

thus risk extinction, or secondly, the population may stabilise at a new, lower level. Only long term field work would provide the answer.

The benefits afforded by terrestrial vegetation along the shores of a pond are of two types. The first, direct benefits, include the utilisation of the habitat as a refuge during the winter. This was certainly the case for Cleaver Pond in the winter of 1984, when the newts (as well as common toads, *Bufo bufo*) were known to have used rabbit burrows in the vegetation as hibernaculae (Verrell, 1985). These burrows were destroyed in the autumn of 1985. The second type of benefit can be termed 'indirect', and includes protection from human interference. Aside from the dumping of domestic and garden refuse, the most common form of interference in Conniburrow is the collection of breeding amphibians by children (the 'small child effect'). It seems unlikely that this effect was more severe for Cleaver Pond than for Marigold Pond in 1986, and thus unlikely that differential interference was the major factor responsible for the sharp decline in smooth newt population size seen at the former site.

In summary, the activities of a local government provided an unfortunate opportunity to assess the importance of the terrestrial habitat surrounding a pond for the smooth newt population breeding and feeding there. Destruction of this habitat resulted in a sharp reduction in the size of the breeding population, probably due to the removal of overwintering refuges. Improved accessibility to the pond, and a concomitant increase in the intensity of the 'small child

effect', probably exacerbated this. It is thus clear that there can be a serious conflict over the interests of those committed to amphibian conservation and those who wish to make ponds safe and accessible public amenities. In such conflicts, it seems probable that the amphibians will be dealt the worst hand.

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### ENZYME (ALDOLASE) ACTIVITY IN HYPEROSMOTIC MEDIA (NaCl AND UREA) IN THE TERRESTRIAL TOAD, *BUFO VIRIDIS* AND FROG *RANA RIDIBUNDA*

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#### ABSTRACT

In this study we examined the adaptation of the enzyme Aldolase from frogs and toads to different temperatures and to hyperosmotic media of 400-1000mOsm/Kg of urea or NaCl. Maximum enzyme activity was found between 200-400mOsm/Kg NaCl, both in enzymes from green toads and from marsh frogs. However, above 500mOsm/Kg, the activity of enzymes from green toads was significantly higher than the activity of enzymes from marsh frogs.

The activity of aldolase from green toads decreased very slowly as the media concentration of urea increased. However, the activity of aldolase from marsh frogs decreased rapidly under the same conditions.

The maximum activity of aldolase from both frogs and toads was at 25°C. The activity of aldolase from green toads was significantly higher than the activity of aldolase from marsh frogs when measured only at high temperatures (35°C). The results of this study support the idea that the biochemical systems of terrestrial amphibia are tolerant to hyperosmotic media.

## INTRODUCTION

The green toad is terrestrial and lives in a wide range of habitats reaching the southern limit of its distribution in Israel, where it penetrates into the desert (Warburg, 1972). Katz (1973) succeeded in adapting green toads to 800mOsm/Kg NaCl and Degani (1985b) adapted the green toad to 800mOsm/Kg urea. Degani *et al.* (1984) found that after three months in the soil, blood plasma concentration reached a maximum of 1400mOsm/Kg with urea concentration at 900mM. Intracellular urea concentration in the muscle of these toads amounted to 500mM urea. The plasma osmolality of *R. ridibunda* did not reach 600mOsm/Kg in any of these conditions (Katz, 1973; Degani, 1985a). *B. viridis* appears to show an ability to tolerate a wide variety of osmotic plasma concentrations, which help it to adapt to a wide variety of osmotic environments.

Urea accumulation in the intra — and — extracellular fluids appear to play a critical role in adaptation of amphibians to terrestrial life (Bentley, 1966; McClanahan, 1967 and 1972; Johns, 1980; 1982; Degani *et al.*, 1981; 1984). Data presented by McClanahan (1972); and Jones (1982) suggested that the rate of urea synthesis increases in *Xenopus laevis*, *Scaphiopus couchi*, *Bufo woodhousei* and *Hyla cadaverina*, when the water potential of the environment decreases. During dehydration, the plasma, urine and intracellular body fluid osmolalities of these amphibians were elevated. This was caused not only by increased urea but also by increased concentrations of  $\text{Na}^+$ ,  $\text{Cl}^-$  and  $\text{K}^+$  (Degani and Warburg, 1984). However, there is no information on the adaptation of enzymes to such high concentrations of urea and NaCl.

The hypothesis examined in this study is whether the enzymes of terrestrial amphibia are more tolerant to high temperatures, and to urea and NaCl concentrations, than aquatic species. In the present study the activity of Aldolase was compared in the green toad (*Bufo viridis*) and the semi-aquatic marsh frog (*Rana ridibunda*).

## MATERIAL AND METHODS

Green toads (*B. viridis*) and marsh frogs (*R. ridibunda*) were collected from the same locality in Northern Israel (Naharia) and were kept in the laboratory in a terrarium containing soil and small vessels of water, at a temperature of 20-23°C.

Samples of muscle were taken (200mg) from the hind-legs of green toads and marsh frogs and the fructose — 1,6 Diphosphate Aldolase activity was determined after homogenization (polytron homogenizer, model Kinematic CH-6010 Krins-LU-Switzerland) in 1ml TEM buffer phosphate pH 7.8.

The assay of activity was performed as described by Gracy *et al.* (1970). Aldolase activity was measured after the enzymes were acclimated for 20 minutes in different solutions of NaCl (from 100 to 1000mOsm/Kg) or urea (from 400 to 1000mOsm/Kg). Enzyme activity was also measured at different temperatures (15°C, 20°C, 25°C, 30° and 35°C) in a

solution of 300mOsm/Kg. Aldolase activity was measured by monitoring the rate of oxidation of NADH at 340nm on a Gilford Spectrophotometer at 25°C. One unit of enzyme activity is defined as 1μmole of substrate (FDP) that is cleaved in one minute at 25°C. Aldolase activity was calculated to mU by the equation:  $100D = 0.0806 \mu\text{mole/ml/min.} = 0.0806 \text{ unit of activity (U)}$ .

## RESULTS

The activity (%) of aldolase during acclimation to NaCl solution up to 1000mOsm/Kg is shown in Fig. 1. Maximum enzyme activity was found between 200-400mOsm/Kg NaCl, both in enzymes from green toads and from marsh frogs. However, above 500mOsm/Kg NaCl, the activity of enzyme from green toads was significantly higher than the enzyme activity of marsh frogs.

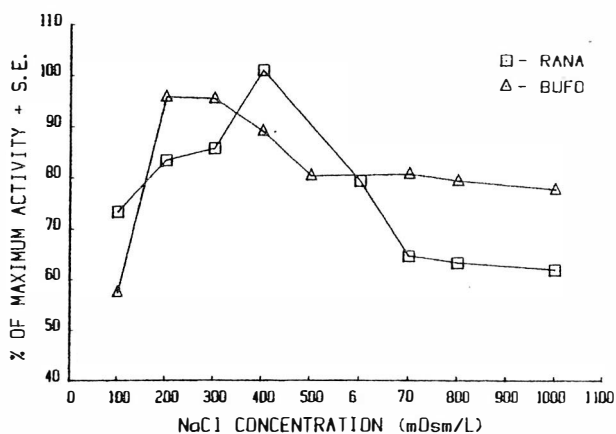


Fig. 1 Comparison between enzyme activity of *Bufo viridis* and *Rana ridibunda* in different NaCl osmotic pressures.

There were no significant differences found between the aldolase activity from green toads or marsh frogs acclimated to 400mOsm/Kg (200mOsm/Kg NaCl and 200mOsm/Kg urea) (Fig. 2). In media of osmolality above 400mOsm/Kg increase due to additional urea caused the activity of aldolase from *B. viridis* to be higher than that of the enzyme from *R. ridibunda*. The activity of aldolase from marsh frogs decreased rapidly after acclimatisation to a high urea concentration, and the activity of aldolase from green toads decreased slowly (Fig. 2). Both enzymes from green toads and from marsh frogs decreased with a linear correlation to increasing media urea concentration (Table 1). The slope of the linear equation for green toads is smaller than the slope of the linear equation for marsh frogs.

The maximum activity of aldolase from both green toads and marsh frogs was found at 25°C (Fig. 3). There was no significant difference between the activity of aldolase from green toads and marsh frogs at temperatures of 15-30°C. However, there is a significant difference only at a temperature of 35°C ( $P < 0.05$ ; t-test). At this temperature the activity of aldolase from green toads was higher than the activity of aldolase from marsh frogs.

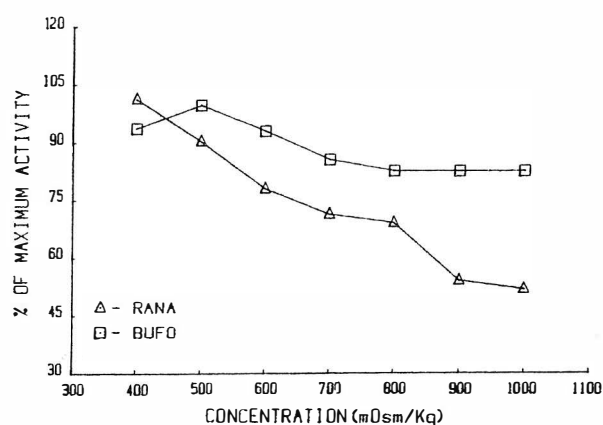


Fig. 2 Enzyme activity from *Bufo viridis* and *Rana ridibunda* in different urea solutions.

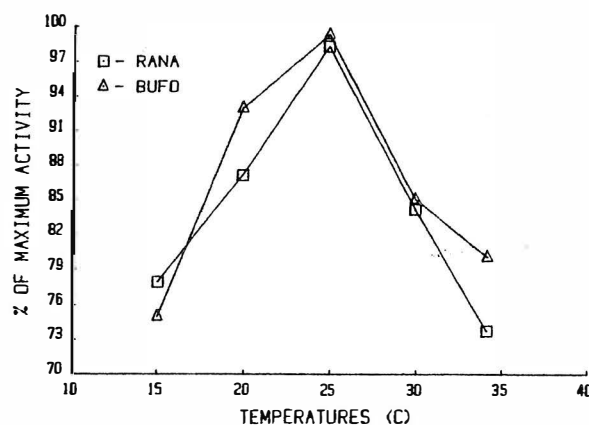


Fig. 3 Temperature effect on enzyme activity from *Bufo viridis* and *Rana ridibunda*.

| <i>Rana</i>           |      |    |        | <i>Bufo</i>           |     |    |       |
|-----------------------|------|----|--------|-----------------------|-----|----|-------|
| Equation              | r    | N  | P      | Equation              | r   | N  | P     |
| NaCl                  |      |    |        | NaCl                  |     |    |       |
| $y = -0.8099x + 1345$ | 0.95 | 11 | <0.001 | $y = -0.5430x + 1259$ | .87 | 13 | <0.05 |
| Urea                  |      |    |        | Urea                  |     |    |       |
| $y = -1.7109x + 2170$ | .91  | 22 | <0.001 | $y = -0.3946x + 1523$ | .71 | 12 | <0.01 |

TABLE 1: Enzyme activity equations of *Bufo viridis* and *Rana ridibunda* acclimated to different solutions according to the most significant correlation.

Notations:

Y = Enzyme activity (mu) and X = osmolality (mOsm/kg).

There are significant differences ( $P < 0.05$ ) between the slopes of both pairs *Rana* : *Bufo* in NaCl, *Rana* : *Bufo* in Urea.

## DISCUSSION

The osmotic concentration recorded in the body fluid of green toads following prolonged dehydration in the soil is the highest reported so far for any terrestrial amphibian (Degani *et al.*, 1984). A similar high osmotic concentration was recorded in the terrestrial spadefoot toad (*Scaphiopus couchi*) (Shoemaker, 1969; McClanahan, 1972) from the Arizona desert. Both *Bufo* and *Scaphiopus* store urea in their body fluids and this allows them to raise the bodily osmotic pressure more efficiently than aquatic species (Balinsky, 1981; Degani *et al.*, 1984).

There are some physiological mechanisms described which increased the plasma osmolality by increasing the plasma  $\text{Na}^+$ ,  $\text{Cl}^-$  and urea during dehydration or saline adaptation. For example by reducing the glomerular filtration rate of the nephron and increasing the influx of  $\text{Na}^+$ ,  $\text{Cl}^-$  through the nephron, bladder and the skin (Jorgensen, 1950; Bentley, 1958; Maetz, 1968). Accumulation of urea occurs slowly and may be due to both urea retention and, possibly to increased synthesis due to a higher concentration of precursors in the urea cycle (Jones, 1980; Balinsky, 1981; Lee *et al.*, 1982).

In this study we found that aldolase activity is tolerant to a high urea concentration only in terrestrial

green toads but not in semi-aquatic marsh frogs. These results might explain the results of previous studies in our laboratory. The accumulation of urea in the plasma of green toad is accompanied by an intracellular space both *in vivo* and *in vitro* (Degani *et al.*, 1984; Degani, 1985a,b). In the green toad from soil the intracellular urea is less concentrated than that in the plasma. (Degani *et al.*, 1984). The urea concentration in the plasma of the green toad was significantly higher than that in the plasma of the marsh frog, both adapted to a hyperosmotic (urea) environment (Degani, 1985a). Crab-eating frogs (*Rana cancrivora*), the only anuran that lives in sea-water had a maximal urea concentration in the plasma of 350mM (Gordan *et al.*, 1961) and this is similar to the urea concentration (265mM) found in the plasma of green toads adapted to sea-water (Katz, 1973). The muscle of the crab-eating frog is not only tolerant to urea, but actually requires it. Thesleff and Schmidt-Nielsen (1962) were unable to observe contractions unless urea was present in the medium, and they routinely added 250mOsm urea to the medium bathing the muscle.

Muscle tissue of green toads seems more tolerant to high urea concentration than the muscle of marsh frogs (Degani, 1985a). In conclusion while these previous studies show that different physiological mechanisms are tolerant to high urea concentrations,

this study shows that in terrestrial amphibians there might also be enzymes which are able to adapt activity to these conditions (Delsone and Whitford, 1973; Licht *et al.*, 1975; Degani, 1985a).

Rapid dehydration or adaptation to salinity causes an elevation of  $\text{Na}^+$  and  $\text{Cl}^-$  in the tissues (Degani and Warburg, 1984) so that the enzymes are exposed to high  $\text{Na}^+$  and  $\text{Cl}^-$  concentrations (Degani, 1985a). In this we found that an enzyme (aldolase) of green toads tolerates a higher concentration of  $\text{Na}^+$  and  $\text{Cl}^-$  than does the same enzyme from marsh frogs and this is maybe a partial explanation of the greater tolerance both to dehydration and to saline environments of the toad compared to the frog.

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