

THE STIMULUS FOR THE WATER-BALANCE RESPONSE TO DEHYDRATION IN TOADS

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(Received 16 January 1965)

Abstract—1. Changes in rates of water exchange similar to those caused by dehydration (enhanced cutaneous uptake and reduced urinary loss) are elicited by injection of hyperosmotic solutions of NaCl or sucrose.

2. Rates of water exchange bear the same relation to plasma sodium concentration in both dehydrated and NaCl loaded toads.

3. Injection of a hyperosmotic urea solution does not affect rates of water exchange.

4. Loss of blood causes toads to take up amounts of water greater than those excreted.

5. Small volumes of plasma from dehydrated toads cause cutaneous water uptake to exceed urine production when injected into hydrated individuals.

INTRODUCTION

AMPHIBIANS that frequent terrestrial habitats typically respond to dehydration by decreasing rates of urinary water loss and increasing the rate at which water can be taken up through the skin. These effects, known as the water-balance response, are thought to be mediated by a neurohypophyseal hormone similar to the anti-diuretic hormones of mammals (see Sawyer, 1956). The water-balance response is apparently adaptive for the exploitation by amphibians of dry environments. After dehydration, the toad *Bufo regularis* is capable of a large increase in its rate of water uptake, whereas a wholly aquatic frog (*Xenopus laevis*) is not (Ewer, 1952). Species of frogs of the genus *Neobatrachus* from dry areas recover water losses more rapidly than congeneric forms from less arid regions (Bentley *et al.*, 1958). A similar correlation between rate of rehydration and aridity of habitat was reported in salamanders by Cohen (1952).

Although the hormonal basis and physiological characteristics of the water-balance response have received considerable attention, little is known of the stimulus which elicits it aside from the fact that it is associated with dehydration. Concentrations of electrolytes in the body fluids increase in dehydrated toads by an amount commensurate with their water loss (Shoemaker, 1964), and experiments reported here were undertaken to determine whether this manifestation of dehydration provides the stimulus for the water-balance response.

MATERIALS AND METHODS

Three species of toads were used in this study. *Bufo marinus* weighing 100–300 g were obtained from commercial suppliers. Specimens of *Bufo cognatus* (40–60 g) and *Bufo valiceps* (25–35 g) were captured in southern Arizona and Houston, Texas,

respectively. Toads were maintained at 20–25°C in pans containing a shallow layer of tap water. They were force-fed beef liver or canned dog food once or twice each week. Toads were not fed for 2 weeks preceding an experiment, to prevent weight losses due to defecation.

The influence of water deficit on rates of water exchange in *Bufo marinus* was investigated by subjecting each of six toads to a series of four dehydration experiments in which water losses of about 4, 8, 16 and 25 ml/100 g were incurred. They were maintained in tap water for several days between dehydrations. Toads were dehydrated at the rate of about 1 ml/(100 g × hr) by placing them in wire cages in the laboratory. Their hydrated weight (bladder empty) was determined initially, and subsequent weight losses were taken to reflect the evaporation of water since no urine or feces was voided. Rates of water uptake were determined from the total weight gained in the first hour after the toads were returned to water. Urine production over the same period was estimated by reweighing each toad after emptying its bladder. Urine was expelled by inserting a glass tube into the cloaca and applying pressure to the lower abdomen. These procedures were used to measure rates of water exchange in all experiments reported here.

The effects of elevated body fluid concentrations on rates of water exchange were examined by injecting hydrated toads intraperitoneally with hyperosmotic solutions. Effects of various solutes were determined by injecting toads (*Bufo cognatus*) with 2 ml/100 g of the following solutions: 1.0 M NaCl, 2.0 M sucrose, 2.0 M urea and 0.11 M NaCl. The same six animals were used to test the effects of each solution, and they were kept in tap water for several days between tests. Similarly, injections of 0.5, 1.5, 2.5 and 3.5 ml of 1.0 M NaCl/100 g were given to each of six *Bufo marinus* to determine the relationship between the magnitude of the solute load and rates of water exchange. Following injection, the toads were placed in dry, covered pans for 1 hr to allow time for the distribution of the solute, and then were returned to water. Rates of water uptake during the first hour were determined, and the amount of urine accumulated in the bladder from the time of injection to the end of the first hour in water was also measured for each toad.

The effect of blood loss on rates of water exchange was tested using *Bufo cognatus*. The same procedures were followed as in the solute-loading experiments except that removal of blood from the heart was substituted for solute injection. One ml of blood/100 g was withdrawn from each of five toads, and twice this amount was removed from four other toads. A needle was inserted into the hearts of six control toads, but no blood was taken. Heart punctures were made using a heparinized 27-gauge hypodermic needle.

The water-balance response of amphibians has been used in the assay of anti-diuretic hormone in mammalian plasma (Buchborn, 1957; Lauber *et al.*, 1959) and this suggested the feasibility of using hydrated toads to detect increased concentrations of a similar hormone in the plasma of dehydrated toads. One to 2 ml of blood was collected from each donor toad (*Bufo marinus*) *via* heart puncture. Centrifugation of each blood sample yielded plasma which was divided into four aliquots and injected intraperitoneally into each of four hydrated assay toads

(*Bufo cognatus* or *Bufo valiceps*). The assay toads were returned to water immediately following the injection, and their rates of water uptake and urine production in the first hour were measured. Plasma from hydrated toads and from toads that had been dehydrated to 80 per cent of their original weight was tested for "water-balance" activity by this procedure.

RESULTS

Dehydration increases cutaneous uptake of water and depresses urinary water loss in *Bufo marinus* (Fig. 1). These results are similar to those reported for *Rana pipiens* (Adolph, 1943) and *Bufo bufo* (Jørgensen *et al.*, 1956). Loading hydrated

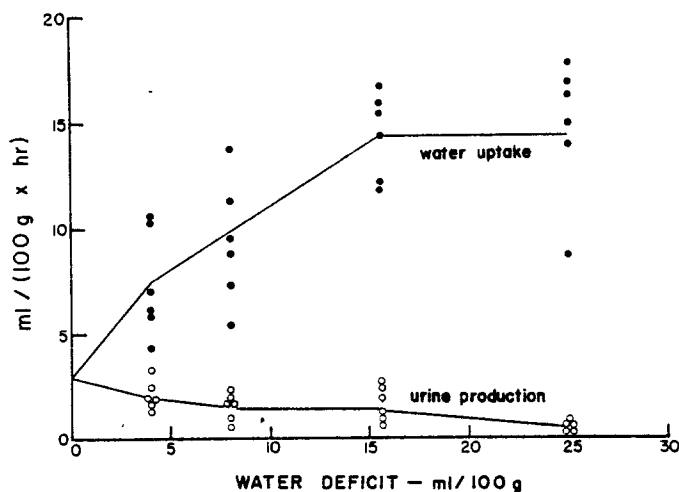


FIG. 1. Effect of dehydration on rates of water exchange in *Bufo marinus*. ●, water uptake; ○, urine production.

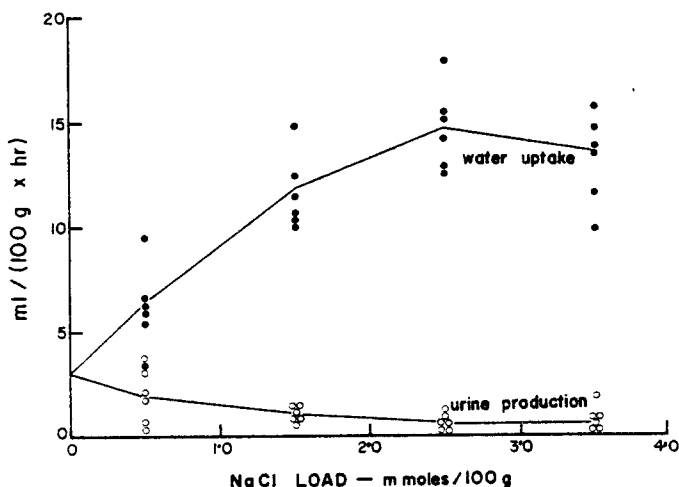


FIG. 2. Effect of sodium chloride loading on rates of water exchange in *Bufo marinus*. Symbols as in Fig. 1. (NaCl values calculated.)

toads with varying amounts of NaCl has much the same effect (Fig. 2). Jørgensen (1948) observed that NaCl loading also elevates rates of water uptake in *Bufo vulgaris* and *Rana temporaria*.

The concentration of sodium in the plasma was not measured directly in toads for which rates of water exchange are reported, but this was readily estimated using data from other similarly treated individuals. The concentration of sodium (C) in the plasma of dehydrated toads was estimated using the equation

$$C = \frac{115 \text{ m-equiv./l} \times 78 \text{ ml/100 g}}{\text{ml lost/100 g}},$$

which describes the relationship between water deficit and plasma sodium concentration in *Bufo marinus* (Shoemaker, 1964). The apparent dilution volume of an

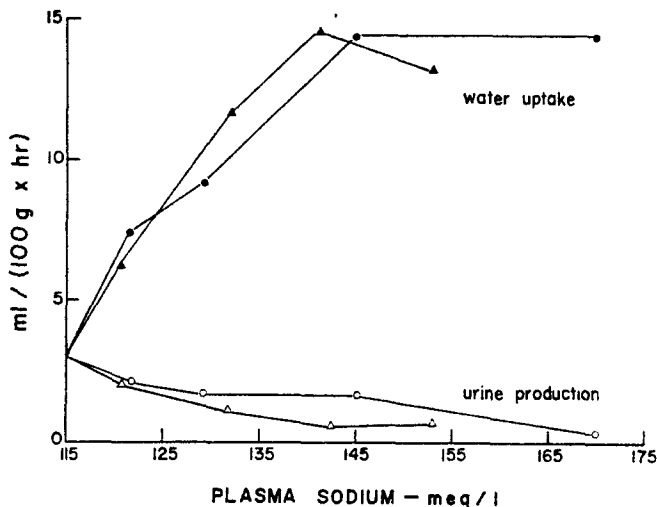


FIG. 3. Rates of water exchange as a function of the concentration of sodium in the plasma of *Bufo marinus*. ●, ○, dehydrated toads; ▲, △, NaCl loaded toads. (Sodium/Cl values calculated.)

administered sodium load was found to approximate the total water content of the animal (78 ml/100 g in hydrated *Bufo marinus*) as has been demonstrated in lizards (Bentley, 1959) and mammals (Conway & McCormack, 1953). An initial plasma sodium concentration of 115 m-equiv./l was used in making these calculations. When rates of water exchange in dehydrated and NaCl loaded toads are plotted as a function of the concentration of sodium in their plasma, the similarity of the effects of these two treatments is apparent (Fig. 3).

The effects of osmotically equivalent amounts of NaCl, sucrose and urea on rates of water uptake and urine production are shown in Fig. 4. NaCl and sucrose were equally effective in eliciting a water-balance response, whereas urea loading

and injection of an equivalent volume of isosmotic NaCl had no effect on rates of water exchange. Statistical comparisons made using the Mann-Whitney *U* test showed the elevation of rates of water uptake by toads injected with 1.0 M NaCl and 2.0 M sucrose over those receiving no injection, 0.11 M NaCl or 2.0 M urea

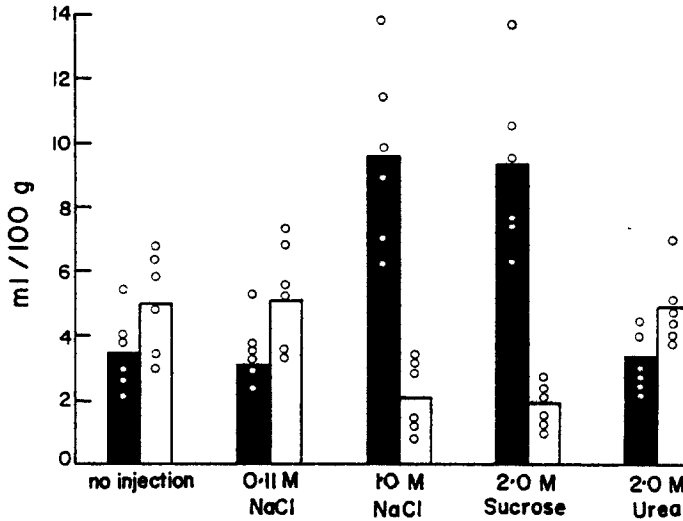


FIG. 4. Effect of various solutes on rates of water exchange in *Bufo cognatus*. Black bars represent means of water uptake in first hour after the toads were returned to water. White bars indicate total urine accumulated from time of solute loading to end of first hour in water (2 hr in all). Individual values are shown as circles.

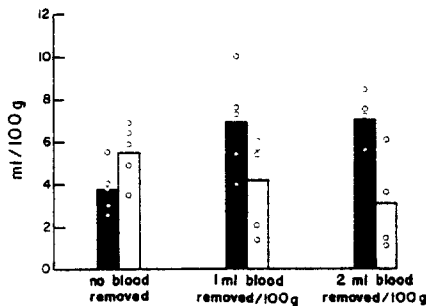


FIG. 5. Effect of loss of blood on rates of water exchange in *Bufo cognatus*. Legend as in Fig. 4.

to be significant at the 0.002 level. The depression of urine production was significant at the 0.02 level in the case of 1.0 M NaCl and at the 0.01 level in the case of 2.0 M sucrose.

Removal of blood also elicited a water-balance response (Fig. 5), but this was less pronounced than that caused by solute loading. Elevation of rates of cutaneous uptake by toads that had lost 1 and 2 ml of blood/100 g was significant at the 0.04

and 0.02 levels, respectively (Mann-Whitney *U* test). The depression of urine production was not statistically significant ($P > 0.1$).

Injection of small amounts of plasma from dehydrated *Bufo marinus* into *Bufo cognatus* and *Bufo valiceps* caused water uptake to exceed urine production in these animals, whereas plasma from hydrated toads had no effect on rates of water exchange in either species (Table 1). The fact that urine production by *Bufo valiceps*

TABLE 1—EFFECT OF PLASMA FROM DEHYDRATED *Bufo marinus* ON RATES OF WATER EXCHANGE* IN *Bufo cognatus* AND *Bufo valiceps*

Water deficit of donor toad	Species used for assay	ml of plasma injected	Water exchange, ml/(100 g × hr)	
			Water uptake	Urine production
None	<i>B. cognatus</i>	0.25	3.5	3.7
20 ml/100 g	<i>B. cognatus</i>	0.10	6.2	2.9
20 ml/100 g	<i>B. cognatus</i>	0.25	8.2	2.5
None	<i>B. valiceps</i>	0.25	3.3	2.9
20 ml/100 g	<i>B. valiceps</i>	0.20	5.2	3.6
20 ml/100 g	<i>B. valiceps</i>	0.20	5.4	3.9

* Rates shown are means for groups of four toads.

injected with plasma from dehydrated toads exceeded the normal rate probably indicates that these animals were already beginning to excrete the water load imposed by their enhanced water uptake. The same phenomenon was observed in *Bufo cognatus* in the second hour after they were returned to water.

DISCUSSION

The belief that the water-balance response of amphibians is mediated by a hormone similar to the antidiuretic hormones of mammals is based on several lines of evidence. A number of investigators, beginning with Brunn in 1921, have found that neurohypophyseal extracts from mammals and amphibians effect changes in rates of water exchange in amphibians similar to those brought about by dehydration (see Sawyer, 1956). It has been demonstrated by Sawyer *et al.* (1961) that the active principle of the amphibian neurohypophysis is an octapeptide similar in structure to mammalian neurohypophyseal hormones. Levinsky & Sawyer (1953) and Jørgensen *et al.* (1956) showed that dehydration causes a reduction in the amount of assayable hormone present in the posterior lobe of *Rana pipiens* and *Bufo bufo*, respectively. This has been interpreted as the result of the release of this material into the circulation. The observation that plasma from dehydrated toads has biological properties similar to those of neurohypophyseal hormones (Table 1) supports this view.

Comparison of the effects of dehydration and solute loading on rates of water exchange indicates that the increased concentration of the body fluids brought about by water loss provides sufficient stimulus for the water-balance response. Changes

in rates of water uptake cannot be explained simply on the basis of the increased osmotic gradient across the skin since initially a 10 per cent increase in the concentration of the body fluids causes a two- or threefold increase in the rate of water uptake. Moreover, at higher concentrations this rate is not increased by further elevation of the osmotic gradient (Fig. 3). Evidently, the increase in cutaneous water uptake is due to changes in the properties of the skin like those brought about by neurohypophyseal hormones. The results summarized in Fig. 4 are reminiscent of those obtained by Verney (1947) when he injected hyperosmotic solutions into the circulation of water loaded dogs. He found that NaCl and sucrose caused a rapid and pronounced decrease in urine production whereas urea had no effect. Verney proposed that osmoreceptors located in the area supplied by the internal carotid artery triggered the release of neurohypophyseal hormones in the dog, and argued that urea would be ineffective in setting up an osmotic gradient across a receptor cell membrane since it enters cells readily. Andersson (1953) injected hyperosmotic NaCl into the hypothalamus of goats and observed an antidiuretic response which supports the osmoreceptor theory. Also, there is a good correlation between serum osmolarity and antidiuretic hormone level in humans (Buchborn, 1957). However, numerous attempts by Ginsburg & Brown (1957) to inhibit diuresis in the rat through intravascular administration of concentrated solutions have been unsuccessful. Bentley (1959) found that NaCl loading causes a decrease in urine production in the lizard *Trachysaurus rugosus*, whereas sucrose loading causes diuresis. Thus the osmoreceptor theory for the release of antidiuretic hormones set forth by Verney appears applicable to toads, but evidence from other groups of vertebrates is inadequate to permit generalization. Anaesthesia, emotional stress, osmotic diuresis and other factors may influence urine production, and when these factors are adequately controlled the osmoreceptor theory may become more generally applicable.

Although it appears unnecessary to invoke the action of volume or pressure receptors in the control of rates of water exchange in dehydrated toads, the response to blood removal indicates their presence. Similarly, hemorrhage causes anti-diuresis in rats (Ginsburg & Brown, 1957), and a marked increase in the concentration of vasopressin in the blood of dogs (Weinstein *et al.*, 1960). Toads that take up excess water after solute loading return to their original weight after several hours, and this also argues for the presence of a mechanism for volume regulation that does not depend entirely on the concentration of the body fluids. The utility of this duality of control over rates of water exchange is apparent since fluid volume changes may, in some cases, be independent of changes in solute concentration.

SUMMARY

The effects of dehydration on rates of water exchange in toads—i.e. increased cutaneous uptake and decreased urinary loss—were duplicated by injecting hyperosmotic NaCl and sucrose solutions. Rates of water exchange vary with the concentration of sodium in the plasma in the same fashion in both dehydrated and NaCl loaded toads. Osmotically equivalent amounts of sucrose are as effective as

NaCl in eliciting the response. Urea loading has no effect on rates of water exchange, thus elevation of the concentration of non-permeating solutes apparently stimulates the release of the neurohypophyseal water-balance hormone. Loss of blood also causes water uptake to exceed urine production but is less effective than solute loading. Small amounts of plasma from dehydrated toads were found to elicit a water-balance response when injected into hydrated individuals.

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