THE SURVIVAL OF *ARTEMIA SALINA* (L.) IN VARIOUS MEDIA

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INTRODUCTION

The range of chemical and physical conditions under which the cells and tissues of Metazoa can function is limited. Yet some animals are capable of living in very odd and extreme environments.

Excellent examples of extreme environments are found in highly saline lakes and pools. These vary considerably in their total osmotic concentration, and in the nature of the solutes present. The ability of *Artemia salina* to live in such brines has long been known (for data on distribution see Daday, 1910; Abonyi, 1915; Stella, 1933; Kuenen, 1939; Barigozzi, 1946; etc.).

It is of interest to know the types of solutes that can be tolerated, particularly as claims have been made that *Artemia* can survive in several most unlikely media. For example, Boone & Baas Becking (1931) state apparently from hearsay: 'It has been known for a long time that *Artemia* is capable of living for days in solutions of potassium permanganate, potassium bichromate, and silver nitrate.' Such statements as these might be taken as evidence that *Artemia* is highly impermeable. A study of the survival of *Artemia* in various media may thus give information concerning the permeability of the animal and possibly also about the regulatory mechanisms.

Previous work on the survival of *Artemia* in various media has been done by Martin & Wilbur (1921) on the adults, and by Boone & Baas Becking (1931); Jacobi & Baas Becking (1933); Baas Becking, Karstens & Kanner (1936) on the hatching and survival of the nauplii. This previous work was mainly concerned with the effects of sodium, potassium, magnesium, and calcium ions, and, where this work overlaps with that presented below, the results are comparable. Corner & Sparrow (1956) have also studied the toxicity of copper and mercury compounds to *Artemia* larvae.

MATERIAL AND METHOD

The actual systematic position of *Artemia salina* is not clear. There appear to be numerous variable races. Daday (1910) regarded all the salt-water forms as belonging to one species *A. salina* (L.), but other authors have claimed that there are several distinct species. Cytogenetical work (e.g. Stella, 1933; Barigozzi, 1946; Goldschmidt, 1952) has shown a complex picture of different genetic races, and it is difficult to apply normal species criteria. In this and subsequent papers *Artemia* has been regarded as a single species, *Artemia salina* (L.).

Dried eggs obtained from the U.S.A. were used. The animals were reared in large aquaria containing gently aerated sea water. The temperature range of the aquaria was maintained to within about 19–24° C. A suspension made by grinding up dried yeast pellets in sea water was occasionally added as food. Self-reproducing cultures have been maintained in this way for several years.

The experiments were done on adults, which were about 10–12 mm. long, and at room temperature (18–19° C. usually). Batches of animals were transferred to various media, and the time until the animals had become moribund was observed. The animals were considered to be moribund when all limb movements had ceased or had become reduced to slow ineffective twitches. Considerable differences were seen between individuals and between experiments done at different times. The actual times quoted are therefore to be treated as giving only the approximate magnitude of survival time.

RESULTS

Sea water

Artemia survives indefinitely in sea water, which may be regarded as a balanced salt solution. After gradual acclimatization, numbers of individuals will survive indefinitely in various dilutions and concentrations of sea water, ranging from 10% sea water up to and including a saturated crystallizing sea-water brine. The majority of habitats where *Artemia* occurs probably have ionic ratios fairly similar to these sea-water brines. The absence of *Artemia* from the sea itself is presumably due to ecological rather than physiological factors.

Distilled water

The survival time of *Artemia* placed in glass distilled or tap water is relatively short, suggesting a fair degree of permeability. The swimming becomes very much slowed after about 12–18 hr., and most are dead or moribund by 24 hr.

Regarding distilled water as a neutral standard (i.e. non-toxic), we can say that those solutes that extend this survival time are favourable to the animal, and those that markedly reduce it are toxic.

Substances prolonging survival time

(1) *Sodium chloride. Artemia* adults survive for several days in pure NaCl solutions (0·5 M-NaCl, which is approximately isotonic with sea water, was used). This result would be expected as NaCl is the major constituent of most natural brines.

(2) *Sodium bromide.* Many individuals survived actively for well over 30 hr. in 0·5 M-NaBr solution.

(3) *Mannitol.* The survival time in a solution of mannitol approximately isotonic with the haemolymph was prolonged several hours over the distilled-water time. This is presumably due to the osmotic effect decreasing water flux through the animal, thus slowing loss of ions.

Substances with slight or slow toxicity

(1) *Certain sodium salts.* Sodium sulphate (0·25 M) allowed active survival for more than 12 hr., though animals were moribund or very slow by 24 hr. Sodium nitrate (M) allowed active survival for about 5–6 hr. Sodium benzene-sulphonate (0·5 M) allowed many to survive actively for about 8 hr.

Vinogradov (1953) states that *Artemia* is found in some Russian lakes where sulphates are present in high concentration, in some cases even forming a saturated solution of Na_2SO_4. As Na_2SO_4 by itself is scarcely toxic, there seems no reason why *Artemia* should not have adapted to these conditions, provided that chloride and other ions are also present.

(2) *Magnesium, calcium, lithium, and choline salts.* In a 0·25 M-magnesium chloride solution animals survived actively for 6–9 hr. In 0·25 M-calcium chloride toxic symptoms appeared a little faster. In 0·5 M-choline chloride the animals were moribund within 2–4 hr. In 0·5 M-lithium chloride the animals were moribund in 2–3 hr.

Rapidly toxic substances

These substances in concentrations similar to those mentioned above would cause death in minutes rather than in hours.

(1) *Dilute acids.* 5% nitric acid killed the animals in 2–3 min.

(2) *Sodium bicarbonate.* Very surprisingly in a M-$NaHCO_3$ solution there was loss of mobility and apparently death within 5 min. As the sodium ion is not toxic, it is probable that pH or narcotic effects due to the bicarbonate ion are responsible.

(3) *Metabolic inhibitors.* Animals placed in sea water plus 2% sodium azide were usually very moribund by 30 min. Animals placed in a 15% solution of ethyl urethane were very much slowed or moribund in 30–60 min.

(4) *Fixatives.* Alcoholic fixatives (e.g. Carnoy and alcoholic Bouin), absolute alcohol, and acetone are lethal within 1 min. Aqueous fixatives are less rapid. Death occurs within 10 min. in Susa and within about 15 min. in aqueous Bouin.

(5) *Silver salts.* Even dilute solutions of $AgNO_3$ were found to be very highly toxic. The animals were removed from sea water, rinsed in distilled water to remove adherent chloride, and then placed in dilute $AgNO_3$ solutions. In a 10 mM./l. solution the animals were moribund in about 5–10 min. Even in a 0·1 mM./l. solution most of the animals were moribund in 20 min. If both ends of the gut were ligatured with fine threads (made by teasing out bolting silk) to prevent swallowing, the response to $AgNO_3$ was not affected. There was no appreciable difference in the survival time as between 10 mM./l. $AgNO_3$, and M-$NaNO_3$ + 10 mM./l. $AgNO_3$ (Na:Ag ratio 100:1).

(6) *Potassium salts.* One of the most remarkable findings of earlier work was the high toxicity of potassium salts to *Artemia* (Martin & Wilbur, 1921; Boone & Baas Becking, 1931; Jacobi & Baas Becking, 1933). In the present work 0·5 M. solutions of potassium chloride, potassium nitrate, and potassium benzene-sulphonate have been used. In all these the animals become moribund within 30 min. A similar

rapid effect was seen when the animals were ligatured at head and anus to prevent swallowing.

A saturated solution of potassium permanganate kills *Artemia* within 15 min. But with this salt the permanganate ion would also be expected to have a toxic action.

Baas Becking and his associates (working on the hatching and survival of nauplii) claimed that the toxic effect of potassium ion (and also that of magnesium and calcium ions) could be antagonized by the presence of sodium ions. However, their experiments were done by mixing the chlorides of two and in some cases more cations in various proportions to give a constant total molarity, and repeating the experiment at a relatively small number of other total molarities. This makes it rather difficult to interpret their results, particularly as the antagonism ratios appeared to vary with changes in the total molarity.

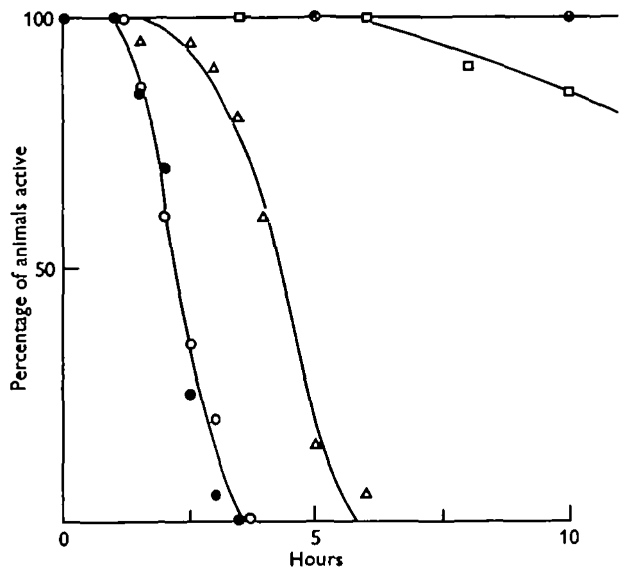

Fig. 1. The survival of *Artemia* in media with various Na:K ratios.

	Composition of media	
	K mM./l.	Na mM./l.
●	100	0
○	100	100
△	100	500
□	100	1000
⊗	100	1800

In the present work on adult *Artemia* a simpler procedure giving a clearer result was used. In these experiments the potassium concentration was not varied. A series of solutions was made up each containing 100 mM./l. KCl, and from 0 to 1800 mM./l. NaCl. Groups of adult animals were transferred from a sea-water culture to these various solutions. The survival time is presented graphically (Fig. 1). The results clearly indicate a competitive or antagonistic effect between sodium and potassium ions, and that for prolonged survival the Na:K ratio must equal or exceed 10.

The ecological consequences of the potassium sensitivity are probably important, and *Artemia* is absent from desert lakes rich in potassium (Boone & Baas Becking, 1931).

DISCUSSION

It is clear that, although *Artemia* can live over a very wide concentration range, it is by no means insensitive to the chemical composition of its environment, and this indicates a degree of permeability. In fact the only media in which *Artemia* can survive for long periods are solutions in which certain sodium salts predominate. Many substances are extremely toxic. These include substances to which *Artemia* has in the past often been regarded as resistant. The speed with which certain toxic substances act suggests that they are entering through, or acting on, a permeable part of the external surface. Further evidence for this, in the case of silver and potassium ions, was obtained in the experiments where the gut was isolated from the medium by ligatures.

However, it is clear that *Artemia* is much less sensitive to the chemical nature of its environment than many other animals, as judged by the concentration range over which the animal can survive or by the rates at which toxic symptoms appear. This is seen, for example, in the work of Corner & Sparrow (1956) who compare the toxicity of various copper and mercury compounds to *Artemia* and some other Crustacea.

In general the results indicate that *Artemia* must possess a fair degree of permeability, and therefore that the ability to survive in concentrated brines must be due to the presence of regulatory mechanisms that actively adjust the composition of the body fluid.

SUMMARY

1. The survival of *Artemia salina* adults in various media has been studied.

2. Prolonged survival is only possible in media in which certain sodium salts (principally NaCl) predominate.

3. Certain substances are found to be highly toxic.

4. It is confirmed that the high toxicity of potassium ions can be antagonized by sodium ions.

5. The results indicate that the animal is appreciably permeable.

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