M ... First died in 17 days, second died in 41 days, three were dead in 57 days, and the remainder died in 66 days.
 0'0010 M ... First died in 33 days, second died in 42 days, and the remainder died in 57 days.
 0'0013 M ... First died in 3 hours, three were dead in 15 hours, and all were dead at the end of 46 hours.
 0'0017 M ... Three died in 3 hours, and the remainder died in 15 hours.

(4) *Ammonium Hydrate*

- 0'0003 M ... One died in 7 days, two died in 22 days, three were dead in 28 days, and all were dead by the 29th day.
 0'0006 M ... First died in 16 days, second died in 17 days, four were dead by the end of 20 days, five were dead in 24 days, and the last died on the 27th day.
 0'0010 M ... Five died in 16 hours, but the last one remained alive for 4 days.
 0'0013 M ... All died in 55 minutes.

(5) *Calcium Hydrate*

- 0'0005 M ... First died in 16 days, second in 21 days, third in 23 days, fourth in 25 days, fifth in 34 days, and the last died on the 41st day.
 0'0007 M ... First died in 20 days, second in 23 days, four were dead in 24 days, fifth died in 27 days, and all were dead in 29 days.
 0'0008 M ... First died in 18 days, second in 20 days, two more died in 24 days, five were dead in 28 days, and the last died in 46 days.
 0'0010 M ... First died in 15 hours, second in 94 hours, third in 7 days, fourth in 14 days, and last two in 17 days.
 0'0013 M ... Four died in 15 hours, and the last two in 94 hours.

(6) *Barium Hydrate*

- 0'00015 M ... First died in 3 days, two more in 14 days, a fourth in 30 days, and the last two in 39 days.
 0'00030 M ... First died in 30 hours, two more in 17 days, a fourth in 18 days, and the last two in 30 days.

(7) *Magnesium Hydrate*

A saturated solution of magnesium hydrate was made in distilled water, but the solubility was so slight that no effect was produced on the tadpole.

TABLE II. ACIDS

Molecular concentration of added chemical in the solution

OBSERVATIONS

(1) *Hydrochloric Acid*

0.0003 M	...	First died in 46 days and the remaining five in 59 days.
0.0006 M	...	All dead in 52 hours.
0.0010 M	...	Five died in 52 hours but one remained alive for 94 hours.
0.0013 M	...	First died in ten hours, a second in 21 hours, and the remaining four in 52 hours.
0.0017 M	...	Two died in 4 hours, a third in 5 hours, two more in 7 hours, and the last died in 10 hours.

(2) *Sulphuric Acid*

0.0003 M	...	First died in 17 days, a second in 23 days, and the remaining four in 24 days.
0.0004 M	...	First died in 29 days, a second in 33 days, and the remaining four in 39 days.
0.0005 M	...	All died within 15 hours.
0.0006 M	...	All died within 15 hours.

(3) *Phosphoric Acid*

0.0006 M	...	First died in 17 days, a second in 20 days, three more in 20½ days, and the last one died on the 21st day.
0.0010 M	...	Five died in 22 hours, and the last one died in 29 hours.
0.0013 M	...	In this experiment only four tadpoles were used, and they all died within 15 hours.
0.0020 M	...	In this experiment only three tadpoles were used, and they all died within 15 hours.

(4) *Acetic Acid*

0.0006 M	...	One died in 36 days, and the remaining five in 48 days.
0.0010 M	...	First died in 14 days, a second in 17 days, a third in 31 days, and the remaining three in 36 days.
0.0013 M	...	Two died in 22 hours, and the remaining four in 45 hours.
0.0015 M	...	All died in 39 hours.
0.0017 M	...	Five died in 30 hours, and the last one in 45 hours.
0.0033 M	...	All died in 16 hours.

(5) *Carbonic Acid*

0.034 M	...	First three died in 17 days, and the remaining three in 20 days.
0.045 M	...	First died in 16 days, a second in 17 days, and the remaining four in 20 days.
0.068 M	...	In this experiment only one tadpole was used, and it died in 16 hours.
0.136 M	...	In this experiment only one tadpole was used, and it died in 10 minutes.

Molecular concentration of added chemical in the solution

TABLE III. ALKALINE SALTS

OBSERVATIONS

		(1) <i>Sodium Bicarbonate</i> (NaHCO_3)
0.0017 M	}	... None of these died within 27 days, when the experiment was discontinued.
0.0023 M		
0.0033 M		
0.0063 M	...	First died in 57 days, and the remaining five on the 75th day.
0.0323 M	...	Two died in 22 days, a third in 23 days, two more in 25 days, and the last in 28 days.
0.0476 M	...	First died in 14 days, second in 15 days, third in 18 days, fourth in 21 days, fifth in 22 days, and the last in 28 days.
0.1000 M	...	In this experiment only one tadpole was used, and it died in 44 hours.

(2) *Sodium Carbonate* (Na_2CO_3)

0.0017 M	...	All lived for 84 days.
0.0023 M	...	First died in 41 days, a second in 59 days, a third in 61 days, a fourth in 69 days, and the last two on the 82nd day.
0.0033 M	...	First died in 15 hours, but the remaining five lived for 67 days.
0.0063 M	...	First died in 19 hours, two more in 45 hours, a fourth in 6 days, a fifth in 14 days, and the last in 23 days.

(3) *Di-Sodium Phosphate* (Na_2HPO_4)

0.0007 M	}	... None of these died within 52 days.
0.0011 M		
0.0015 M	}	... None of these died within 57 days.
0.0031 M		
0.0047 M	...	All dead by the 42nd day.
0.0232 M	...	One dead in 16 days, a second in 20 days, a third in 21 days, two more in 24 days, and the last in 28 days.
0.0465 M	...	In this experiment only one tadpole was used and it lived for 3 days.

Molecular concentration of added chemical in the solution

TABLE IV. ACID SALTS

OBSERVATIONS

		(1) <i>Mono-sodium Phosphate</i> (NaH_2PO_4)
0.0015 M	}	... None of these died during the continuation of the experiment, which lasted 57 days.
0.0025 M		
0.0035 M		
0.0050 M		
0.0373 M	...	First two died in 17 days, and a third in 18 days, a fourth in 19 days, a fifth in 21 days, and the last in 23 days.
0.0746 M	...	In this experiment only one tadpole was used, and it died at the end of 41 hours.

(2) *Sodium bisulphate* (NaHSO_4)

0.0005 M	...	Two died in 26 days, two more in 29 days, and the last two in 39 days.
0.0010 M	...	All died in 15 hours.
0.0020 M	...	All died in less than 18 hours.

TABLE V. NEUTRAL SALTS

Molecular concentration of added chemical in the solution

OBSERVATIONS

(1) *Sodium Chloride*

0.100 M	...	First died in 4 days, a second in 6 days, a third in 7 days, and the remaining three in 9 days.
0.125 M	...	In this experiment only one tadpole was used, and it died in 42 hours.
0.250 M	...	In this experiment only one tadpole was used, and it died in 2 hours.

(2) *Potassium Chloride*

0.038 M	...	Three died in 9 days, and the remainder in 14 days.
0.044 M	...	First died in 6 days, two more in 7 days, another two in 8 days and the last one in 9 days.
0.051 M	...	First two died in 5 days, two others in 6 days, and the last two in 7 days.
0.125 M	...	In this experiment only one tadpole was used, and it died in 3 hours.

(3) *Ammonium Chloride*

0.033 M	...	One died in 12 days, and the remaining five one day later.
0.050 M	...	First two died in 21 hours, a third in 24 hours, a fourth in 76 hours, a fifth in 6 days, and the last in 7 days.
0.100 M	...	In this experiment only one tadpole was used, and it died in less than 17 hours.

(4) *Calcium Chloride*

0.083 M	...	First died in 45 hours, a second in 69 hours, two more in 4 days, and the last two in 7 days.
0.100 M	...	Two died in 44 hours, and the remaining four in 92 hours.
0.250 M	...	In this experiment only one tadpole was used, and it died in 1 hour and 10 minutes.

(5) *Barium Chloride*

0.0033 M	...	All died in 30 hours.
0.0066 M	...	All died in 30 hours.

(6) *Magnesium Chloride*

- 0.033 M ... First died in 12 days, a second in 13 days, and the remaining four in 16 days.
 0.050 M ... Two died in 40 hours, and the last four in 46 hours.
 0.071 M ... All died in 40 hours.
 0.100 M ... All died in 24 hours.

(7) *Sodium Sulphate*

- 0.033 M ... One died in 10 days, three more in 14 days, a fifth in 15 days, and the last one in 16 days.
 0.050 M ... First died in 7 days, a second in 8 days, a third in 10 days, a fourth in 12 days, and the last two in 13 days.
 0.066 M ... Five died in 4 days, and the remaining one in 7 days.

(8) *Sodium Acetate*

- 0.050 M ... First died in 7 days, a second in 15 days, two more in 21 days, a fifth in 40 days, and the last one in 42 days.
 0.063 M ... Three died in 7 days, and the remaining three in 14 days.
 0.077 M ... First died in 4 days, three more in 7 days, and the last two in 8 days.
 0.100 M ... Three died in 5 days, and the other three in 6 days.

(9) *Copper*

A strip of pure copper placed in distilled water killed six tadpoles in less than 15 hours.

(10) *Silver*

Six tadpoles placed in a dilute solution of colloidal silver died in less than 24 hours.

(11) *Platinum*

Six tadpoles placed in a dilute solution of colloidal platinum lived for more than 30 days.

SUMMARY OF RESULTS GIVEN IN TABLES I TO V

Taking that strength of solution for which at least half the tadpoles used in the experiments died within forty-eight hours, the following may be stated as the lethal concentration of each of the solutions used. In a few cases the values can only be taken as approximate, because at the upper limit the concentration was too suddenly increased, and this was not appreciated until it was too late in the season to obtain a fresh supply of tadpoles :—

TABLE VI

Lethal concentration			Lethal concentration		
Substance	Molecular	Ionic ¹	Substance	Molecular	
(a) ALKALIES :			(c) ALKALINE SALTS :		
Calcium hydrate ...	0·0013	0·0026 (?)	Sodium bi-carbonate ...	over	0·1000
Sodium hydrate ...	0·0017	0·0017	Sodium carbonate ...		0·0063
Potassium hydrate ...	0 0013	0·0013	Di-sodium phosphate ...	over	0·0465
Ammonium hydrate ...	0·0010	0·0010 (?)	(d) ACID SALTS :		
(b) ACIDS :			Mono-sodium phosphate ...	over	0·0746
Sulphuric acid ...	0·0005	0·0010	Sodium bi-sulphate ...	under	0·0010
Hydrochloric acid ...	0·0013	0·0013	(e) NEUTRAL SALTS :		
Acetic acid ...	0·0013	0·0013 (?)	Sodium chloride ...		0·1250
Phosphoric acid ...	0·0010	0·0010 (?) ²	Potassium chloride ...	under	0·1250
Carbonic acid ...	0·0500	(?)	Ammonium chloride ...		0·0500
			Calcium chloride ...		0·1000
			Magnesium chloride ...		0·0500
			Sodium sulphate ...	over	0·0660
			Sodium acetate ...	over	0·1000

1. These are calculated on the assumption of total dissociation, which would almost be the case at these dilutions, where it is doubtful it is marked (?)

2. Calculated as dissociating to yield, H , H_2PO_4 .

An examination of the preceding summarized table shows that the substances tested may be divided roughly into two classes, which exhibit toxic effects at quite different concentrations, viz., (1) the acids and alkalies, and in a less degree certain acid and alkaline salts, which kill at concentrations lying between 0·001 and 0·002 molecular, and (2) the neutral salts which require a concentration at least fifty times as great (0·050 to 0·125 molecular) before acquiring an approximately equal toxic action.

It is probable from this that the action upon the cells in the two sets of cases is different in character, and that in the case of the acids and alkalies the effect is due to a chemical action of the hydrogen or hydroxyl ion respectively upon the protoplasm, while in the case of some of the neutral salts the action is a physical one due to osmotic pressure.

Leaving out of account for the present one or two cases, such as ammonium, barium, and magnesium salts where there seems to exist a special toxic action, it may be pointed out that the concentrations at

which all the alkalies and acids kill lie at the same order of magnitude, and, as in the extremely dilute solutions in which the reactions are occurring the alkalies and acids may be taken as practically completely dissociated, this shows that the action is mainly due to the hydrogen or hydroxyl ion respectively, the other ion being comparatively inert. Further, the figures show that the hydrogen and hydroxyl ion are practically of equal power, which points to a combination between these ions respectively and the proteid of the cell.

Provided the combination between hydrogen or hydroxyl ion and the proteid of the cell were more stable than that between the proteid and the various constituents of the nutrient plasma, with which the cell proteid or protoplasm carries on the metabolism, then such a combination would furnish an explanation of the decrease, and finally the complete stoppage of the metabolic processes and therewith of the life of the cell.

It has been shown by Moore and Roaf¹, that the action of anaesthetics in limiting the activity of the protoplasm is in all probability due to the formation of chemical compounds or adsorption products between the proteid of the protoplasm, and the anaesthetic. So long as the concentration or pressure of the anaesthetic is kept above a certain level these compounds or physical aggregates persist and decrease the power of the protoplasm to undertake the normal processes of metabolism, which must involve a temporary combination or aggregation of tissue proteid and nutrient molecule before the latter can undergo chemical change and oxidation. When the anaesthetic is pushed beyond a certain level of concentration, the metabolic processes are so much hindered that life becomes impossible.

Similarly, acid intoxication leads to unconsciousness or coma, because the acid products reaching the cells form more stable compounds with the proteid of the protoplasm than do the normal nutrient materials, and so lead as the concentration is increased to stoppage of metabolism and of the supply of energy necessary for carrying on the vital processes of the cell.

1. *Roy. Soc. Proc.*, Vol. LXXIII, 1904, p. 382-412, and B, Vol. LXXVII, 1905, p. 86-102.

There is little doubt that the low concentrations of acid or alkali which have been shown to lead to death in the above experiments produce their effects in a similar fashion by forming relatively stable compounds or aggregations with the cell protoplasm, which are formed even at very low ionic concentrations.

It has been pointed out by Moore¹ that the protoplasm of the cell must be regarded as in labile equilibrium with the nutrient materials which are supplied to it so that it can enter and pass out of combination, physical or chemical, with the various constituents. The point of equilibrium at each instant depends on the concentration, or osmotic pressure, of each constituent in the cell for the time being. In order that this power of the cell may remain in action it is necessary that there shall not be present in the cell (at any rate beyond given concentrations) any constituents which enter into stable combination with the protoplasm, otherwise combination with such constituents will occur to the exclusion of the labile metabolic combinations.

An example may make the above view clearer. Haemoglobin enters into labile combination with oxygen to form oxy-haemoglobin, and according to the osmotic pressure or concentration of the oxygen in the plasma, the point of equilibrium shifts, and combination or dissociation occurs. Similarly, carbon monoxide combines with haemoglobin to form carboxyhaemoglobin, but the combination is much more stable, and the equilibrium point lies much closer to complete formation of carboxyhaemoglobin at much lower pressures of carbon monoxide. Now if haemoglobin be subjected *simultaneously* to the action of given osmotic pressures of oxygen and carbon-monoxide in the plasma, there will be at any instant given proportions of the haemoglobin in combination with oxygen and carbon-monoxide respectively, the amount depending upon the two osmotic pressures and the relative stabilities of the two compounds. On account of the higher stability of the carbon-monoxide compound, a comparatively low concentration of carbon-monoxide in the plasma will enormously

1. *In Recent Advances in Physiology and Bio-Chemistry*, edited by Leonard Hill, Arnold, 1906, pp. 148-159.

diminish the amount of oxy-haemoglobin, and correspondingly diminish the oxygen carrying power of the blood. This is the explanation of carbon-monoxide poisoning.

Exactly similar reasoning holds in the case of the protoplasm of the living cell, and the materials in the nutrient medium with which it reacts; the nutrient proteid, carbohydrate, and fat on the one hand, and toxic materials, such as anaesthetics, hydrogen, or hydroxyl ions on the other. The toxic materials limit and finally stop the reaction between the cell and its nutrient substances, by forming more stable compounds with the protoplasm of the cell. For each toxic substance the equilibrium point between nutrient matter and toxic substance in their reaction with the protoplasm will depend upon relative concentrations or osmotic pressures, and the degree for each of stability of combination¹. Since the concentration of the nutrient materials may approximately be taken as constant, it follows that for each toxic substance there is a given concentration at which it becomes fatal to the life of the cell, and the lower the fatal concentration the higher accordingly must be the stability of the combination between it and the cell protoplasm. So that the figures given above in the table might be regarded as coefficients of the affinity of each substance for combining with cell protoplasm².

Moore and Roaf³ have given experimental evidence along different lines for the existence of such combination between proteid and anaesthetic in the case of a large number of anaesthetics.

The evidence in the literature of combinations between proteid and alkali or acid is too well known to require detailed description.

Barrett⁴ in the case of Paramoecium, has brought forward evidence from measurements of conductivity and electrical potential that added acids or alkalies pass into combination with the tissue of the organisms which is convincing, although, as shown in a succeeding section, part of the observed changes may be ascribed to excretory substances thrown out by the organisms.

1. Shown by the constant (k) of the equilibrium equation.

2. The question whether the combination is due to a chemical compound, or to a physical adsorption or aggregation may be left open, and is not vital to the issue.

3. *Loc. cit.*

4. *Zeit. f. Allgemeine Physiol.*, Vol. IV, 1904, p. 438, and Vol. V, 1905, p. 10; also, *British Medical Journal*, 1906, Vol. I, p. 129.

On these grounds the conclusion may be drawn that various substances or ions in solution which affect cells do so by combining with the cell substance to form compounds or aggregates of varying stability which interfere with the play of activity between the cell substance and its normal nutrient constituents; and that the effect is proportionate (1) to the concentration of the toxic substance, and (2) to its combining power with the cell substance.

Secondly, in the case of the alkalies and acids the main factor is the concentration of the hydrogen or hydroxyl ion, and the other ion has little effect.

The neutral salts only commence to show injurious or lethal effects at a much higher concentration where the osmotic pressure begins to exceed that of the organism, and as the effects are bound up with changes in volume of the organism which always precede them the phenomena are discussed in a separate section.

SECTION B. EFFECTS OF NEUTRAL SALTS UPON THE VOLUME OF THE ORGANISM

One of the most striking results in the whole research was the wonderful diminution in volume of such a complicated organism as the tadpole, which was compatible with life, and the fact that marked diminution in volume was obtained while the concentration of the salt in the surrounding fluid was still obviously much below that of the tissue fluids of the organism itself.

The latter fact is of high interest in view of the usually accepted view that the living cell does not shrink in volume until the osmotic pressure of the surrounding fluid is equal to that of the cell contents.

This, in fact, has hitherto formed an axiom in measuring the osmotic pressure of living cells, such as the blood corpuscles, frog-muscle, etc. It has been assumed that, if a living cell neither shrinks nor swells when placed in a given saline medium, that then the said medium is isotonic, or of equal osmotic pressure, with the cell contents.

The results of our experiments with living tadpoles, show clearly however, that this position must be reviewed, and that certain animal

cells and tissues may decrease in size long before the osmotic pressure without is equal to the osmotic pressure within.

In the case of the tadpole, and probably in the case of other living organisms, the criterion of unaltered volume of the cell does not appear to be equality of osmotic pressure within and without, but rather a constant and fixed difference of pressure within and without, to which the cell appears to accommodate itself, and which is normal to the organism.

Further, the particular organism with which we have worked appears to be able to live with widely different osmotic pressures in the external medium, and to be able to accommodate itself to those pressures, still preserving, by shrinkage in volume, a positive difference between the internal and external pressures.

We have seen tadpoles living and *active* for days, although shrunk to approximately one-third of their normal size, in solutions of different neutral salines, which even then only began to approximate in osmotic pressure to the normal osmotic pressure of the tissues of the adult frog.

It is remarkable that the concentrations of saline at which death of the tadpoles began to occur fairly rapidly, coincided roughly with the normal osmotic pressure of the adult organism. Thus, the lethal concentration of sodium chloride and potassium chloride for tadpoles was nearly the same, approximately 0.125 M, corresponding to a depression of freezing point of 0.4332° C.,¹ while the osmotic concentration of frogs' blood is 0.1093 M NaCl ² corresponding to a depression of freezing point of 0.3789° .

This result is of interest as giving an indication of how the living cells in this particular organism react to increased osmotic pressure in their external medium. The cells do not remain of constant volume, as the osmotic pressure of the external medium is run up from that of tap water to the concentration of the internal medium or above it; but there is a change of volume throughout, although the osmotic pressure in the external medium is a long way

1. Calculated from data given by HAMBURGER, *Osmotischer Druck*, Wiesbaden, 1902, Vol I, p. 83.

2. *Ibid*, p. 181.

below that of the tissue fluids. By the time the osmotic pressure in the external medium is approximately that of the internal medium the tadpole may have shrunk to approximately one-third of its normal volume.

Finally, the death limit lies, especially when the salt used for raising the osmotic pressure in the outer medium is foreign to that chiefly constituting the medium of the cell, just above that of the internal medium, assuming that the osmotic pressure in the tissues of the tadpole is approximately that of the adult frog.

It hence appears clear that the living cells in the tadpole possess a regulatory mechanism which tends to preserve a constant difference in osmotic pressure between the external and internal media, as shown by the shrinkage long before the external medium possess the osmotic pressure of the internal medium, and that it is only after this mechanism has been overcome by the osmotic pressure of the external medium becoming higher than that of the internal medium that any foreign ions in the external medium enter the cell in appreciable quantity, and lead to the death of the cell and of the organism.

Thus, comparing the action of sodium chloride and potassium chloride, as shown in Table V, it is not until 0.1 M concentration is attained that either salt begins to show marked poisonous effects although the tadpoles in both solutions have at this concentration shrunk to mere pigmies of their former selves, but at 0.125 M concentration the potassium chloride kills in three hours, while the sodium chloride takes forty-two hours to kill.

Now, it is well known that the potassium ion once it enters the cell in much more than normal concentration rapidly kills, hence it is evident that it is only when the concentration or osmotic pressure without is equal to that within that the potassium ion begins to enter rapidly, and the potassium ion, being more poisonous than the sodium ion, leads more rapidly to a lethal result.

It is probable that the conditions in all living cells are not the same, the blood cells exist in a fluid, the plasma, of constant composition and osmotic pressure, and have developed under conditions in which the osmotic pressures within and without are equal ; but in

other cells, as shown by these experiments, there is no necessity for equality of osmotic pressure within and without, but rather there is a mechanism for preserving a constant difference of osmotic pressure between the internal and external media of the cell.

Hence, while it may be safe to take the constancy of volume as a criterion of equality of osmotic pressure in the case of the blood cells, it is quite otherwise in the case of other cells. Even in the case of mammalian tissues it probably requires further investigation before assuming that a solution, in which the tissue neither gains nor loses weight, is iso-osmotic with the tissue fluids.

SECTION C

EFFECT OF WEAKER SOLUTIONS ON RATE OF DEVELOPMENT

TABLE VII

(a) WEIGHTS

Nature of added chemical	Molecular concentration of added chemical in the solution	Average weight of each tadpole at end of 27 days	Nature of added chemical	Molecular concentration of added chemical in the solution	Average weight of each tadpole at end of 27 days
Tap water	—	0.107 g	Sodium carbonate	0.0017 M	0.098 g
Sodium	0.0003 M	0.123 g		0.0023 M	0.073 g
hydrate	0.0006 M	0.098 g		0.0033 M	0.072 g
	0.0010 M	0.093 g	Di-sodium	0.0007 M	0.148 g
	0.0013 M	0.116 g*	phosphate	0.0011 M	0.130 g
Potassium	0.0003 M	0.128 g		0.0015 M	0.127 g
hydrate	0.0006 M	0.078 g		0.0031 M	0.117 g
	0.0010 M	0.090 g		0.0047 M	0.125 g
Sodium bi-	0.0017 M	0.100 g	Mono-sodium	0.0015 M	0.147 g
carbonate	0.0023 M	0.108 g	phosphate	0.0025 M	0.137 g
	0.0033 M	0.100 g		0.0035 M	0.122 g
	0.0063 M	0.103 g		0.0050 M	0.113 g
			Hydrochloric acid	0.0003 M	0.130 g

* In this only three remained alive, and as probably the weakest died first, the weight of the remaining ones would be above the average.

(b) STAGES OF DEVELOPMENT

Nature of added chemical	Molecular concentration of added chemical in the solution	OBSERVATIONS
Sodium carbonate	0.0017 M	One reached the stage, when four legs appear, in 40 days; a second in 41 days. One tail almost gone in 42 days.
	0.0023 M	One reached four-legged stage in 59 days.
Sodium bicarbonate	0.0063 M	One reached four-legged stage in 45 days.
Di-sodium phosphate	0.0007 M	Four reached four-legged stage in 40 days, and a fifth in 41 days. Three tails had almost disappeared in 42 days, and five in 45 days.
	0.011 M	One reached four-legged stage in 40 days, and a second in 41 days. One tail had almost disappeared in 40 days.
	0.0015 M	One reached four-legged stage in 40 days, a second in 42 days. One tail almost gone in 42 days.
	0.0031 M	Two reached four-legged stage in 42 days.
	0.0047 M	One reached four-legged stage in 40 days, a second in 41 days, and a third in 42 days.
Mono-sodium phosphate	0.0015 M	Three reached four-legged stage in 40 days, and a fourth in 41 days.
	0.0025 M	One reached four-legged stage in 40 days, and a second in 46 days.
	0.0035 M	One reached four-legged stage in 42 days, a second in 45 days, and a third in 46 days.
	0.0050 M	One reached four-legged stage in 40 days, and a second in 41 days.
Hydrochloric Acid	0.0003 M	One reached four-legged stage in 41 days, and a second in 42 days.

From the table it is seen that the rate of growth is greater in the weakest solution used of sodium hydrate and potassium hydrate (0.0093 M) than in tap water. There is no increase with the alkaline carbonate. Both phosphates cause an increase in all strengths up to about 0.05 M. The tadpoles only lived in the least concentrated solution of hydrochloric acid used (0.0003 M), but having survived this concentration, the organisms developed more rapidly than in tap water.

The results obtained in the case of mono-sodium phosphate and hydrochloric acid differ from those obtained by Moore, Roaf, and Whitley¹ in observations upon *Echinus esculentus*, but it must be remembered that we are here dealing with a much more complicated organism possessing a specialized excretory mechanism, and hence there is probably a neutralizing action on the part of the organism. The great increase in growth with the phosphates points to a special stimulating action of these salts upon cell-division and growth, the acid phosphate absorbed being probably converted into alkaline phosphate by the activity of the ammonia formed by the organism (see Section D).

SECTION D. EFFECTS OF EXCRETORY PRODUCTS FROM THE ORGANISMS UPON THE EXTERNAL MEDIUM

In considering the effects of ions in varying concentration, present in the external medium, upon the living organism, as we have been doing in the preceding sections, it must not be forgotten that there is also to be considered the action of the organism, or its excretory products, upon the external medium. Such action may easily alter the ionic concentrations in the external medium, unless the volume of the external medium is kept large, compared to the volume of the organism, and the amount of their excreta, and the water be regularly changed.

Thus, we have seen in a preceding section, that the effects of the ions are due to their forming compounds or aggregates, of varying degree of stability, with the cell protoplasm, until an equilibrium point is attained in each individual case. As a result of this aggregation, the different ions and dissolved substances will be taken up, each in definite amount, by the protoplasm from the solution, and it is only when the volume of solution compared to volume of organism is large that such uptake will not appreciably effect the concentrations of dissolved substances in the external medium. Also, the excretion of bodies by the action of the cell's metabolism may neutralize added reagents in the external medium, and cause changes in ionic

1. *Loc. cit.*

concentrations. This factor may be guarded against by frequent changing of the external medium.

By preserving a large volume of fluid, compared to the volume of the tadpoles used, and by frequent changing, we have endeavoured to secure that the concentrations of the salts and ions tested by us should remain throughout each experiment close to the concentrations initially made up.

We thought it well, however, to investigate the amount of change produced by tadpoles in media similar to those we had experimented with in the preceding sections. For this purpose, one litre of solution was used in each experiment, and in one of two similar vessels, 24 tadpoles were placed, while the other was used as a control, and had no tadpoles added. The media tested in this way were 0·0005 M hydrochloric acid, and 0·0005 M sodium hydrate, and the duration of the experiment was in each case twenty-four hours. At the end of this period, the 24 tadpoles were removed, and the following determinations made :—(1) The change in reaction to di-methyl-amido-azo-benzol, and to phenol-phthalëin, as contrasted with the controls, by titration with deci-normal alkali or acid ; (2) the amount of ammonia formed by the tadpoles, estimated by using Nessler's reagent ; (3) the amount of carbon-dioxide formed and going to neutralize caustic alkali, and so reduce hydroxyl ion concentration in the case of the alkaline medium ; this was estimated by passing a current of air free from CO_2 through the acidified solution, drying by pumice impregnated with dried copper sulphate, absorbing by soda lime and weighing. The results are shown in the following table :—

Reagent added to medium	Weight of tadpoles	Change of reaction in c.c. of deci-normal solution to		Ammonia formed in c.c. of deci-normal solution	Carbon-dioxide formed in c.c. of deci-normal solution
		(a) di-methyl	(b) phenol-phthalëin		
0·0005 M hydrochloric acid	2·38 grams	1·2 c.c. less acid than control	0·8 c.c. less acid than control	0·59 c.c.	—
0·0005 M sodium hydrate	2·26 grams	0·8 c.c. more alkaline than control	1·6 c.c. less alkaline than control	0·54 c.c.	0·4 c.c.

NOTES.—Since di-methyl is practically unaffected by carbon dioxide, the loss of acid in the acid medium must be made up of combinations of organisms with acid and ammonia. The total loss being 1·2 c.c. and the amount of ammonia excreted 0·59 c.c., the amount of acid lost in the organisms by combination, probably with protoplasm, is $1·2 - 0·59 \text{ c.c.} = 0·61 \text{ c.c.}$ In the experiment with sodium hydrate the gain in alkalinity is 0·8 c.c., and the amount of ammonia is only 0·54 c.c., and since there must have been some amount taken up by the tadpoles, it follows that there must have been an excretion of other basic bodies by the tadpoles.

The results on such small quantities cannot possess quantitative accuracy, but show that the ionic concentrations in the external medium undergo slight changes, due in part to absorption by the organisms, and in part to excretion.

On account of the second of these two factors it is obviously impossible to follow accurately by exact physical methods, such as determinations of electrical conductivity and potential the proportions in which the organism takes up or combines with ions present in the external medium, as has been attempted recently by Barrett.¹

Interesting and valuable as such experimental observations undoubtedly are, they do not give quantitatively the amount of combination of added ions with the protoplasm, because the excretory products given off at the same time by the organisms also alter the electrical conductivity and potential to hydrogen electrodes of the medium, and since it is difficult or impossible accurately to discount this effect, the change in conductivity or potential do not afford a measure of combination between protoplasm and the added ions in the external medium.

The combinations between ions and protoplasm, and the degree of stability of these is, hence, better measured by the physiological effects.

SUMMARY AND CONCLUSIONS

1. For the organism experimented with, the ions which possess the most powerful action are the hydrogen and the hydroxyl ion, which kill in concentrations of 0.001 to 0.002 M. The degree of toxicity is of the same order of magnitude for these two ions. The most probable explanation of this result is that these ions form more stable compounds or aggregations with the protoplasm than do the ions of the neutral salts. In the case of salts, which, by hydrolysis or dissociation in solution, alter the concentrations in hydrogen or hydroxyl ions in the medium, such as the carbonates and phosphates of the metals of the alkali group, and acid sodium sulphate, the degree of toxicity is roughly proportional to the concentration in hydrogen or

1. *Loc. Cit.*

hydroxyl ion. Thus, sodium bi-carbonate requires the same concentration as a neutral salt (over 0.1000 M) before it kills ; sodium carbonate corresponding to its higher concentration in hydroxyl ions, kills at 0.0063 M ; while sodium bi-sulphate, which is almost completely dissociated, corresponding to its high concentration in hydrogen ions, kills at less than 0.0010 M.

2. Neutral salts, such as sodium chloride, potassium chloride, and calcium chloride, when present along with traces of other ions, as in tap water, do not kill until the osmotic pressure rises above that of the fluids of the organism. But, when the concentration becomes hypertonic, compared with that of the body fluid of the organism, then even these salts rapidly commence to be fatal. So that there is a long range of concentrations where such salts, under our conditions of experimentation, are comparatively harmless, and then a short range in which they become rapidly fatal. The length of this short range varies with the ions of the neutral salt, the potassium ion, for example, after isotonicity has been passed, being much more rapidly fatal than the sodium ion. This we take as an indication that the ions of the neutral salt used do not rapidly increase in concentration within the cells of the organism until the isotonic point has been passed.

3. The organism does not remain constant in volume until the surrounding medium has become isotonic with the internal medium, but diminishes constantly in volume as the concentration of the outer medium is increased, although this is still very hypotonic. In the neighbourhood of the isotonic point the volume may be only about one-third of the normal volume. Hence, the fact of unchanged volume when living cells are placed in different media cannot always be taken as an indication of isotonism between such solutions and the cell fluids.

It appears from this that the organism we have used tends to preserve not equal osmotic pressures of inner and outer media, but possesses a regulating mechanism which preserves a higher pressure within than without up to the point at which the outer pressure approximately equals the normal internal pressure ; beyond this point the mechanism is upset, and the lethal effects rapidly appear.

Some neutral salts contain ions with specific poisonous effects upon protoplasm, and kill on this account when the outer medium is still very hypotonic, such as salts of all the heavy metals, barium salts, and ammonium salts.

4. A colloidal solution of platinum, as concentrated as we were able to prepare it, did not appear to have the slightest effect upon tadpoles, while a very dilute colloidal silver solution killed in less than twenty-four hours. The latter effect might, however, be due to a trace of silver in solution.

5. At much lower concentrations than those which give lethal results, certain ions possess a favouring action upon growth. Of those salts which we have tested, this action is most pronounced in the case of the phosphates. A less amount of increase is seen with potassium and sodium hydrates, and also with the lowest concentration of hydrochloric acid used (0.0003 M), the tadpoles perishing with all the higher concentrations.

6. The excretory products given out by the organisms render it experimentally impossible to determine, by physical methods, the exact amount of combination between the organisms and the ions of the added solutions in the external medium.

In conclusion, we wish to express our thanks to Professor B. Moore for his valuable assistance and constant advice during the course of these experiments.