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HERPETOLOGICAL JOURNAL, Vol. 2, pp. 18-23 (1992)

SODIUM CHLORIDE AND POTASSIUM CHLORIDE TOLERANCE OF DIFFERENT STAGES OF THE FROG, *MICROHYLA ORNATA*

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(Accepted 18.6.90)

ABSTRACT

Short term effects of different concentrations of NaCl and KCl on embryos and tadpoles of the frog *Microhyla ornata* were studied. Both NaCl and KCl caused significant reduction in swelling of the perivitelline space (PVS), an effect very similar to that reported for acidic pH. Tadpoles were observed to be somewhat more resistant to both NaCl as well as KCl, as compared to the embryos. KCl was found to be more toxic than NaCl. A typical teratogenic effect was observed in KCl treated embryos which showed swollen head coelom, whereas NaCl caused incomplete closure of the neural tube.

INTRODUCTION

Amphibian embryos may be exposed to different salinities during the period of their embryonic development. Later the tadpoles also have to face varying environmental conditions. The reasons for variation in salinity are many. Intermittent rainfall often leads to drying of temporary rain-water pools thereby increasing the salinity (Munsey, 1972). It is also likely that the breeding sites on the coastline may be affected by tidal inundation. Thus, the salinity of the medium is an important factor in the developmental ecology of amphibians.

Some work has been done regarding the effects of salinity on breeding and development of a few amphibians (Ely, 1944; Ruibal, 1959; Beebe, 1985). Considerable work has been done on salt tolerance of the embryos, tadpoles and adults of the Indonesian frog *Rana cancrivora* (Gordan et al., 1961; Gordan and Tucker, 1965; Dunson, 1977). This frog is known to tolerate high salt levels in the ambient medium. However, no information is available regarding any of the Indian species of frog. In this study we estimated both the NaCl and the KCl tolerance limits at different stages of development of the frog *Microhyla ornata*.

MATERIALS AND METHODS

Spawn was collected from natural ponds/temporary rain-water pools during the early morning hours (07.00-09.00hr), brought to the laboratory, manually "dejellied" using forceps, and exposed to the various concentrations of NaCl and KCl at late gastrula stage (Gosner stage 11/12, Gosner, 1960).

Eight day old (stage 24), and hind-limb stage (stage 39) tadpoles collected from the field were also exposed in a similar manner. The media were prepared by adding 10% NaCl or KCl stock solution to aged tap-water to obtain the desired concentration of NaCl or KCl. The physico-chemical parameters of the aged tap-water were: pH 7.5-7.8, total hardness <75 ppm (as CaCO₃), total alkalinity <60 ppm (as CaCO₃). The experiments were run at room temperature which varied between 23 and 27°C. The Na and K content of this tap-water were negligible. Ten embryos or tadpoles were picked up randomly and released in a 250 ml capacity glass bowl containing 100 or 200 ml of medium. Larger tadpoles were exposed in 3 l capacity glass troughs with 1 l of medium. At least 30 embryos or tadpoles were exposed to each concentration to study the effects of the two salts. Aged tap-water served as a control medium. This water was kept in a large polyester container for 15 to 30 days and aerated using an electrical aerator pump.

The embryos and tadpoles were observed every 24 hours for a total period of 96 hours from the time of exposure. Mortality data of the embryos and tadpoles was recorded. Morphometric measurements were taken as described earlier (Padhye and Ghate, 1988). Embryos were fixed in Bouin's fluid, embedded in BDH cerasin wax and 7 µm sections produced using routine techniques. Stains were haematoxylin and eosin. LC 50 values were calculated by Reed-Muench method (Woolf, 1968).

RESULTS

The control embryos showed normal development and differentiation of head, trunk and tail region at 24 hours. The embryonic perivitelline space (PVS) was swollen and the egg diameter with vitelline membrane (VM) increased by about three times to that of the egg at the late gastrula stage. At 48 hours all embryos hatched normally and were found attached to the inside of the vessel with the help of the suckers. At 96 hours the tadpoles were actively swimming and showed well developed head with a typical pigment pattern on the dorsal side.

TOXIC AND TERATOGENIC EFFECTS ON THE EMBRYOS

As far as the toxic effects were concerned the experimental embryos exposed to 0.2% NaCl did not show any mortality during 96 hours of exposure. In 0.3% NaCl some mortality occurred at 96 hours, however, at 0.4 and 0.5% NaCl mortality was observed at 72 hours. The embryos exposed to 0.6% NaCl died within 48 hours, while those in 0.7% NaCl died within a few hours showing total mortality in 24 hr.

Compared to NaCl, KCl appeared to be more toxic. Embryos exposed to 0.1% KCl showed slightly stunted growth while there was total mortality of the embryos within 96 hours at 0.2% and higher concentrations. At

0.4%, KCl was totally lethal within 72 hours while 0.5% KCl was lethal to all the exposed embryos within 48 hours. The LC 50 values at different periods of exposure for both NaCl and KCl are given in Table 1.

Hours of exposure	LC50 values	
	in % NaCl	in % KCl
24	0.6482 (0.6226-0.6748)	>0.5
48	0.5604 (0.5335-0.5887)	0.3548 (0.3233-0.3894)
72	0.4222 (0.3931-0.4535)	0.2732 (0.2497-0.2989)
96	0.2711 (0.2493-0.2947)	0.1414 (0.1312-0.1524)

TABLE 1. Toxicity of NaCl and KCl to late-gastrula stage embryos of *M. ornata*. Figures in parentheses indicate 95% confidence limits.

The prominent teratogenic effect, observed in the embryos treated with the higher concentration of NaCl was incomplete closure of the neural tube at 48 hours. At 0.5% some embryos (>50%) showed anteriorly open neural tube while all the embryos exposed to 0.6% NaCl showed almost completely open neural tube (Fig. 1). Histological observations revealed that in these cases the organisation of the neural cells within the neural tube was totally disturbed. The neural folds were formed, however, but they failed to close. These embryos did not survive. At lower concentrations the neural tube closed normally. A photographic record of the neural tube of the control and experimental embryos is presented in Figs. 2-6. No such effect was seen with KCl treatment.



Fig. 1 Control embryo (at the right) showing closed neural tube. Two curved embryos (NaCl treated) at the left showing incompletely closed neural tube (arrows).

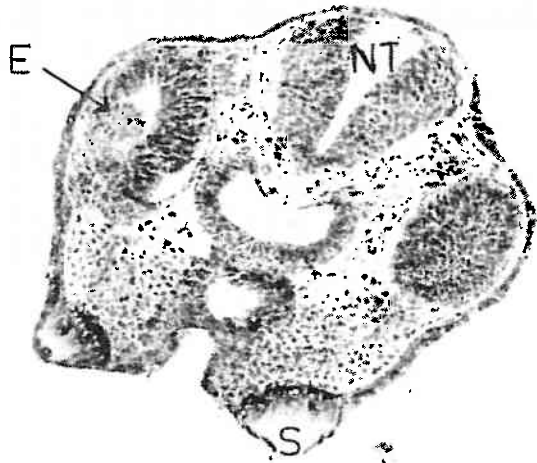


Fig. 2 T.S. of control embryo passing through the sucker region (S). See the well-developed neural tube (NT). Differentiation of eyes (E) has also started.

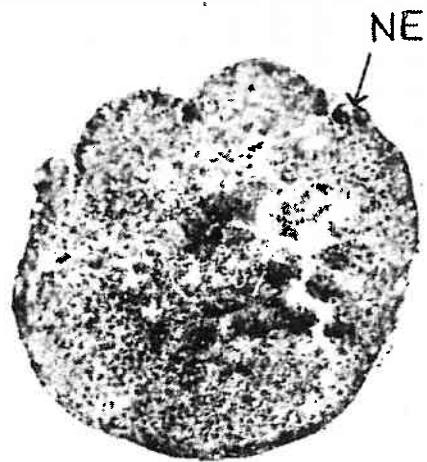


Fig. 5 T.S. of another NaCl treated embryo with abnormal neural tube. Note highly disorganised mass of embryonic cells. It is apparent that the non-neural ectoderm (NE) has failed to expand and enclose the neural cells.

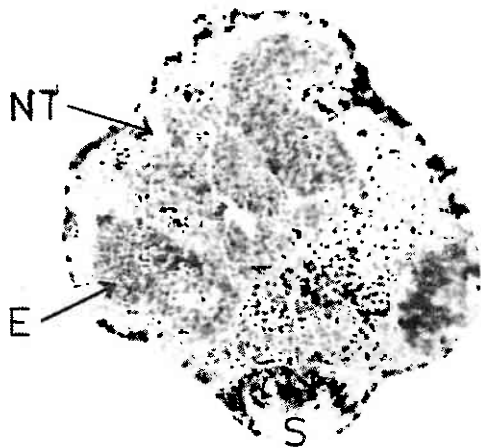


Fig. 3 T.S. of an experimental embryo (NaCl-treated) through the sucker region. Note abnormal formation of the neural tube (NT) and eyes (E).

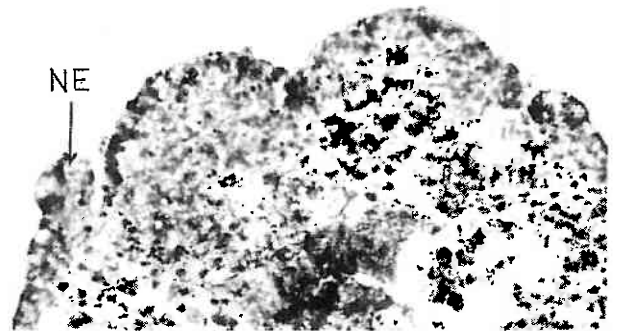


Fig. 6 Close-up of the above experimental embryo showing disorganised neural cells with no evidence of tube formation.



Fig. 4 Close-up of the neural tube showing its organisation in a control embryo.

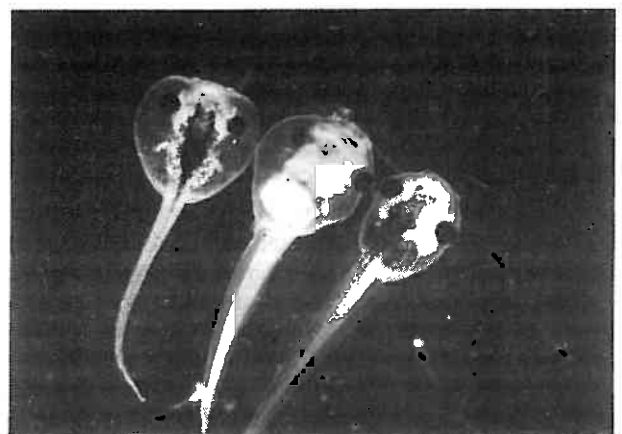


Fig. 7 Control (right) and experimental tadpoles at 96 hours. Note swollen head region in tadpoles due to continuous treatment of KCl from gastrula stage onwards.

The pigmentation in embryos was reduced gradually with the increase in the concentration of both NaCl and KCl, however, KCl was more potent in its effect in this regard. At the highest tolerated concentration of NaCl the embryos were almost pale white and the pigment was present only in eyes and suckers. However, at and beyond 0.2% KCl, even the eyes and suckers did not show much pigmentation in the surviving tadpoles.

The general development of the embryos was almost normal up to a concentration of 0.2% NaCl. At the higher concentrations the embryos showed distinct microcephaly, retarded growth as evident from total length and "swollen belly" due to reduced yolk absorption. KCl-treated embryos showed only slightly stunted growth up to 0.1% concentration. At 0.2% KCl there was retardation of growth and the entire head coelom of the tadpole was swollen (Fig. 7). This gave unusual buoyancy to the head region of the tadpole and the tadpoles then floated with their ventral side upwards. This effect was not observed in embryos treated with NaCl, at least during 96 hours of exposure.

Tail-fin erosion was a distinct feature of the tadpoles surviving after embryonic exposure to 0.3% NaCl and above. The reduced tail-fin had a wavy margin.

The experimental embryos, both KCl and NaCl-treated, showed prominent reduction in swelling of the PVS after 24 hr exposure as compared with the controls (Fig. 8). In 0.6% NaCl and 0.5% KCl the egg diameter with VM remained almost the same as that of the egg at the late gastrula stage. This caused curving of the body axis of the embryos within the intact VM, due largely to inadequate space. Even at lower concentrations both NaCl and KCl caused significant reduction in normal swelling of the PVS. After hatching such tadpoles showed curved body axis and abnormal swimming. At lower concentrations (up to 0.2%) these embryos showed only partial curvature of the body axis, the abnormality which was not apparent after hatching. No significant effect on hatching was observed, at least at lower concentrations, although hatching was invariably delayed by a few hours to one day.

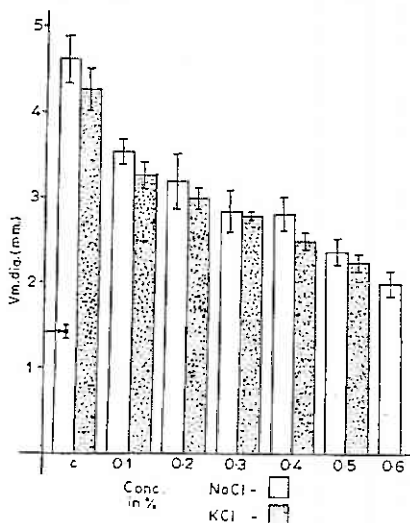


Fig. 8 Bar diagram showing diameter of PVS of the control and experimental embryos at 24 hours exposure to different concentrations of NaCl and KCl. The mark inside the control (C) bar, indicated by an arrow, shows the diameter at the beginning of the experiment. The values listed are mean and standard deviations of at least 20 observations.

TOXIC EFFECTS ON THE TADPOLES

Under similar treatment conditions 8 day old tadpoles tolerated up to 0.3% NaCl and did not show mortality in 96 hours. At a concentration of 0.4% and above, however, mortality was observed. The hind-limb as well as fore-limb stage tadpoles tolerated up to 0.6% NaCl without any mortality up to at least 48 hr.

KCl was found to be toxic at and above 0.2% to 8 day old tadpoles. Total mortality was observed at 0.3% KCl in 96 hours, while 0.4% KCl and above caused immediate mortality. Hind-limb as well as fore-limb stage tadpoles showed slight increase in the tolerance. They could tolerate 0.2% KCl without any mortality while 0.4% KCl was lethal in 96 hours. The 96 hour LC 50 values of these salts for different stages of the tadpoles are given in Table 2.

Stage	96 hour LC 50 values	
	in % NaCl	in % KCl
Late gastrula stage	0.2711 (0.2493-0.2947)	0.1414 (0.1312-0.1524)
8 day old tadpoles	0.5027 (0.4623-0.5466)	0.1593 (0.1397-0.1817)
Hind-limb stage tadpoles	0.6929 (0.6014-0.7985)	0.2539 (0.2325-0.2773)

TABLE 2. Comparative toxicity of NaCl and KCl to different developmental stages of *M. ornata*. Figures in parentheses indicate 95% confidence limits.

DISCUSSION

Experimental exposure of the embryos of *Microhyala* to NaCl and KCl solutions revealed that the potassium salt is more toxic than the sodium salt. It is possible that potassium ions interfere with a large number of biochemical reactions and hence are more toxic. There is no comparable data regarding the effects of KCl on amphibian embryos, however, it is known that KCl is more toxic than NaCl even to freshwater fish and molluscs (McKee and Wolf, 1963).

A very prominent effect of treatment of NaCl and KCl was prevention of swelling of the PVS of the embryo. It is known that the PVS, a space between the embryo proper and the VM, enlarges as development proceeds. This enlargement, or swelling, is due to slow flow of water into the PVS and it is necessary for normal development of the embryo (Krogh, 1939). The size of the PVS is said to be determined by osmotic gradients across the egg membrane (Holtfreter, 1943). It is possible therefore that the reduction in the PVS in the embryos exposed to salt solutions is related to changes in these osmotic gradients, especially because the degree of reduction in size is proportional to salt concentration (Fig. 8). Even the so-called hatching enzyme is involved in altering the properties of the VM, the properties that control inflow of water into the PVS, and the salt solutions may affect the enzyme activity thereby reducing the inflow of water. Even altered pH of the surrounding medium has the same effect on the PVS of the amphibian embryo, as has been discussed earlier (Padhye and Ghate, 1988). The curling of the body axis is due to reduction in the PVS, as the space is inadequate for growth of the embryo.

Another important effect noted was on the neurulation process. The embryos exposed to near lethal concentration of NaCl showed an open neural tube. KCl did not induce this defect. The only other published report regarding effect of salt solution on the neurulation process in frogs is that of Ruibal (1959). In the case of *Rana pipiens*, Ruibal (1959) observed that the neural tube failed to close when the embryos were exposed to 0.5-0.6% salinity.

The process of neurulation is an extremely complex event in early development. The normal process of neurulation requires certain changes in the neural and non-neural ectodermal cells, especially changes in the volume and shape of certain cells (Karfunkel, 1974). It is also known that non-neural ectodermal cells (epidermis) help in raising and closing the neural folds during neurulation (Schroeder, 1970; Brun and Garson, 1983). It is likely that NaCl inhibited one of these processes because the cells involved did not undergo the required changes or that the cells were killed due to lethal salt concentration. A change in the behaviour of the cells such as cell-to-cell adhesion may also affect the neural tube formation. It is interesting to note here that concentrations greater than 90 mM NaCl (approximately 0.53%) and 50 mM KCl (approximately 0.37% KCl), were shown to inhibit cell adhesion in dissociated amphibian gastrula (presumptive ectodermal) cells while lower concentrations actually promoted cell adhesion (Komazaki, 1989). This shows that ions in the external medium can affect cell adhesion. Why potassium did not have a similar effect on the formation of the neural tube is inexplicable at present.

Other effects of the treatment of salt solutions on the developing embryos, such as retardation of growth, reduction of pigmentation, microcephaly etc., were similar to those described by Ruibal (1959). These effects suggest that both salts severely interfere with the biochemical events of early development. Yolk absorption seems to be affected, leading to the formation of the embryos with "swollen belly".

The embryos that hatched as tadpoles in 0.2% and 0.3% KCl showed a swollen head region. This deformity indicates that osmoregulation in these tadpoles is affected. Again no such effect was observed with NaCl. There are no published reports of the effects of KCl on early development of frog embryos, however, mercuric chloride produces identical effects in *M. ornata* (Ghate and Mulherkar, 1980). Mercury is believed to be causing this defect through inhibition of enzymes like ATPase which are involved in osmoregulation. It is possible that K ions also inhibit ATPase, especially Na-K dependent ATPase that are known to occur in membranes.

As far as the exposure of the tadpoles to different salt concentrations was concerned, the tadpoles were found to be slightly more tolerant to low salt concentration. Similar observations were made by Beebee (1985) in the case of *Bufo calamita* embryos and tadpoles exposed to saline media. At this stage the tadpoles possess functional kidneys and skin, the organs that are involved in salt and water regulation. Such organs are not available to the developing embryos and hence the embryos are probably more sensitive to salt solutions than the tadpoles.

What the foregoing discussion points out is that the embryos of *Microhyla* are sensitive to NaCl and KCl solutions and that increase in the salt concentration of the water bodies in which *Microhyla* breed may affect successful reproduction of this frog. The results also point out that K and Na ions are quite different in their action, some of the effects produced being very specific for a given ion. Normally Na and K concentration of temporary rain water pools (the favoured site for breeding in this frog) is below 5 to 10 ppm and desiccation may not always raise it to alarming levels. Tidal inundation of the breeding ponds is, however, possible at several places over the range of the species.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. S.N. Navalgundkar, Principal, Modern College, for encouragement and facilities. Thanks are due to Prof. M.R. Marathe, Head, Physics Department, for making available the word processing facility and to Mr. S. Kashikar for helping the authors during the final preparation of the manuscript. Mr. Narendra Naidu, who helped during preparation of the photographs also deserves thanks. We also gratefully acknowledge Prof. R. Ruibal for reading the preliminary draft and his suggestions. A.D.P. thanks U.G.C. New Delhi for awarding JRF.

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HERPETOLOGICAL JOURNAL, Vol. 2, pp. 23-26 (1992)

PLASMA CONCENTRATIONS OF ALDOSTERONE AND ELECTROLYTES IN *GALLOTIA GALLOTI* (SAURIA: LACERTIDAE)

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(Accepted 29.7.90)

ABSTRACT

Plasma concentrations of aldosterone, sodium and potassium were measured in the lizard, *Gallotia galloti*. Aldosterone concentrations in control animals were 29.48 ± 8.65 ng/dl, which falls within the range reported for this hormone in mammals. Peripheral sodium and potassium concentrations were 132.81 ± 2.28 and 5.77 ± 0.32 meq/l, respectively. Plasma aldosterone and sodium were negatively correlated. A positive relationship could be established between potassium concentration and aldosterone levels. Acute or chronic administration of exogenous aldosterone increased the circulating levels of this hormone, being maximal in chronically treated animals. However, although plasma concentrations of aldosterone were augmented by acute administration, the sodium concentration in the plasma was only elevated by chronic treatment. No further changes to potassium concentration could be observed under primary hyperaldosteronism conditions. The extent to which aldosterone may be implicated in the regulation of sodium and potassium transport in reptiles and its possible action on postrenal structures of electrolyte transport are discussed.

INTRODUCTION

Adrenal corticosteroids have been implicated in the control of osmoregulation in reptiles (Bentley, 1976; Bradshaw, 1975; Callard & Callard, 1978), but their precise modes and loci of action await elucidation. Aldosterone has been isolated from reptilian adrenal tissue after *in vitro* incubation of this tissue, and it is also now known that this steroid is secreted into the plasma (Sandor, 1972; Vinson, Whitehouse, Goddard & Sibley, 1979). To date, only a few indications of the plasma level of this steroid hormone are available. Bradshaw & Grenot (1976) found that in a North African terrestrial agamid, *Uromastix acanthinurus*, and in the large omnivorous skink from Western Australia, *Tiliqua rugosa*, plasma levels were 36.04 and 31.74 ng/dl, respectively. However, Nothstine, Davis & DeRoos (1971) have reported a higher concentration of 760 ng/dl plasma in post-caval plasma from the caiman. The physiological significance of aldosterone in reptiles is not at all clear. Early studies in lizards, such as *Amphibolorus ornatus* and *Dipsosaurus dorsalis*, in which hypophysectomy and dexamethasone treatment were associated to an increased tubular reabsorption of sodium ions (Bradshaw, 1972; Bradshaw, Shoemaker and Nagy, 1972), are difficult to interpret because these treatments would also be expected to decrease plasma

aldosterone (Bradshaw, 1978). However, Rice, Bradshaw and Prendergast (1982) found that in adrenalectomized *Varanus gouldii* sodium and glucose concentrations fell after the operation, and potassium concentrations rose, as has been observed in mammals. The fall in aldosterone concentrations in *Varanus gouldii* as a result of salt-loading is associated with a marked decrease in fractional reabsorption of sodium and chloride ions by the kidney, and an increase in the rate of potassium secretion, suggesting an obvious mineralocorticoid effect of aldosterone. In an effort to elucidate the physiological role of aldosterone in reptiles, peripheral plasma concentrations were measured in relation to electrolyte concentrations under several treatments in an omnivorous lacertid endemic to the Canary Islands, *Gallotia galloti*.

MATERIAL AND METHODS

ANIMALS

A total of 18 adult male and female *Gallotia galloti* lizards were trapped between May and September 1989 in the zone of Tegueste (Tenerife, Canary Islands, Spain). Lizards were transported to the laboratory and acclimatized in a large indoor terrarium. Mean body weight of experimental animals was 39.34 ± 1.23 g. Food and water were provided *ad libitum*.