

## THE EFFECT OF OSMOTIC AND ION-OSMOTIC STRESSES ON THE MORTALITY OF RAINBOW TROUT (*SALMO GAIIRDNERI*)

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**Abstract**—1. The possibility that costs of ionic and osmotic regulation are different in fish was assessed using mortality rates of rainbow trout exposed to an ionically active substance, sodium chloride and a non-electrolyte, mannitol, in the ambient water.

2. A 24-hr  $LC_{50}$  of 175 mOsm was calculated from data for fish exposed to sodium chloride concns of 400, 450, 500, 550, 600, 650 and 700 mOsm.

3. Similarly, a 24-hr  $LC_{50}$  of 767 mOsm was calculated from data for fish exposed to mannitol concns of 400, 500, 600 and 700 mOsm.

4. The relatively higher  $LC_{50}$  for fish exposed to increased ion and osmotic concns suggest the costs of ionic regulation may be significantly greater than those associated with osmotic regulation.

### INTRODUCTION

Discrepancies exist in the literature concerning the energy cost of ion-osmoregulation in fish. Some experimental data obtained for fish in various salinities indicate this cost to be a significant part of its total metabolic rate. Using data on oxygen consumptions some investigators have estimated the cost of ion-osmoregulation to be substantial, representing 20–26% of the total metabolic expenditure in fish (Rao, 1968; Farmer & Beamish, 1969). The rationale behind analyses on the basis of the measurement of oxygen consumption is that ion-osmoregulation involves active transport, which requires energy. The cost of regulating can be calculated by measuring rates of oxygen consumption over a range of external ion-osmotic concns and comparing these rates with that at blood iso-osmoticity. However, some investigators feel that this approach does not give reliable information about the energy cost of osmoregulation (Potts & Parry, 1964). Thermodynamic estimates support the conclusions that osmoregulation is an insignificant part of the animal's total metabolic rate and further assume the ionic costs to be similarly small. Potts (1954) calculated the minimum work required for ion-osmoregulation on the basis of an animal's permeability, its surface area, the respective concns of its blood, urine and external medium. The energy requirement for the freshwater crayfish (Potts, 1954) and for the eel (*Anguilla dieffenbachii*) (Shuttleworth & Freeman, 1974) were calculated as 0.3 and 0.1%, respectively, of the total metabolic rate. All these experiments have been performed using different salinities to vary osmotic pressure. It is possible that costs of ionic and osmotic regulation are different. Were this correct, then an ionic stress of a given osmolality should differ from an equivalent non-ionic osmolality

stress. Such stress differences might be identified through differences in mortality between osmotic and ionic solutions of the same osmolality.

Separation of the environmental ionic and osmotic components can be accomplished chemically. Both ionic and osmotic concns can be changed using a salt which ionizes in an aqueous solution. Whereas the osmotic concn alone can be changed by using some non-electrolyte as the solute in test solutions. However, no background data exist as to the feasibility of using a sugar compound as the osmotically active solute in osmotic studies of fish. In fact, very little work exists for any aquatic animal on manipulating the ionic and osmotic components independently. An exception is Venkataramiah's (1977) work on brown shrimp (*Penaeus aztecus*) in which animals were tested in various ion-free seawaters. He used specially blended artificial sea-salts which had one major cation excluded, for example, Ca-free mix, K-free mix, etc. Only one ion was missing at a time, the others being present in the normal ratio. The osmotic concn of each salinity medium was maintained the same as normal seawater by the addition of TRISMA-8.3 1Tris (hydroxymethyl) aminomethane buffered to a pH of 8.30.

The objective of the present study was to determine the feasibility of using mannitol as an osmotically active solute to separate the osmotic and ionic components of ion-osmotic regulation. This determination was based on mannitol's relative toxicity to sodium chloride and hence the qualitative differences between osmotic and ion-osmotic regulation in aquatic animal.

### MATERIALS AND METHODS

Hatchery-reared rainbow trout (*Salmo gairdneri*), total length 15–20 cm, were acclimated for 2 weeks and tested at a temperature of  $15 \pm 1^\circ\text{C}$ . After being fed twice daily to satiation they were fasted for one week prior to experimentation. Fish were exposed to several concns of sodium

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chloride and mannitol in 16-polypropylene tanks each holding 10 fish in 12 l. of water. Throughout the experiments the water or test solutions were replaced daily to prevent build-up of waste products. All fish were held in tanks in fresh water for 24 hr prior to the start of each trial. Seven sodium chloride concns were chosen from 400–700 mOsm at 50 mOsm intervals on the basis of preliminary observations (Kostecki, 1979). Mannitol concns were chosen to produce the same osmotic effects as the corresponding concns of sodium chloride. On the basis of preliminary observations, indicating low toxicity, the mannitol intervals were greater at 100 mOsm. A freshwater control group was included. Ten fish were used in the control and each concn of mannitol and sodium chloride.

The mannitol and sodium chloride concns were made up by the addition of the appropriate chemical to deionized water. A trace amount of calcium (1.5 mEq/l) was added to the sodium chloride and mannitol tanks equivalent to the freshwater control levels.

The number of deaths in each tank was recorded hourly for the first 6 hr, and thereafter the observation interval was gradually increased to every 6 hr by the third day. Death was defined as a complete absence of muscular activity. Cumulative mortality and times of death were plotted on probit paper to obtain the time to 50% mortality ( $TL_m$ ) (Sprague, 1969). These calculated  $TL_m$  values were then plotted as functions of concn to obtain dose–mortality curves.

### RESULTS

Dose–mortality curves are shown in Fig. 1 for the ionic and osmotic stressors. No deaths occurred in the control group. For comparative purposes, the 24-hr  $LC_{50}$  (lethal concn at which 50% of the animals die in 24 hr) were calculated by extrapolation from Fig. 1 as 767 and 175 mOsm for mannitol and sodium chloride, respectively. Fish were more sensitive to an ion-osmotic stress than to an equivalent pure osmotic stress. For example, at an osmolality of 400 mOsm no

deaths occurred in 60+ hr for fish exposed to mannitol, however, trout survived only 16 hr in the sodium chloride.

### DISCUSSION

In order to show the relative magnitude of the osmotic and ionic regulatory problems faced by fish, mannitol was used as a non-ionic, osmotic determinant contrasting with the use of salt solutions. This method assumes that mannitol does not readily pass through cell membranes or, if it does, that it does not interfere with the animal's normal metabolic activity. Mannitol has been used as a solute in media for botanical experiments and does not appear to enter living cells or be metabolized in plants that do not normally contain mannitol (Ferguson & Street, 1958; Thimann *et al.*, 1960; Groenewegen & Mills, 1960). Mannitol's place in animal work has been in functional kidney research. It has been used to measure the rate of glomerular filtration and the volume of extracellular fluid (Berger *et al.*, 1947; Windhager *et al.*, 1959; Wong *et al.*, 1979) and in hemodialysis (Raja *et al.*, 1976).

Work with the utilization of mannitol in animals has given conflicting results. Lafon (1937) noted that mannitol is only slightly utilized by mice, if at all. Dominguez *et al.* (1947) indicate that there may be some metabolism of mannitol in human beings. However, all the studies indicating metabolic utilization of mannitol are based on feeding experiments or the i.v. injection of mannitol. The movement of mannitol into animal cells appears to be unlikely. Windhager *et al.* (1959) notes that the movement of mannitol across the tubule of *Necturus* is slight. It therefore seems reasonable to assume that in the present study mannitol was not biologically active.

Rainbow trout are euryhaline teleosts which sur-

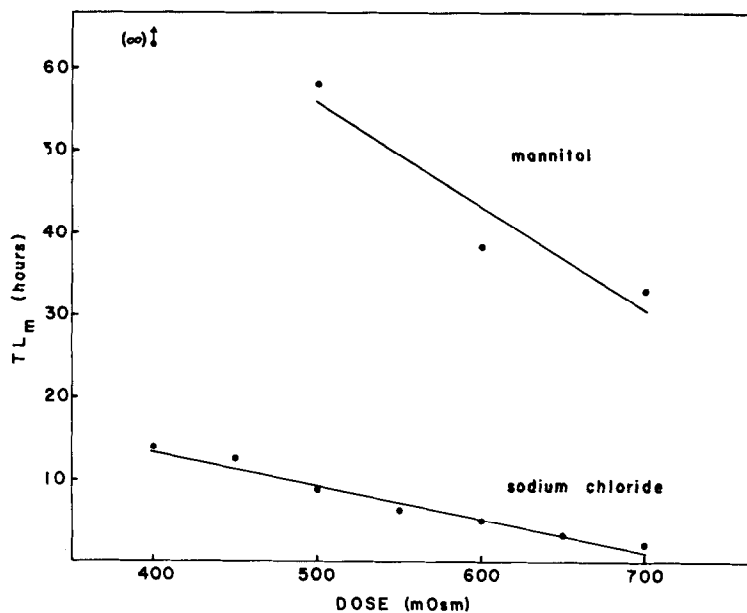


Fig. 1. Dose–mortality curves for rainbow trout exposed to various concns of mannitol and sodium chloride. Lines were fitted by eye.

vive well in both freshwater and saltwater, but are less tolerant of rapid transfer to high salinities ( $\geq 2/3$  seawater) (Bath & Eddy, 1979). These fish normally live in freshwater with a very low ionic concn. They have survived for more than two weeks in isosmotic mannitol (+1.5 mEq/l  $\text{Ca}^{2+}$ ) or sodium chloride (+1.5 mEq/l  $\text{Ca}^{2+}$ ) media (Kostecki, unpublished observation).

In the present study at higher concns, the fish began to die rapidly when exposed to the ionic-osmotic gradient (sodium chloride), but very much more slowly with respect to the osmotic gradient (mannitol). Mortality therefore appears to be due to the effects on ionic rather than osmotic regulation.

Ionic and osmotic regulation cannot be separated into two distinct sets of regulatory mechanisms. A teleost fish in hyperionic, hypoosmotic water (i.e. high salinity) gains ions but losses water. It regulated by drinking, taking up water and monovalent ions, and excretes the excess monovalent ions extrarenally. Some divalent ions are taken up by drinking and excreted renally. Therefore osmotic regulation is not possible without ion transport. Osmotic regulation deals with an osmotic problem that may necessitate ionic regulation. Ionic regulation imposes an osmotic problem on the animal which may necessitate osmotic regulation.

At hyperosmotic mannitol concns the fish presumably lose both water and ions, but the fish cannot regulate in the normal manner by drinking the medium as they do in the high salinity situation. There are relatively few monovalent ions available (only trace amounts of  $\text{Cl}^-$ ) for the transport of water across the gut. If the fish drinks in the hyperosmotic mannitol medium it is increasing its osmotic water loss problem. Perhaps these fish do not drink the medium but rely on changes in permeability and reduction in urine flow to deal with the osmotic change. That is, low water permeability and renal loss minimize the osmotic problem. With high salinities water permeability may decrease but the permeability for ions may be high. Then the ions would still enter more freely and impose a high cost on the fish for their excretion, in turn resulting in a higher death rate in sodium chloride compared to equivalent mannitol osmotic concns.

At any given osmolality more fish died in the sodium chloride than in the mannitol, indicating that the ion-osmotic stress was greater than the osmotic stress. These results may serve to explain the discrepancies existing between the data on oxygen consumption and the thermodynamic calculations. It is reasonable to expect that regulatory metabolic costs increase as the stress increases implying that ionic regulation is metabolically more important than osmotic regulation. Kostecki (1979) has shown that the energy costs of the osmotic component of ion-osmotic regulation is an undetectable fraction of the total metabolic rate in rainbow trout. Furthermore, oxygen consumption measured for osmotic regulation approximated the expected levels calculated from thermodynamic theory. Therefore, the thermodynamic calculations accurately indicate the cost of osmotic regulation, but probably not the ionic regulatory costs. Thus, ionic regulation may be responsible

for the metabolic increases found in the oxygen consumption experiments at different salinities.

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