Comparative Biochemistry and Physiology

ADAPTATION OF THE TIGER SALAMANDER, *AMBYSTOMA TIGRINUM*, TO ARID HABITATS

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Abstract—1. During long-term dehydration, the tiger salamander, *Ambystoma tigrinum*, is able to tolerate high plasma fluid concentration (> 550 mOsmole/l.).

2. An increase in plasma solute concentration followed by the production and accumulation of urea accounted for increased plasma concentration during dehydration.

3. The "benevolent bladder" hypothesis proposes that differential reabsorption of water and salts regulates plasma concentration during long-term dehydration.

INTRODUCTION

THE ADAPTATIONS of amphibia which allow survival in arid habitats have been considered recently (Bentley, 1966a; Ruibal *et al.*, 1969; Shoemaker *et al.*, 1969; McClanahan, 1972). All these studies dealt with anurans. The few studies in which adaptations of terrestrial salamanders to their habitat were considered, dealt primarily with lungless salamanders, Plethodontidae, from moist habitats (Heatwole & Lim, 1961; Spight, 1967; Spotila, 1972). Studies of this type prompted Bentley (1966) to make the generalization that "Urodeles do not live in conditions as arid as those experienced by many anurans".

The tiger salamander, *Ambystoma tigrinum*, inhabitating the Chihuahuan desert of southern New Mexico (Webb & Roueche, 1971) is certainly an exception to Bentley's statement. During the terrestrial phase of its life cycle, *A. tigrinum* is able to survive in the same stressful conditions encountered by desert anurans.

The present study was conducted to investigate the physiological adaptations of *Ambystoma tigrinum* to arid habitats.

MATERIALS AND METHODS

The salamanders used in this study were collected as larvae from an ephemeral cattle tank, 30 miles northwest of Las Cruces, Dona Ana County, New Mexico. Individuals of various sizes transformed rapidly in a laboratory holding tank at 20 ± 1°C. Only fully transformed individuals were used in this study.

Salamanders used in the plasma osmolarity studies were divided into four groups. In the control group, total plasma osmolarity and plasma urea concentrations were determined immediately upon removal from the holding tank. The remaining groups were placed in a large wooden box containing soil of 8% water by weight (see Walker & Whitford, 1970, for

description of soil). The box was partitioned into three sections, and each section had one artificial subterranean chamber with a maximum depth of 40 cm which was connected to the surface by a burrow. Salamanders placed in the box readily entered the artificial burrow and proceeded to dig further into the walls of the burrow forming their own chambers. The first group was removed from the soil after 1 month, the second after 3 months and the final group after 9 months. The soil box was kept at room temperature, $20 \pm 5°C$, and natural photoperiod during the course of the study. All salamanders were weighed to the nearest 0·1 g immediately before being placed in soil and after removal from soil. The soil, which was allowed to dry during the course of the experiment, was sampled from a section as the salamanders were removed. Moisture content of the soil at each sample period was determined gravimetrically.

A blood sample was collected from each salamander in the following manner. A sodium heparin solution (0·2 ml/salamander) was injected into the abdominal vein. After a 30-min incubation period at 4°C, a small abdominal incision was made and blood was collected in capillary pipets. The blood was immediately transferred to plastic microcentrifuge tubes and centrifuged for 1–2 min. The plasma was then transferred to small sample osmometer tubes and total plasma osmolarity was determined using an Advanced Instruments Model 3L Freezing Point Depression Osmometer. The concentration of plasma urea was then determined using a Biochemica Test combination kit for urea.

RESULTS

Plasma urea concentration in *A. tigrinum* increased significantly $(P < 0.05)$ during the course of the study (Fig. 1). There was a significant increase in total
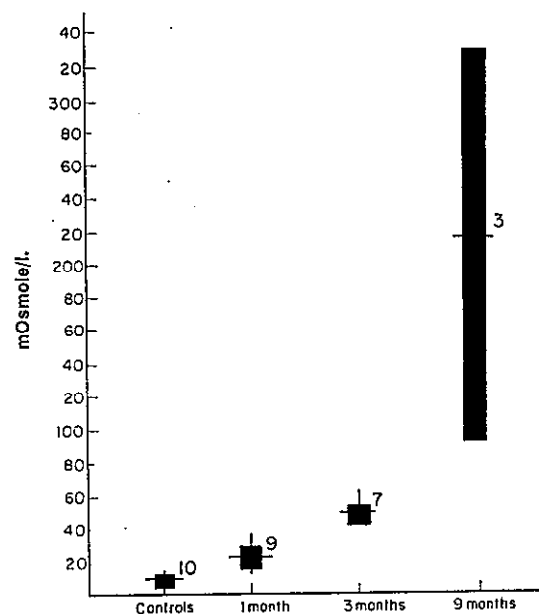

FIG. 1. The alteration of urea concentration (mOsmole/l.) of *A. tigrinum* during long-term dehydration in the soil. The vertical line is the range; the horizontal line the mean. Solid bars on each side of the mean are 95 per cent confidence intervals. The number adjacent to the mean is the sample size.

plasma concentration of salamanders in the soil compared to controls (Fig. 2). There was no significant increase in total plasma concentration between salamanders removed at 1 and 3 months. In salamanders removed after 9 months in the soil, total plasma concentration was significantly higher than the 1 or 3 month animals (Fig. 2).
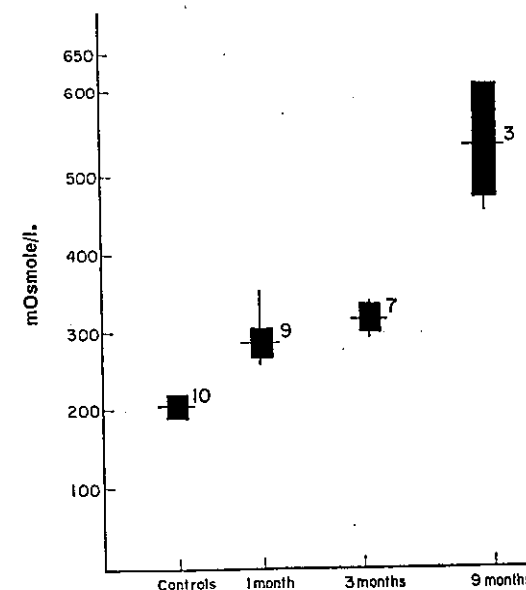
FIG. 2. The alteration of total plasma osmolarity (mOsmolc/l.) (excluding urea) of *A. tigrinum* during long-term dehydration in the soil. Method of presentation is the same as Fig. 1.

The equation (Shoemaker, 1964): $C = C_0 V_0 / V_0 - D$ (where C is the final concentration of solute; C_0, initial concentration of solute; V_0, water content in ml/100 g of fully hydrated animal and D, water loss in ml/100 g) was used to determine if the increase of urea concentration and total plasma osmolarity were attributable to water loss and subsequent concentration of solutes in the salamander. This equation is based on two assumptions (1) the total amount of solute in a body compartment remains constant during dehydration and (2) water in a compartment acts as a solvent. If fluid is removed from the bladder prior to dehydration (standard weight), the concentration of body fluids begins as water is lost by the animal. If bladder fluids are not removed prior to dehydration, concentration of body fluid is delayed. When bladder reserves of water are exhausted concentration of body fluid begins (McClanahan, 1967; Shoemaker, 1969). Bladder fluid was not removed in the present study.

Experimental results were very different from those predicted by Shoemaker's equation (Figs. 3 and 4). The expected delay in body fluid concentration was not

FIG. 3. Values estimated for total plasma osmolarity of *A. tigrinum* from observed data (solid line) and values calculated by Shoemaker's equation (dashed line). C, Final concentration (mOsmole/l.); D, water deficit (ml H_2O loss/100 g).
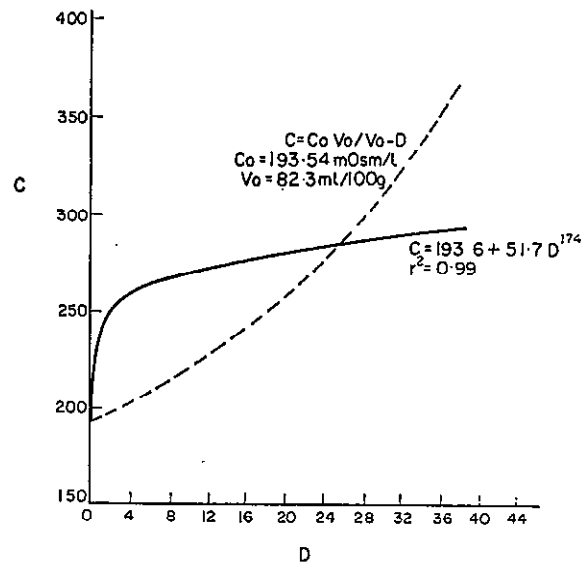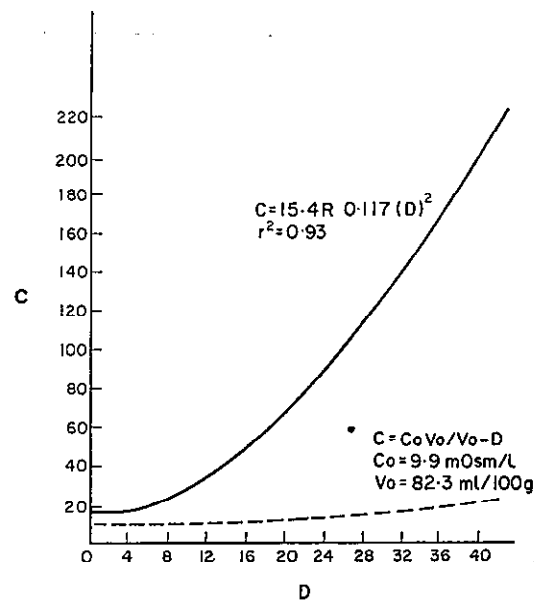

FIG. 4. Values estimated for plasma urea concentration of *A. tigrinum* from observed data (solid line) and values calculated by Shoemaker's equation. Method of presentation is the same as Fig. 3.

apparent. Instead, a large initial increase in total osmolarity (urea excluded) was observed during the first month, followed by no significant increases from 1 to 9 months. Using a paired t-test, the difference between the observed and the values predicted by the equation were highly significant ($t = 6.86$, $N = 29$, $P < 0.01$). The observed plasma urea concentration was significantly higher than the predicted ($t = 14.07$, $N = 29$, $P < 0.01$) (Fig. 4).

When the salamanders were removed from the soil it was observed that some of them had constructed burrows. These burrows were lined with a thin dense layer of moist soil and were very different in appearance from the surrounding sandy soil. Animals removed directly from these burrows appeared to be in excellent condition after 9 months in the soil. During the final days of the experiment some individuals left their underground shelter and moved about on the surface. Once in contact with air they dehydrated rapidly and died. McClanahan (1972) in the laboratory and Ruibal (1969) in the field have described movements to the surface by spadefoot toads. The reason for such behavior is not known.

DISCUSSION

Some of the adaptations which allow anurans to survive in arid regions, such as utilization of water reserves in the bladder (Ruibal, 1962; Bentley, 1966a), tolerance of high internal osmotic concentrations and accumulation of urea (Bentley, 1966a; Shoemaker, 1969; McClanahan, 1967, 1972) and survival at high levels of water loss (Thorson & Svihla, 1943; Main & Bentley, 1964) have now been demonstrated for tiger salamanders, *A. tigrinum* (Alvarado, 1972; present study). These adaptations and other factors such as similarity of oxygen consumption rates and respiratory gas exchange patterns in temperate anurans and lunged urodeles (Whitford, 1973), suggest a very conservative physiological evolution in the amphibia. Urea accumulation during dehydration has been shown in the aquatic anuran *Xenopus* (Balinsky *et al.*, 1961). It was suggested that this mechanism was used by early amphibia in resisting water stress by storage of nitrogenous wastes as urea and thus reducing urinary water loss. As McClanahan (1972) has pointed out the same mechanism reduces an unfavorable osmotic gradient between an amphibian and a dehydrating environment. The other adaptations may also date back to early amphibia and may have been retained in both anurans and urodeles in response to evolution under the burden of a permeable skin.

Alvarado (1972) demonstrated that body fluid concentrations of rapidly dehydrated tiger salamanders could be predicted by the equation $C = C_0 V_0 / V_0 - D$ (see Results), which was originally developed for dehydrated anurans (Shoemaker, 1964). In the present study, it was found that this equation was unsuitable for predicting body fluid concentrations of salamanders after long-term dehydration (1–9 months). It is possible that one of the basic assumptions, that total solutes in a body compartment remain constant, may not be valid in this case.

The "benevolent bladder" hypothesis may help to explain the unexpected alteration of plasma osmolarity. It must be assumed that urine is produced by *Ambystoma* during long-term dehydration and stored in the bladder. Further,

solutes, probably Na+, are reabsorbed while water is retained in the bladder. This would result in a higher total plasma osmolarity than predicted by Shoemaker's equation. As plasma osmolarity increased beyond a critical level the release of water from the bladder would serve as a control of plasma osmolarity. The pattern of high initial concentration of plasma followed very minor increases during long-term dehydration displayed by *Ambystoma* in this study, seems to follow the above scheme.

Possible mechanisms required for the above system have been demonstrated in amphibia. McClanahan (1972) found that in spadefoot toads a dilute bladder urine was formed during dehydration. Aceves *et al.* (1970) have shown that transformed *A. mexicanum* under terrestrial (though not dehydration) conditions have higher Na+ concentration in the bladder fluid than in the ureters. They concluded that water was being more rapidly removed from the bladder than was Na+. Shoemaker (1964) observed a similar concentration of bladder fluids in the toad *Bufo marinus* during dehydration. Adult *Ambystoma* in aquatic habitats have been observed to have higher urine concentration in the cloaca than the bladder (Alvarado & Kischner, 1963) and to be actively reabsorbing Na+ across the bladder (Bentley, 1966b).

While the above system may be hormonally controlled, information on hormonal influence in urodele water balance is inconclusive (Bentley, 1971). Gilles-Baillien (1969) had demonstrated that the sensitivity of the bladder in the terrestrial tortoise, *Testudo hermanni*, to neurohyposeal hormones increased when the tortoise was in hibernation. It is possible that a similar situation may occur in urodeles during long term water stress.

It is apparent from a comparison of Figs. 3 and 4 that at least two major alterations of body fluid concentrations, an initial increase in plasma osmolarity and a subsequent increase in urea concentration, occur during long-term dehydration of *A. tigrinum*. The concentration of plasma solutes appears to represent a fairly rapid mechanism for conserving water and producing a favorable osmotic gradient with the soil environment. Upon reaching a certain high level, the plasma concentration (excluding urea) is closely regulated, perhaps by water reabsorbed from the bladder. This form of regulation by the bladder would be directly homologous to the delay of fluid concentration demonstrated for *Ambystoma* by Alvarado (1972) and for anurans by Shoemaker (1964) and McClanahan (1967). Once regulation of plasma concentration begins, this mechanism can no longer be considered to either conserve water or produce a favorable osmotic gradient. A second mechanism takes over these important functions as the production and accumulation of urea becomes the primary means of concentrating body fluids. *A. tigrinum* is able to tolerate high concentrations of urea in its plasma and appears to increase urea production as a function of increasing water stress. This is in agreement with McClanahan's (1972) findings that spadefoot toads have increased urea concentration in drier soils.

While there is no question about adaptive significance of the physiological mechanisms used by amphibia to conserve water and maintain favorable osmotic

gradient, alteration of the fossorial microenvironment may represent another very important mechanism. Ruibal (1969) observed that the soil in the spadefoot toad burrow was very different than the surrounding soil. An alteration of soil lining the burrows used by *A. tigrinum* was observed in the study. Such alteration of the soil might significantly reduce the amount of water lost by an amphibian to the soil. Both the physiological mechanisms involved with survival during long-term dehydration and the relationship of the fossorial amphibia to their soil microenvironments are presently under investigation.

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Key Word Index—Ambystoma tigrinum; osmolarity; urea; benevolent bladder hypothesis; adaptation; burrow; water deficit; amphibian; salamander; long-term dehydration.

ROLE OF ALKALINE PHOSPHATASE IN INTESTINAL WATER ABSORPTION BY EELS ADAPTED TO SEA WATER

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Abstract—1. Water movement from mucosa to serosa was measured in everted intestines isolated from sea water-adapted or fresh water-adapted eels.

2. Water movement was enhanced by raising the pH of the mucosal medium, and reduced when inhibitors of alkaline phosphatase were added to the mucosal surface.

3. The role of intestinal alkaline phosphatase in salt and water absorption is discussed in relation to sea water adaptation of the eel.

INTRODUCTION

When eels are transferred from fresh water to sea water, they drink sea water and absorb it from the intestine, and the rate of water absorption is particularly high during the first week of sea water adaptation both *in vivo* (H. Oide & Utida, 1968) and *in vitro* (M. Oide & Utida, 1967). Concomitantly, the alkaline phosphatase activity of the intestinal mucosa increases (Utida & Isono, 1967; Utida *et al.*, 1968; Oide, 1970). Since there is always a considerable quantity of clear, alkaline fluid in the intestine of teleosts living in sea water (Smith, 1930; Hickman, 1968), it is conceivable that alkaline phosphatase is involved in salt and water absorption in sea water-adapted eels.

In the present work, the effects of pH and of inhibitors of alkaline phosphatase on water movement were examined in the isolated intestine of the eel in order to elucidate the role of alkaline phosphatase in water absorption.

MATERIALS AND METHODS

Japanese cultured eels, *Anguilla japonica*, weighing about 200 g, were obtained from a commercial fishpond and kept without feeding in a fresh water tank at 20°C for about a week before use. Some eels were then transferred to a sea water tank (20°C) and kept there for 7 days (sea water eels), unless otherwise mentioned, and the others were kept in the fresh water tank for the same period (fresh water eels).

Sampling and determination of ionic concentration and pH of body fluids

Eels were lightly anaesthetized with 0·05% MS 222 (Sandoz). Blood was drawn by cardiac puncture and serum was obtained as described elsewhere (M. Oide & Utida, 1967). After opening the abdomen, a sample of abdominal fluid was taken with a pipette and the intestine was isolated. The intestinal fluid was then collected in a test-tube. Concentrations