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SALINITY ADAPTATION IN THE SALAMANDER *BATRACHOSEPS*

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SUMMARY

(1) *Batrachoseps attenuatus* and *B. major* were successfully acclimated to 600 m-osmol NaCl and 400 m-osmol sucrose solutions.

(2) Accumulation of sodium and an increased rate of synthesis of urea provide substantial increases in plasma concentrations of these solutes. Sodium concentrations in excess of 230 mM and urea concentrations in excess of 200 mM indicate that these are the two major solutes (plus anions) responsible for elevation of osmotic concentration in *Batrachoseps*.

(3) *Batrachoseps* exhibits a water balance response upon dehydration (greater than twofold increase in cutaneous uptake, 50% reduction in urine production). Urine production, estimated from bladder contents, was significantly reduced in salamanders acclimated to sucrose solutions compared to animals acclimated to tap water or saline of equivalent osmotic concentration.

(4) Plasma urea concentration was equivalent to urine urea concentration when *Batrachoseps* was kept in tap water and during short term saline acclimation. After long term saline acclimation, urine urea concentration was one-fourth the plasma urea concentration.

INTRODUCTION

Relatively few species of amphibians can tolerate salinities above 300 m-osmol on a long term basis. Those that can, serve to illustrate both the range of physiological capacities for osmoregulation and the variety of environments inhabited by amphibians.

The pattern of successful adaptation by adult amphibians to hypersaline solutions is consistently one of elevating total osmotic concentration above the external concentration by uraemia and/or hypernatraemia (Gordon, Schmidt-Nielsen & Kelly, 1961; Gordon, 1962; Katz, 1973; Romspert, 1976). The findings of Licht, Feder & Bledsoe (1975) apparently represents a different strategy for elevating osmotic concentration in a genus of salamanders, *Batrachoseps*, in response to saline stress. *Batrachoseps* is reported to elevate total osmotic concentration by moderate elevation of Na^+ and Cl^-, with little or no elevation of urea, and by the addition of an unknown solute, representing one-third of the total osmotic concentration.

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Members of the genus *Batrachoseps* are small, slender salamanders found on the West Coast of the United States. Populations have been found near the tide line (Licht *et al.* 1975), but most live in moist terrestrial environments (Stebbins, 1966). We investigated the remarkable saline tolerance of these salamanders in order to determine the solutes responsible for elevating osmotic concentration and to determine the factors necessary for effective osmoregulation in non-saline terrestrial environments.

MATERIAL AND METHODS

Collection and maintenance

Batrachoseps attenuatus were collected from Berkeley and from Orange County, California and *B. major* were collected from Los Angeles and Orange Counties, California during the spring of 1977. Salamanders were kept unfed in covered 250 ml plastic containers lined with moist paper towels to minimize drinking of the medium, at 15 °C in constant darkness, prior to and during experiments. The animals were normally in captivity less than 1 week before the experiments began.

Protocol

Salamanders were exposed to NaCl solutions in two chronic and two acute experiments. Chronic treatment involved increasing the NaCl concentration of the solution soaking the paper towel in 50 mM increments every 3–4 days. Towels were changed with each new solution. One group of ten salamanders was sampled after 16 days (4 days at 200 mM). The second group of six salamanders was sampled after 25 days (6 days at 250 mM). There was no mortality in the chronic saline experiments. For acute saline experiments one group of ten animals was placed directly onto 250 mM-NaCl soaked towels and the seven surviving salamanders were sampled after 4 days. Another group was kept in 250 mM-NaCl for 5 days, followed by 325 mM-NaCl for 2 days. Three of four animals survived. Animals were also exposed to sucrose solutions following the same protocol as for the chronic saline treatment but with 100 mM increments, the final concentration being 400 mM sucrose maintained for 7 days. Survivorship was 60% with all deaths occurring the day before sampling. Controls were kept with towels soaked in tap water, remoistened every 3–4 days, and towels were changed every 10 days.

Each salamander was weighed daily to the nearest 0·01 g after emptying the bladder by suprapubic pressure. The volume of bladder contents was measured in the tap water, acute 250 mM saline, chronic 250 mM saline, and sucrose salamanders by weighing before and after emptying the bladder.

Sampling

Plasma was obtained by collecting blood directly into heparinized capillary tubes immediately after decapitation. Urine was also collected for analysis on the last day. Samples were either analysed immediately or frozen for later analysis.

Analysis

Most plasma samples were analysed for Na^+, urea and osmotic concentration. The small samples obtained from the acute 325 mM saline group were tested only for urea. Urine was analysed for urea and osmotic concentration. Sodium was determined by flame photometry (Coleman) on 0·5–1 μl samples. Urea was assayed by the phenol hypochlorite and phenol trichloroisocyanuric acid procedures after urease incubation on samples less than 1 μl. Appropriate blanks were run simultaneously (Fawcett & Scott, 1960; Weiser, Schweizer & Hartenstein, 1976). Osmotic concentration (milliosmolal) was measured as reduction of vapour pressure (Mechrolab vapour pressure osmometer) or reduction of melting point (Clifton nanolitre osmometer). Water content was determined by drying carcasses at 105 °C for at least 1 week.

Water balance response

Rates of cutaneous water uptake and urine production were measured in hydrated and dehydrated *Batrachoseps*. Salamanders were placed on paper towels moistened with tap water at 22 °C. Bladders were emptied at the start of each trial. Water uptake was taken as the change in weight during the measurement period before the bladder is drained. Urine production is the difference in weight before and after draining the bladder (Shoemaker, 1965; Hillyard, 1975). Experiments consisted of three consecutive 20 min measurement periods with hydrated salamanders. The salamanders were then dehydrated in dry containers for 2·5 h under a fume hood to a mean of 78% of the standard weight (range 71–84%), and tested for an additional three consecutive 20 min periods during rehydration on moist towels.

Predicted plasma concentrations

Predicted plasma sodium, urea and osmotic concentrations were calculated after the method of Shoemaker (1964) using a water content of 78% for fully hydrated animals, and extrapolating from tap water experimental values. The significance of departures from these lines is explained in the following sections.

RESULTS

There were no physiological differences measured between *Batrachoseps attenuatus* and *B. major*, and both species are treated together in the following analysis.

There was no significant difference in the rate of weight loss between salamanders kept in tap water or salt solutions (Fig. 1). Survivorship was 100% in both chronic saline and tap water treatment groups (25 days). Salamanders kept in sucrose lost weight at a greater rate than either chronic saline or tap water animals, even when hypo-osmotic solutions of the non-absorbable solute were used. From the percent weight lost and the percent water content we calculate that water accounts for greater than 95% of the weight lost in both the tap water and chronic saline acclimated salamanders.

Bladder volumes for the tap water and saline treatment groups are indistinguishable (Table 1). Bladder volumes in sucrose treated animals were only 50% of those on tap water or saline. This indicates only that saline acclimated animals could still fill the

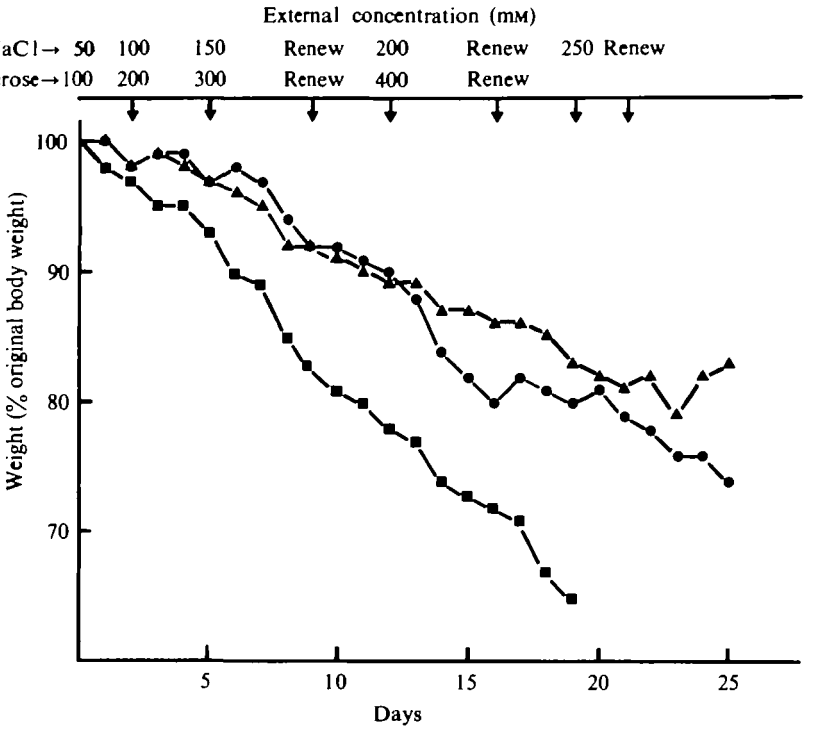

Fig. 1. Weight loss of salamanders maintained in tap water ▲, NaCl ●, and sucrose ■. Changes in external concentrations are also indicated.

Table 1. *Plasma sodium, urea and osmotic concentrations, urine urea and osmotic concentrations, and bladder volumes of* Batrachoseps – $\bar{X} \pm$ S.E. (N)

Experimental condition	Plasma			Urine		
	Na^+ (mM)	Urea (mM)	Total solutes (m-osmol)	Urea (mM)	Total solutes (m-osmol)	Bladder volumes (ml/g)
Tap water	110±7·0 (10)	48±8·2 (10)	339±8·9 (13)	53±9·8 (7)	82±12·4 (7)	0·034±0·003 (5
Acute 250 mM-NaCl 4 days	205±5·5 (6)	121±6·2 (6)	584±8·6 (4)	131±8·6 (6)	—	0·030±0·004 (6)
Acute 325 mM-NaCl 6 days	—	77±16·4 (3)	—	93±11·9 (3)	—	—
Chronic 200 mM-NaCl 16 days	190±7·8 (8)	163±3·3 (10)	645±21 (10)	82±14·9 (8)	425±35·6 (8)	—
Chronic 250 mM-NaCl 25 days	236±7·3 (4)	218±19 (4)	673±11 (4)	126±20·1 (4)	473±8·8 (4)	0·031±0·003 (6)
Sucrose 200 mM 19 days	138±2·0 (2)	154±33 (3)	—	—	—	0·016±0·001 (5)

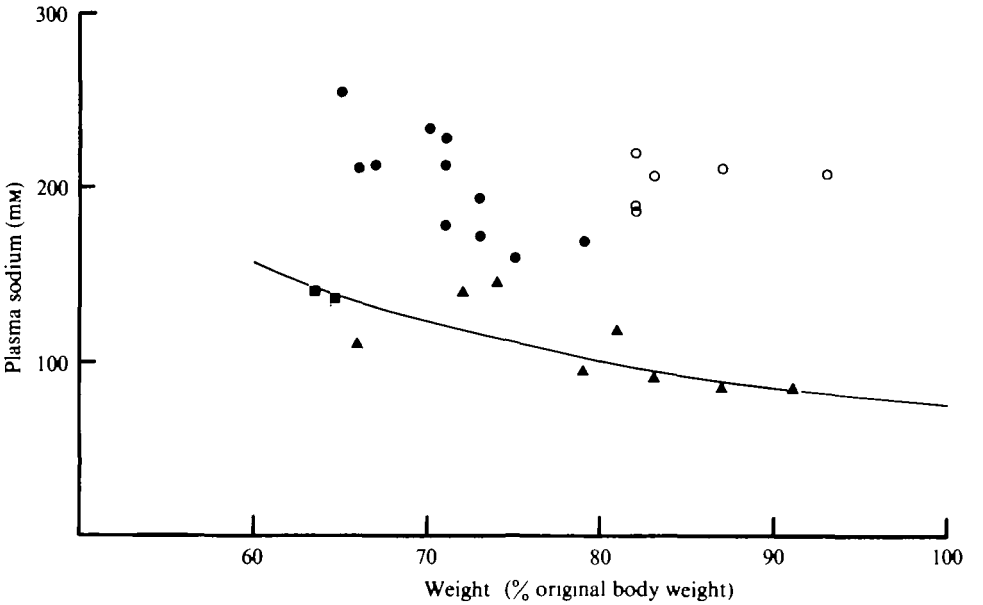

Fig. 2. Plasma sodium concentrations of salamanders in tap water, NaCl, and sucrose. Symbols as in Fig. 1, except ● indicate chronic saline experiments, and ○ indicate acute saline experiments. Line predicts sodium concentrations with no accumulation from the environment.

bladder at least once a day, but that sucrose acclimated animals could not, possibly due to changes in cutaneous water flux.

Solute concentrations are presented in Table 1 and are graphically compared on the basis of percent of original body weight (Figs. 2, 3, 4) because of the range of weight loss experienced due to the chronic nature of the experiments. With chronic saline treatment, the salamanders' plasma sodium concentrations (Fig. 2) are greater than would be predicted from loss of body water alone, but are in all cases less than the external environment. The plasma urea concentrations of saline acclimated animals are greater than would be predicted from the known loss of body water and assuming that the synthesis of urea equals that lost by excretion (Fig. 3). Therefore, both sodium uptake and accumulation of urea (either by increased synthesis or decreased excretion) are characteristic of the process of saline acclimation in *Batrachoseps*. As a consequence, saline plasma osmotic concentration (Fig. 4) is also greater than predicted from the loss of body water alone. Plasma osmotic concentration was considerably above the external medium concentration, often by 100–150 m-osmol.

By doubling the mean plasma sodium concentration (to account for electrically balancing anionic solutes) and adding to it plasma urea levels, all but 65 m-osmol of the total plasma osmotic concentration are accounted for in both saline and tap water treatment groups. There is therefore no indication that solutes other than Na^+, Cl^- and urea are important in the process of acclimation to salt solutions.

Plasma and urine urea concentrations closely approximated each other in tap water and acute saline treatments (Fig. 5). Urinary urea was considerably lower than plasma urea concentrations in chronic saline treatments, indicating that urea is actively reabsorbed in such situations.

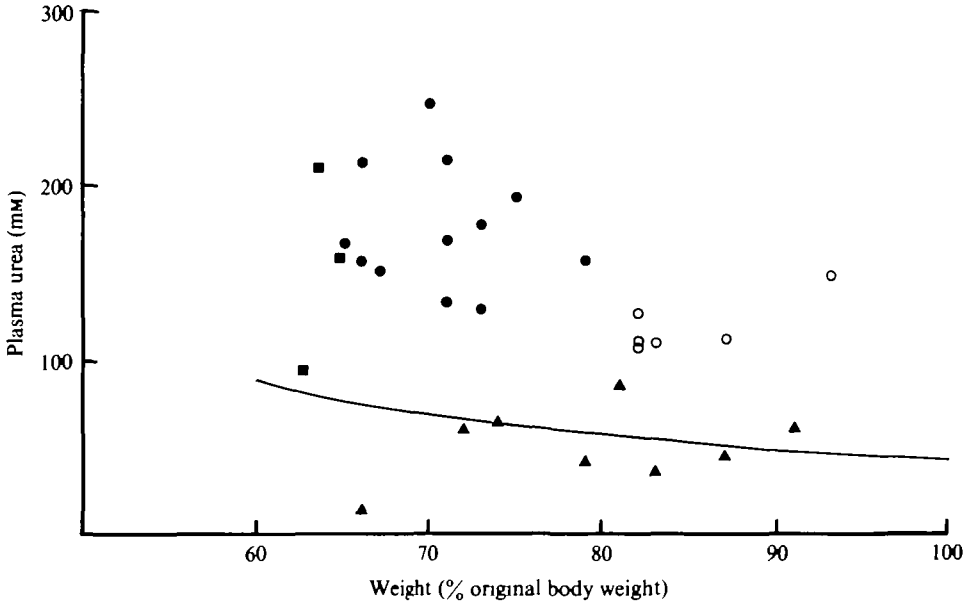

Fig. 3. Plasma urea concentrations. Symbols as in Fig. 2. Line predicts urea concentration when synthesis equals excretion for the duration of the experiment.

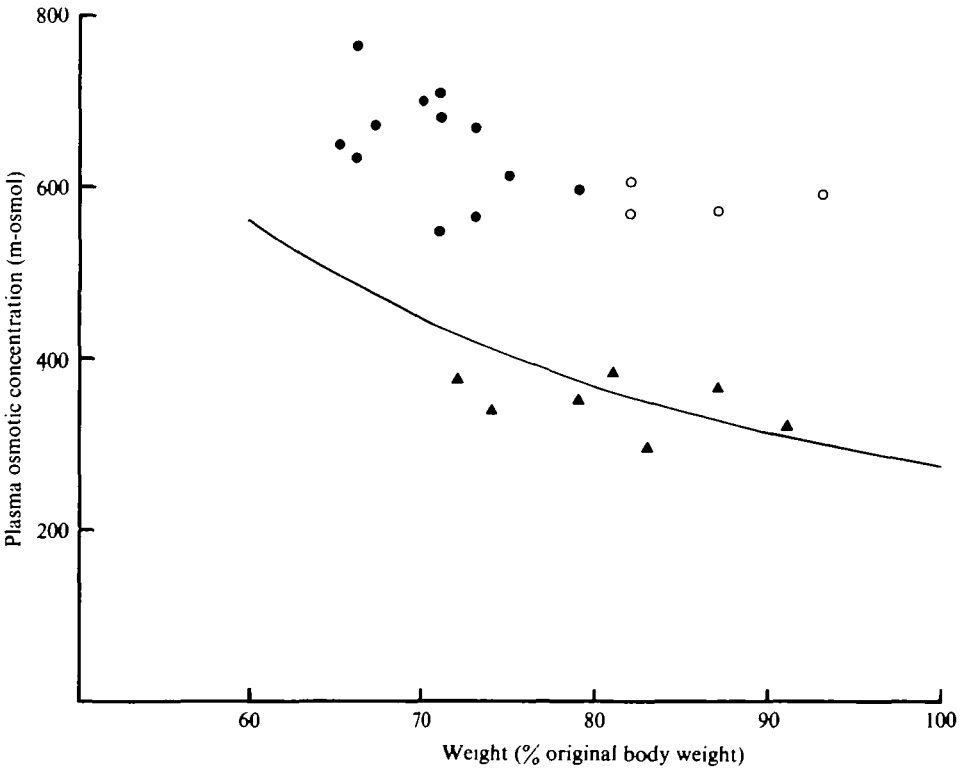

Fig. 4. Plasma osmotic concentrations. Symbols as in Fig. 2. Line predicts concentration when urea synthesis equals excretion and no accumulation of sodium from the environment occurs.

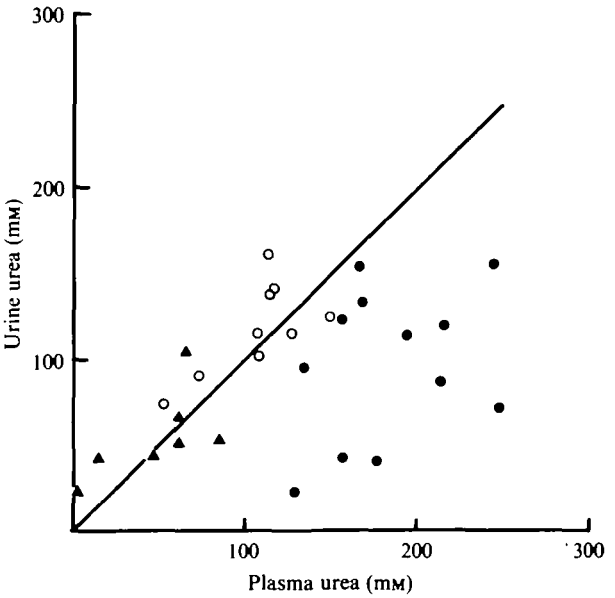

Fig. 5. Plasma and urine urea concentrations. Symbols as in Fig. 2. Line indicates equivalence of plasma and urine concentrations.

Table 2. *Water balance response of* Batrachoseps, $\bar{X} \pm$ S.E. (N), *except % weight lost upon dehydration,* $\bar{X}$ (*range*)

Experimental condition	% weight lost upon dehydration	Water uptake (% initial wt/h)	Urine production (% initial wt/h)
Hydrated	—	3·48 ± 0·43 (6)	2·75 ± 0·44 (6)
Dehydrated	22 (16–29)	7·84 ± 0·87 (5)	1·58 ± 0·20 (5)

Batrachoseps increases cutaneous water uptake twofold during the first hour of rehydration (and nearly 3-fold during the first 20 min) after dehydration to about 80% of their original weight. Urine production is reduced by 50% during this time (Table 2).

DISCUSSION

Batrachoseps attenuatus and *B. major* have an exceptional ability among amphibians to tolerate saline environments. There was no mortality when the salamanders were chronically exposed to solutions of NaCl up to 250 mM. Survival is greatly reduced when salamanders are allowed to drink saline (Licht *et al.* 1975). It should be noted that no animals were collected in localities where they would be expected to encounter saline, so that animals from near the ocean may tolerate even greater salinities. The performance of our experimental animals at 250 mM indicates that they could have been acclimated to higher concentrations of NaCl.

The method of saline adaptation observed here is similar in several respects to that displayed by the most eurhyaline anuran known, *Rana cancrivora* (see Gordon *et al.*

1961; Schmidt-Nielsen & Lee, 1962). Plasma sodium increases but always remains slightly below the sodium concentration of the environment. The major solute increase allowing for maintenance of a hyperosmotic state is attributable to urea. Glomerular filtration rate and urine volumes decrease in *Xenopus laevis* and *Rana cancrivora* when kept in saline (McBean & Goldstein, 1970; Schmidt-Nielsen & Lee, 1962). Although bladder contents were the same in tap water and saline acclimated *Batrachoseps*, we cannot conclude that water uptake and urine production remained the same in these two treatments. It is likely that the osmotic influx of water was not as greatly reduced as in *X. laevis* and *R. cancrivora* because of the larger osmotic gradient seen in *Batrachoseps*. Contrary to the findings of Licht *et al.* (1975) there is no evidence of an unknown solute contributing a major portion of the increased osmotic pressure.

When a non-absorbable solute (sucrose) was substituted for NaCl in the external environment, salamanders reduced urine volume and also lost weight (body water) at a much greater rate than did saline or tap water animals. Plasma Na^+ did not rise above the value expected from concentration effects alone (Fig. 2). Even though urea accumulation occurred, the inward gradient for water probably was quite small in comparison to the saline acclimated animals. Sodium uptake, whether active or passive, therefore represents an important response to maintaining osmotic equilibrium.

The use of different media (NaCl in the present study, sea water in Licht *et al.* 1975) is probably not a factor related to urea accumulation. Previous studies of *Rana cancrivora* and *Xenopus laevis* indicate that urea accumulation occurs to an equal extent in both media (Balinksy, Dicker & Elliott, 1972; Colley *et al.* 1972; Gordon *et al.* 1961; Schlisio, Jurss & Spannhof, 1973).

Although only a few species of urodeles have been investigated, not all of those can elevate plasma urea levels in response to saline. *Ambystoma mexicanum* kept in 30% sea water for a month only achieves plasma concentrations of 11 mM urea (Ireland & Simons, 1977). *Ambystoma tigrinum*, however, accumulates urea (up to 200 mM) when maintained in soil for 9 months (Delson & Whitford, 1973).

Batrachoseps, and a few other salamanders, can increase cutaneous water flux by 2–3-fold with a constant gradient in response to dehydration. Several terrestrial salamanders, including *Triturus vittatus*, *Salamandra salamandra* (Warburg, 1971), *Plethodon punctatus*, *P. hoffmani* (Brown, Hastings & Frye, 1977) and *Aneides lugubris* (Hillman, 1974) can increase cutaneous water flux upon dehydration, whereas some aquatic urodeles (*Necturus maculosus*, *Amphiuma means*, and *Siren lacertina*) cannot (see Bentley, 1971). The rates of water uptake of terrestrial anurans upon dehydration, however, appear to be greater than rates for urodeles when compared on a surface specific basis (Bentley, Lee & Main, 1958; Claussen, 1969).

It is instructive to compare mean urea accumulation rates in the chronic and acute saline experiments. Assuming an initial urea concentration of 48 mM and accounting for water loss, it can be calculated that the acute group (4 days) accumulated urea at a rate of 7·5 μmole/g/day whereas the chronic group (25) accumulated urea at 2·4 μmole/g/day. Net urea synthesis is probably greater in the initial stages of saline acclimation, because the rate of urea excretion will be approximately the same in the chronic and acute groups, or possibly less in the chronic group due to reabsorption of urea.

Several studies have shown that urine urea concentrations in anurans are typically equal to plasma urea concentrations because of increased permeability of the bladder to urea following antidiuretic hormone release (Schmidt-Nielsen & Lee, 1962; Chew, Elliott & Wong, 1972; Maffly *et al.* 1960). *Batrachoseps* apparently is capable of reabsorbing urea, either at the kidney or bladder, when chronically acclimated to saline. This reduces urine urea to a minimum of one-fourth the level of plasma urea and may have important consequences in a long term nitrogen budget.

Because the majority of *Batrachoseps* live away from saline habitats, it is reasonable to assume that mechanisms similar to those described here allow these animals to osmoregulate effectively during times when their soil habitat is not saturated with water and the soil water potential approaches or exceeds normal body fluid potentials. If dehydration is slow, urea accumulation with no net Na^+ uptake may be sufficient for successful osmoregulation. The presence of an active cutaneous Na^+ uptake system, as has been described for another plethodontid salamander (Hillman, 1974), may also play a role in terrestrial osmoregulation.

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