THE TOXICITY OF DISSOLVED METALLIC SALTS TO *POLYCELIS NIGRA* (MULLER) AND *GAMMARUS PULEX* (L.)

BY J. R. ERICHSEN JONES
Department of Zoology, University College of Wales, Aberystwyth

(*Received* 17 *December* 1936)

(With Ten Text-figures)

A. INTRODUCTION AND DISCUSSION

THE study of the toxicity of metallic salts to aquatic animals has been mainly confined to vertebrates, the amount of work done with invertebrates being comparatively small. Warren (1900) studied the effect of sodium chloride on *Daphnia magna* and expressed the relationship between the concentration of the solutions and the time they took to kill the animal by a graph called the "survival curve". Between concentrations of 0·9 and 6·0 per cent this curve was a rectangular hyperbola. Below and above these values results were "indefinite".

Ostwald (1905) working on the toxicity of sea water to *Gammarus pulex* also obtained a curve whose middle portion was a rectangular hyperbola, and Pittenger & Vanderkleed (1915) who investigated the possibility of standardizing drugs physiologically by determining the survival times of goldfish in the solutions concluded that in all cases the survival curve of an aquatic animal in a toxic solution should be a rectangular hyperbola. That is, the concentration plotted against the reciprocal of the survival time should give a straight line. This they called the "fatality curve".

Clayberg (1917) investigated the toxicity of aqueous solutions of chloroform and ether to the sunfish and also concluded that the survival curve was a rectangular hyperbola, but Powers (1917), who made a more comprehensive study of the toxicity of a large variety of salts, acids, alkalis and drugs on the goldfish, concluded that in all cases the middle portion, only, of the fatality curve was straight. Powers appears to have been the first worker to observe that for all toxic substances there is a more or less well-defined lower limit of concentration below which the survival time becomes indefinite and protracted. To this he gave the term "threshold concentration".

The actual way in which the toxic substance killed the animal appears to have attracted little attention. This question was investigated by Carpenter (1927, 1930) who showed that the minnow (*Leuciscus phoxinus*), when immersed in solutions of salts of lead, copper, zinc and other heavy metals, died as a result of the metal combining with some constituent of the mucus secreted by the gills to form an insoluble and impermeable coating over the gill membranes and body surface. This film so impeded respiration that the fish died, virtually from suffocation.

Osterhout (1922) observed that living plant cells immersed in solutions of salts exhibited a steady rise in electrical conductivity, culminating in the death of the plant, when the conductivity rose to that of the surrounding medium. This rise in conductivity was assumed to result from the penetration of current carrying electrolyte into the living cells. Most of Osterhout's experiments were performed with *Nitella* and *Laminaria*.

Heilbrunn (1928) in his discussion of the action of various salts on living protoplasm observes that the weight of permeability evidence favours the theory that salts diffuse into cells and that there is every reason to suppose that some salts enter more rapidly than others. Heavy metal salts, in particular, according to Heilbrunn, produce coagulation of the protoplasm of living cells. It was observed that copper chloride solutions caused coagulation of the protoplasm of sea-urchin eggs, as was clearly shown by centrifuge tests.

The present work is a study of the toxic action of some variety of metallic salts upon the planarian *Polycelis nigra*, being mainly a study of the relationship between survival time and concentration. *Polycelis* has proved to be excellent material as it is highly sensitive and survival times are short and easily determined. Large numbers of individuals of approximately the same size are readily obtainable, and its hermaphroditism is a further factor rendering biological standardization easy.

Each survival curve has been worked out with a single batch of animals obtained from the same source on the same occasion, and where precise comparison between survival curves is made the experiments for both curves were all performed concurrently with the same batch of animals. This was found necessary as the sensitivity varies considerably with the season, the locality from which the material is obtained and the time it is kept in the laboratory.

The same precautions were observed in the case of *Gammarus pulex* and here mature males, only, were selected for experiment.

B. EXPERIMENTS WITH *POLYCELIS NIGRA*

When immersed in solutions of metallic salts *Polycelis* exhibits several more or less well-defined stages of behaviour. For a few seconds after immersion the animal remains quiescent (Fig. 1 *a*). Then it extends and for some time glides about actively by means of its cilia (*b*). Later, somewhat suddenly, the power of gliding is lost and for some time the animal writhes and coils actively (*c*). In some cases an intermediate stage between (*b*) and (*c*) is seen in which the animal glides on the hind half of the body, the fore part raised and coiling from side to side. Stage (*c*) passes into a period of spasmodic extension and contraction (*d*) which becomes more and more intermittent until the animal becomes inert. At this stage (*e*) stimulation with a brush may invoke feeble movement. When no such response results the animal is assumed to be dead and this stage marks the end of the survival time. In some cases disintegration of the body constitutes a final phase (*f*).

In all but the most dilute solutions of highly toxic salts stages (*a*) and (*b*) are practically non-existent, the power of gliding being lost immediately after immersion. In dilute solutions of less toxic salts stage (*b*) occupies a considerable portion of the survival time. In concentrated solutions of highly toxic salts the only movements observed after immersion are one or two violent extensions and contractions; the

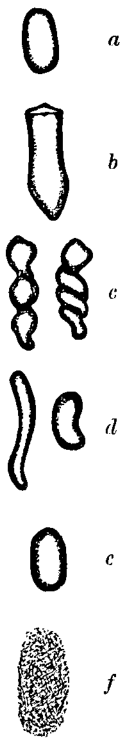

Fig. 1. Stages of behaviour of *Polycelis nigra* in toxic salt solutions.

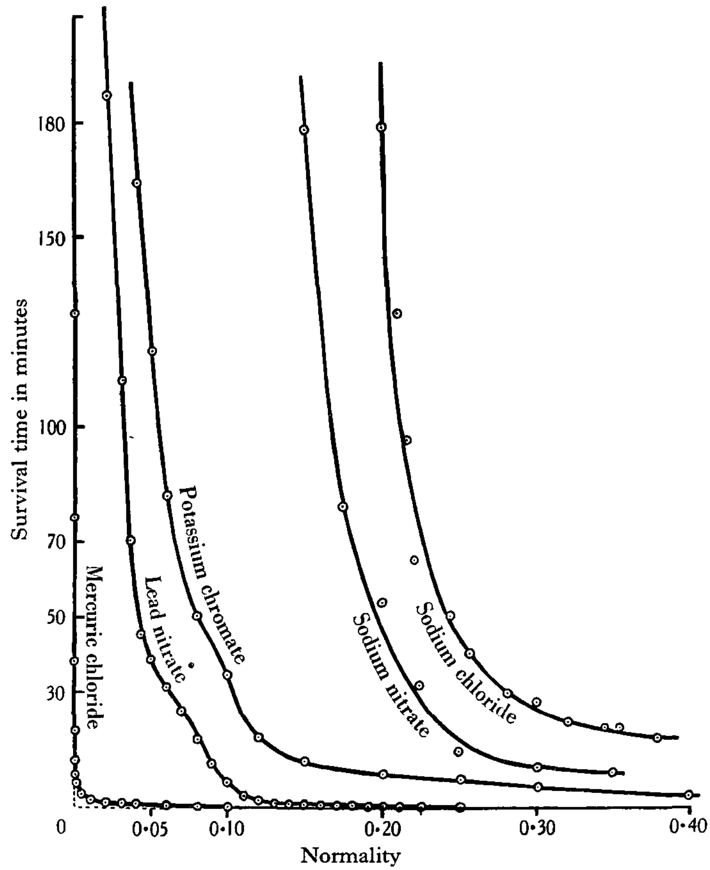

Fig. 2. Survival curves for *Polycelis nigra* in lead nitrate, potassium chromate, mercuric chloride, sodium nitrate and sodium chloride.

proboscis is violently extruded, often separating from the body, the body wall becomes distorted and puckered and may rupture.

All metallic salts are toxic to *Polycelis* if sufficiently soluble, and may be classified into four categories:

(1) Salts which are toxic but are not electrolytes, e.g. mercuric chloride.

(2) The chlorides, nitrates and sulphates of the alkali and alkaline earth metals which are only toxic at relatively high concentrations and practically harmless below 0·125*N*.

(3) Salts of the heavy metals, which are highly toxic at low concentrations and whose toxicity can be attributed mainly to the cation.

(4) Salts such as potassium cyanide, potassium chromate and potassium oxalate where the anion is highly toxic, the cation being comparatively harmless.

Survival curves for *Polycelis* in some of these salts are given in Fig. 2. In general each plotted point is the mean of five determinations at that concentration. All experiments were conducted at a temperature of 16–17° C. It will be seen that in the case of sodium chloride the curve begins to soar vertically upwards at 0·20*N*, indicating arrival at a threshold concentration. Powers states that 0·25*N* is the threshold concentration for *Carcassius* in sodium chloride. At concentrations below

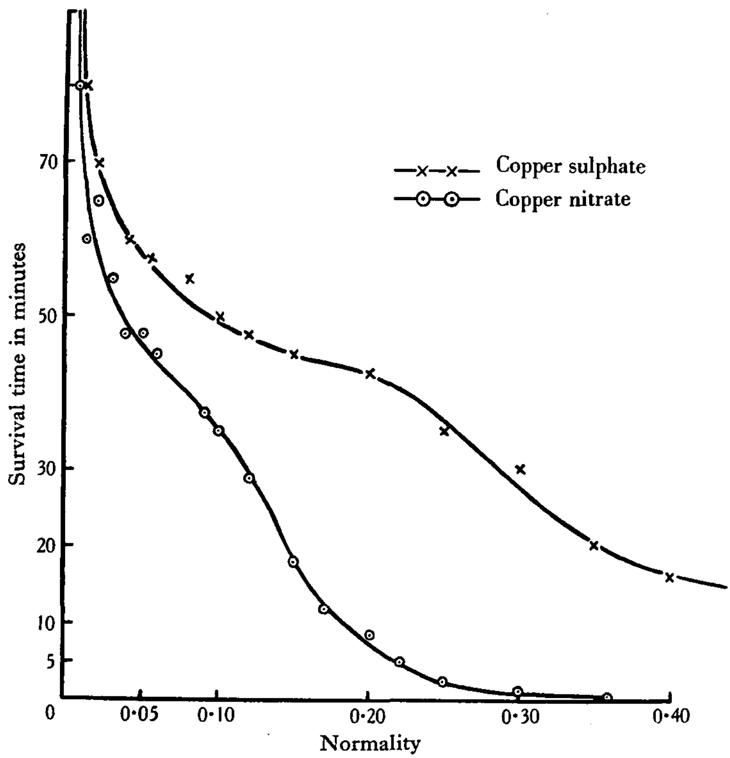

Fig. 3. Survival curves for *Polycelis nigra* in copper nitrate and copper sulphate.

this *Polycelis* survives about as long as it survives in clean tap water. Sodium nitrate similarly ceases to be definitely toxic at 0·15*N*.

In the case of potassium chromate and lead nitrate the survival times remain short at much lower concentrations. Lead nitrate maintains its toxicity down to a concentration of 0·00003*N* where the survival time reaches about 70 days which is approximately the survival time of controls in tap water. Mercuric chloride is still more toxic, down to a concentration of 0·02*N* the survival time is less than 60 sec. and at 0·00002*N* is only 3 hours. At high concentrations death is almost instantaneous.

The death of *Polycelis* in these solutions is not due to any factor preventing the animal receiving oxygen from the water. This is evident from the fact that it survives many days in water boiled for some time and cooled out of contact with the air, though, as might be expected, its sojourn in this medium is characterized by complete torpidity. On restoration to aerated water it becomes active once more.

On the contrary it seems most probable that death in these heavy metal salt solutions results from fixation of the tissues. The more usual fixatives are acids,

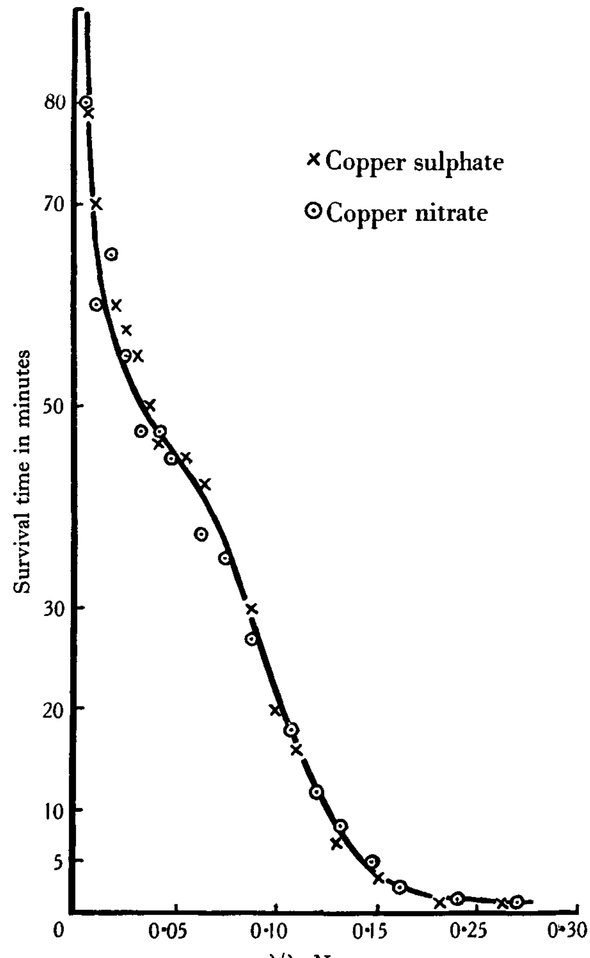

Fig. 4. Survival curves for *Polycelis nigra* in copper sulphate and copper nitrate.

salts of heavy metals or a mixture of both, and operate by precipitating intracellular proteins in a chemically inert state, the liquid living protoplasm becoming permeated with a reticulum of solid structure turning it into an elastic but solid gel (Gray, 1931).

As sodium chloride and similar salts are only toxic in hypertonic solution it is probable that, in such solution, death is primarily due to the withdrawal of water from the body by the surrounding medium. Also, it is possible that the ions diffuse into the body and alter the viscosity of the protoplasm.

As sodium nitrate ceases to be toxic at 0·15N it would appear that at lower concentrations the toxicity of lead nitrate results from the activity of the lead ion only. At concentrations above isotonicity the osmotic pressure of the solution furnishes an additional toxic factor and as sodium nitrate is definitely more toxic than sodium chloride it would appear that the nitrate ion itself must have some toxicity at high concentrations, this acting as a third factor.

At concentrations above 0·10N lead nitrate solutions are partially hydrolysed with formation of free hydrogen ions. The *p*H of lead nitrate solutions reaches 3·4 at 0·25N. The toxicity of the solutions does not appear to be in any degree

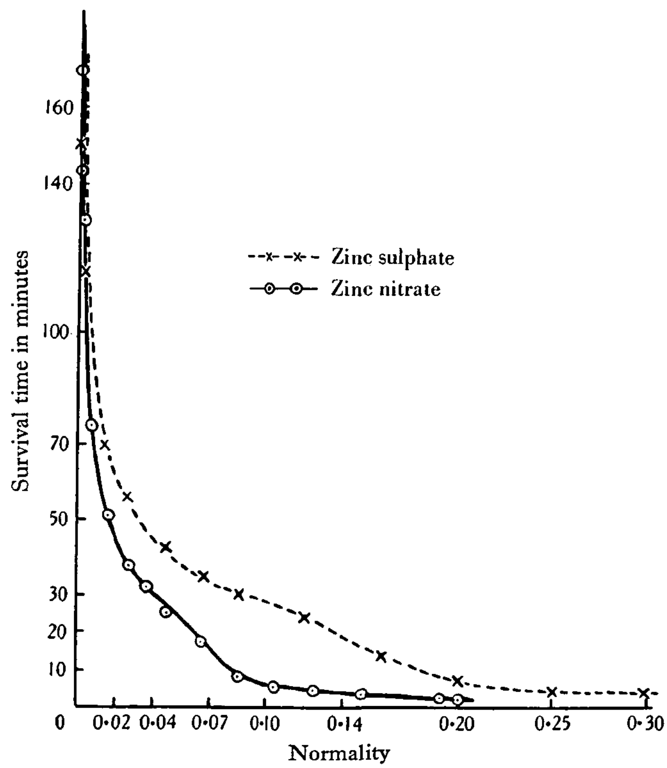

Fig. 5. Survival curves for *Polycelis* in zinc nitrate and zinc sulphate.

influenced by this acidity as buffering up the *p*H to 7·0 has not the slightest effect on the survival time.

Survival curves for *Polycelis nigra* in copper nitrate and copper sulphate are shown in Fig. 3. Each plotted point is the mean of five determinations in 50 c.c. of solution at 16–17° C. The curves are similar in shape to that for lead nitrate and here again toxicity is well maintained at low concentrations; at 0·0001 N the survival time is less than 6 hours.

The high toxicity of copper salts at low concentrations has been noticed by other workers. Ludwig (1927) gives data showing that *Paramecium* is killed by copper sulphate solutions at a concentration of 0·000001 molar, and Powers (1917) gives the survival time for *Carcassius* as under 6 hours in concentrations as low as 0·00000028 N. The writer's experiments with *Gastrosteus aculeatus* give a threshold concentration for copper as low as $5 \times 10^{-7} N$.

It will be noticed that the curves for copper nitrate and copper sulphate are strongly divergent, the sulphate being markedly less toxic at all concentrations than

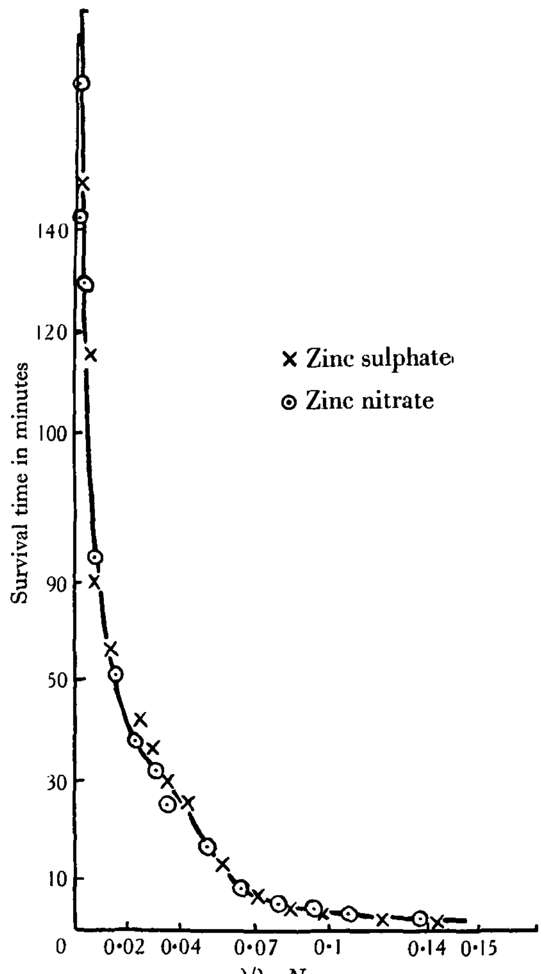

Fig. 6. Survival curves for *Polycelis* in zinc nitrate and zinc sulphate.

the nitrate. This is not due to difference of pH as at all concentrations there is little pH difference between the two salts, nor is it due to differing degree of toxicity of the anions as the divergence is well marked at concentrations below 0·15 N where the anions NO_3 and SO_4 have no appreciable toxicity.

It has been shown by the writer (1935) that in the case of the stickleback (*Gastrosteus aculeatus*) the survival curves for copper nitrate and copper sulphate are divergent when survival time is plotted against normality but coincide reasonably well when the survival time is plotted against the concentration multiplied by the electrical conductance ratio at that concentration ($\lambda/\lambda_0 N$). The same result was obtained with the nitrates and sulphates of zinc, cadmium and nickel, and it was therefore concluded that the conductance ratio can be regarded as a measure of the activity of the solution.

The same relationship holds in the case of *Polycelis* as is shown in Fig. 4. Here survival time is plotted against $\lambda/\lambda_0 N$ and it will be seen that the curves become approximately coincident, showing that the toxicity is determined by the conductance ratio and normality rather than by the normality alone.

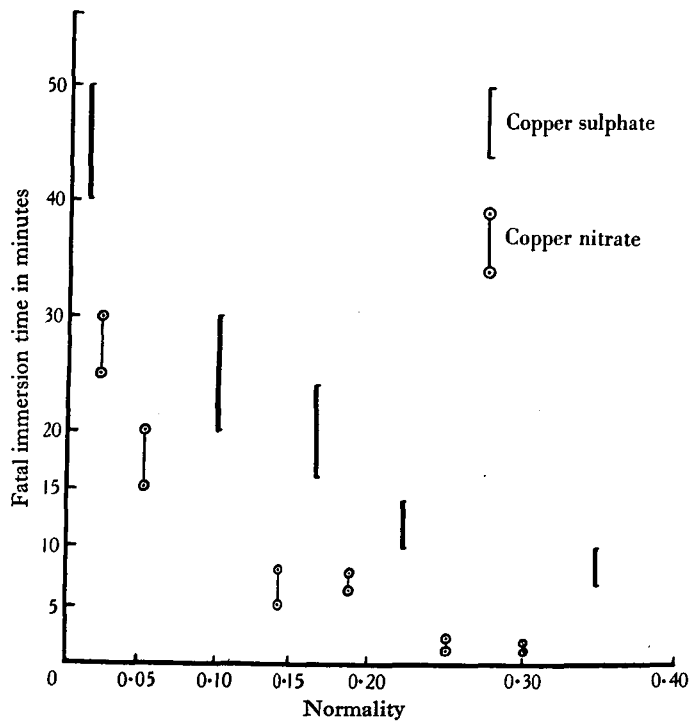

Fig. 7. Fatal immersion times for *Polycelis nigra* in copper nitrate and copper sulphate.

An exactly similar result was obtained with zinc salts, as is shown in Figs. 5 and 6.

It must be regarded as a matter of some interest that the same relationship holds for two animals so widely dissimilar in structure as *Gastrosteus* and *Polycelis*, where the actual mechanism of toxicity is essentially different.

Series of experiments have been performed with *Polycelis* to ascertain the maximum immersion time the animal can withstand at various concentrations with subsequent recovery when removed to clean water. The following is a typical example. Six *Polycelis* were immersed simultaneously in 50 c.c. of 0·35N copper sulphate. In 6 min. one was removed, washed in two changes of clean water and finally left in a Petri dish of clean water. The remaining animals were similarly treated at intervals of 1 min., the last having 11 min. immersion. On examination, after 24 hours, the animals given 6 and 7 min. immersion were alive and active, while those immersed 8 min. or longer were dead and disintegrated. This gives what may be termed the "fatal immersion time" at this concentration, i.e. 7–8 min at 0·35 *N*.

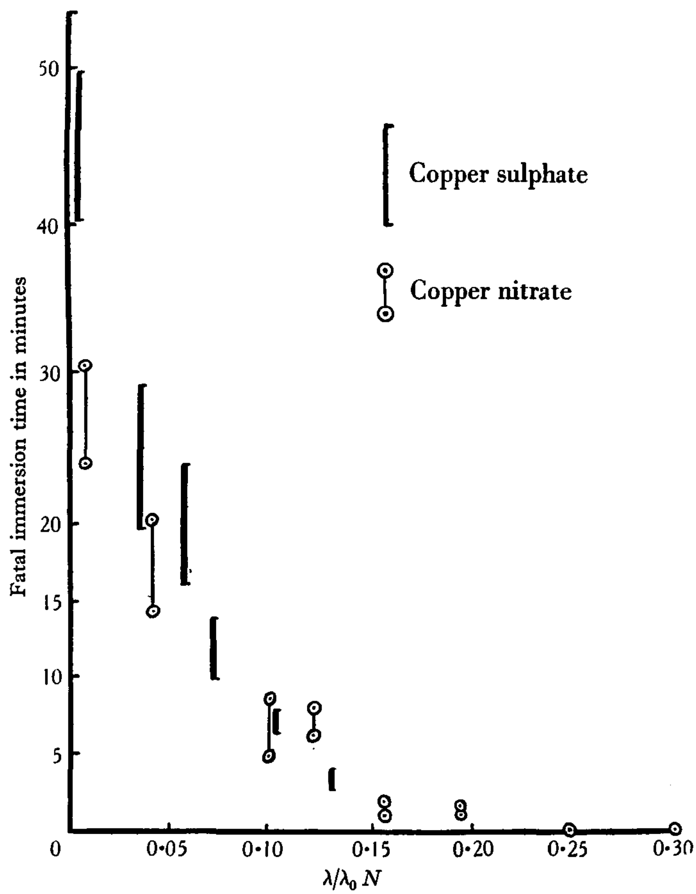

Fig. 8. Fatal immersion times for *Polycelis nigra* in copper nitrate and copper sulphate.

From many series of experiments on these lines the following conclusions have been drawn:

In the case of lead nitrate and zinc nitrate the fatal immersion time is at all concentrations approximately the same as the survival time. Provided the animal is removed from the solution before it becomes inert, it recovers.

With copper salts the fatal immersion times are always much less than the survival times, the animal failing to recover even if removed from the solution some time before movement ceases. Thus in 0·05 *N* copper nitrate 35 min. immersion is necessary to render *Polycelis* inert, but animals removed after 20 min. immersion, though active when removed, were dead in 24 hours.

On the contrary in the case of sodium chloride the animal recovers after being left in the solution nearly twice the period required to render it inert. The "death" of *Polycelis* in sodium chloride is therefore only a state of inertia from which the animal can recover if removed to fresh water.

These immersion time experiments reveal an interesting difference in the mode of toxic action of the salts of lead, copper and sodium and this is a question deserving further investigation.

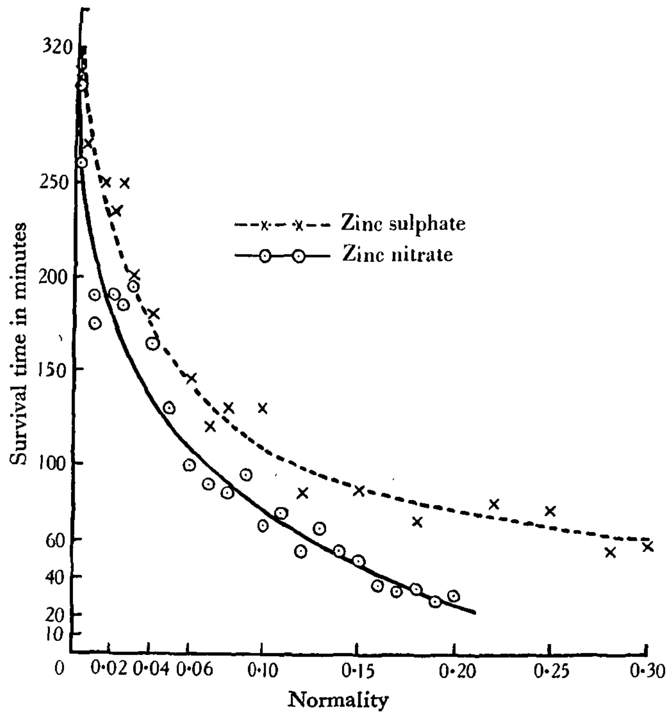

Fig. 9. Survival curves for *Gammarus pulex* in zinc nitrate and zinc sulphate.

Finally it may be noted that the relationship between toxicity and conductivity already referred to is reasonably well shown by the fatal immersion times. In Fig. 7 fatal immersion times for copper sulphate and copper nitrate are shown. Animals immersed for times indicated by the lower limits of the vertical lines recovered while those immersed for times indicated by the upper limits failed to do so. For example, *Polycelis* immersed 15 min. in 0·05 copper nitrate recovered, but 20 min. immersion resulted in eventual death.

Vague as these values are, they certainly reveal a much lower toxicity on the part of the sulphate. In Fig. 8 the same values are plotted against $\lambda/\lambda_0 N$ and here the two series of data coincide reasonably well, so that estimation of toxicity by this method also reveals the relationship between the conductivity and toxicity of salts.

C. EXPERIMENTS WITH *GAMMARUS PULEX* (L.)

Aquatic arthropoda that breathe atmospheric oxygen are extremely resistant to the toxic action of dissolved salts, and their survival times even at very high concentrations are very long and variable. Thus eight *Notonecta glauca* placed in a 0·10N solution of lead nitrate lived respectively $4\frac{1}{2}$, 8, 9, 10, $10\frac{1}{2}$, 11, 13 and 14 days.

Those that make use of oxygen dissolved in the water, for respiration, are more sensitive, probably because their integument is less impermeable. Of these forms

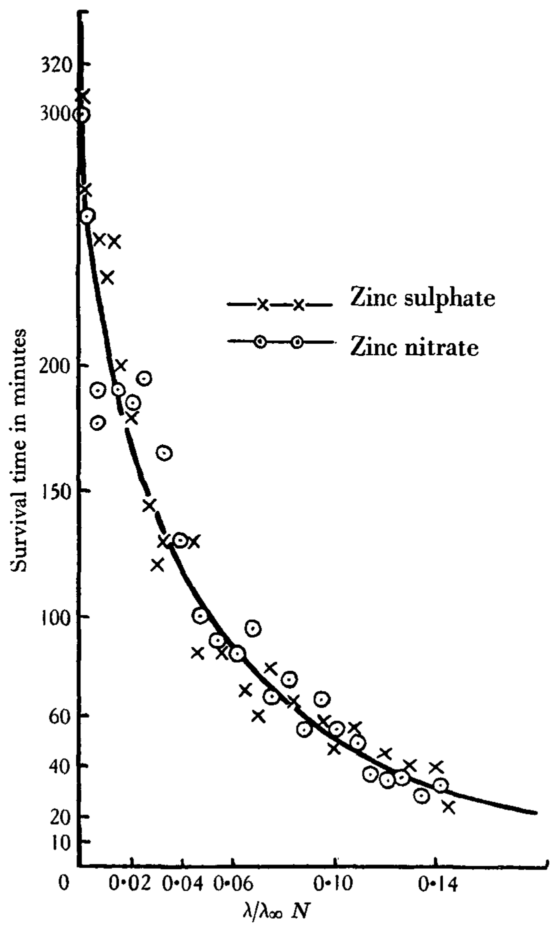

Fig. 10. Survival curves for *Gammarus* in zinc nitrate and zinc sulphate.

Gammarus pulex has been found to be the most suitable for the study of survival time curves, in sensitivity it is comparable with *Polycelis* and it is readily obtained in large numbers.

When placed in toxic salt solutions *Gammarus* swims continually for the greater part of the survival time; towards the end of the survival time swimming ceases but the pleopods continue to move irregularly. Cessation of this movement marks the death point.

Experiments with copper nitrate and copper sulphate resulted in an extraordinary survival curve. From concentration nil to $5 \times 10^{-4} N$ the survival time drops with extreme rapidity to 58 min. and then remains at approximately this value up to a concentration of $0 \cdot 15 N$. Above this concentration the survival time decreases very slowly. With such great variation of concentration making such little difference in the survival time it is not surprising to find that the curves for sulphate and nitrate are coincident whether plotted against normality or $\lambda/\lambda_0 N$.

With zinc salts the curves are normal in shape and here illustrate once more the relationship between conductivity and toxicity. Fig. 9 shows the divergence between the curves for zinc nitrate and zinc sulphate when plotted against normality. Fig. 10 shows the coincidence that results from plotting time against $\lambda/\lambda_0 N$. Each point in these curves is the mean of four observations. Thus the relationship holds for a third aquatic animal differing greatly in structure and physiology from *Polycelis* and *Gastrosteus*.

SUMMARY AND CONCLUSIONS

1. A brief review is given of previous work on the toxic effect of aqueous solutions of metallic salts on living protoplasm and fresh-water animals.

2. The behaviour of *Polycelis nigra* in aqueous solutions of metallic salts is described, survival curves for this animal in some variety of salts are given, and the mode of toxic action of these salts is discussed.

3. It is shown that in the case of heavy metal salts the toxic effect at concentrations below isotonicity is due almost entirely to the cation, the toxicity of the anion being relatively small. At concentrations above isotonicity the anion and the osmotic pressure of the solution act as additional lethal factors.

4. Salts of the metals of the alkalis and alkaline earths are shown to be comparatively harmless below isotonicity, with the exception of salts with a toxic anion such as potassium chromate. This salt is highly toxic at low concentrations.

5. It is shown that in the case of the nitrates and sulphates of the heavy metals, the toxicity is determined by the product of the normality and the electrical conductance ratio at that normality.

6. Recovery experiments with *Polycelis* are described and it is shown that these illustrate the above relationship between toxicity and conductivity.

7. Survival curves for *Gammarus pulex* in salts of copper and zinc are described. Those for zinc exhibit the same relationship.

The work was carried out partly at the Laboratory of Experimental Zoology, University of Cambridge, under the supervision of Dr J. Gray, and partly at the Department of Zoology, University College of Wales, Aberystwyth, under the Supervision of Prof. R. D. Laurie.

REFERENCES

CARPENTER, K. E. (1927). *Brit. J. exp. Biol.* **4**, No. 4.
—— (1930). *J. exp. Zool.* **7**, No. 4.
CLAYBERG, H. D. (1917). *Biol. Bull. Wood's Hole*, **32**.
GRAY, J. (1931). *Experimental Cytology*. Camb. Univ. Press.
HEILBRUNN, L. V. (1928). *The Colloid Chemistry of Protoplasm*. Berlin.
JONES, J. R. E. (1935). *J. exp. Biol.* **12**, No. 2.
LUDWIG, W. (1927). *Z. vergl. Physiol.* **6**.
OSTERHOUT, W. J. V. (1922). *Injury, Recovery, and Death in Relation to Conductivity and Permeability*. Philadelphia.
OSTWALD, W. (1905). *Univ. Calif. Publ. Physiol.* **2**.
PITTENGER, P. S. & VANDERKLEED, C. E. (1915). *J. Amer. pharm. Ass.* **80**.
POWERS, E. B. (1917). *Illinois biol. Monogr.* **4**, No. 2.
WARREN, E. (1900). *Quart. J. micr. Sci.* **43**.