Water Economy
in the Life of a Terrestrial Anuran,
the Toad *Bufo bufo*

By C. BARKER JØRGENSEN

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By C. BARKER JØRGENSEN

Synopsis

Amphibian water economy was studied in male toads, acclimated to a simulated terrestrial habitat, a plastic box furnished with a water bath and a shelter, a halved plastic flower pot. Records were kept of location chosen by the toads, whether inside or outside the shelter or in water. Location was correlated with state of hydration, including amounts of urine stored in the bladder. In 76 % of observations the toads stayed in the dry compartment, and mostly crowding inside the shelter. Deprivation of the water depots in the bladder by catheterization did not affect compartment preference, whereas removal of the shelter substantially increased occupation of the water bath. Standard hydration was the same whether maintained by cutaneous influx and/or resorption of water from the urinary bladder down to bladder contents corresponding to about 3 % of the body mass. Spontaneous deviations from mean hydration varied individually from 4 % to 9.5 % of the body mass. Catheterization of the bladder resulted in negative water balance the following day in from 15 to 69 % of observations, varying individually. The effect on water balance vanished within 2 to 5 days after deprivation of the water depots. In undisturbed toads in the presence of the shelter, water balance was negative in only 2 out of 34 observations (both recorded 4 days after emptying of the bladder), against 7 out of 31 observations in the absence of the shelter. The mean content of urine in the bladder of toads in water varied from less than 50 % of the bladder capacity in the presence of the shelter to about 70 % in its absence. Toads occupying the dry compartment practically exclusively lost water by evaporation. In the presence of the shelter the mean amounts of urine stored in the bladder corresponded to from 1.5 to 2.4 times the daily mean evaporative losses, against less than 1 in the absence of the shelter. When dehydrated toads returned to water a quasi steady state in water balance was established within 4-5 hours at a level below the standard, which was approached asymptotically during the following 2-3 days. The cutaneous permeability to water varied with both environmental and internal factors. Leaving long-term water-acclimated toads dry for about 1 hour increased the water permeability by a factor of about 3. Dehydration increased water permeability proportionally to the loss of body water. The decline in water influx in rehydrating toads can be described as logarithmic functions of time. In the presence of the shelter the ratios between time spent in water and time needed for replenishment of evaporative losses varied individually from about 1 to 5. In the absence of the shelter little of the water absorbed was voided (i.e., used for excretion) during the stay in water. Urine production in water-acclimated toads transferred to a dry environment or to isotonic saline decreased to one third or less the rate at steady state in water and returned to this level upon retransfer to water in the absence of significant changes in hydration of the body. During rehydration in dehydrated toads polyuria might develop at remaining water deficits that resulted in anuria during dehydration from the fully hydrated state. It is concluded (1) that terrestrial-acclimated toads maintain normal hydration by means of the bladder depots; (2) that hydration behaviour, "drinking", basically is anticipatory, independent of the actual water economy; and (3) that integration of water influx through the skin and/or bladder wall and urine production depends upon nervous mechanisms that may operate to maintain water balance in the absence of deviations from normal hydration.

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Contents

Introduction

As the name implies, amphibians are basically adapted to live both in water and on land, but patterns of behaviour among genera and species range from purely aquatic to purely terrestrial. Between these extremes, all degrees of predominance of aquatic or terrestrial habits exist.

Toads of the genus *Bufo* belong to the terrestrial end of the range. Typically, toads spend longer periods, days or weeks, in water only during breeding. On land, toads, as most other amphibians, loose water by evaporation from the skin at rates similar to those from a water surface (Rey, 1937; Spotila and Berman, 1976; Tracy, 1976). Body water lost by evaporation may be replenished by water absorbed through the bladder wall from the depot in the urinary bladder (Bentley, 1966; Ewer, 1952; Overton, 1904; Rey, 1937; Ruibal, 1962; Sawyer and Schisgall, 1956; Shoemaker, 1964). The partitioning of time spent in dry and wet environments may therefore vary with the rate of cutaneous evaporation and exploitation of the bladder capacity for water storage.

The rate of evaporation is determined by physical factors, such as relative humidity of the ambient air and convection. The rate is thus greatly influenced by the microhabitat selected, as well as other behaviour. Typically, resting toads and other anurans lower the head and keep the limbs close to the body when exposed to a dehydrating environment, a posture that minimizes the evaporative area (Putnam and Hillman, 1977; Shelford, 1913; Thorson and Svihla, 1943).

Refilling of the bladder depot and rehydration of the body in case of water deficits is by means of osmotic water uptake through the skin. With few exceptions, e.g., the arboreal frog *Phyllomedusa sauvagei* (Shoemaker, 1987), amphibians do not drink, and in fresh water oral water intake is insignificant compared with the cutaneous intake (Durig, 1901; Jørgensen, 1954; Scheer *et al.*, 1974). In *Bufo* spp, cutaneous water uptake is mainly through the highly vascularized pelvic patch, and osmotic influx of water occurs when the patch is in contact with a substratum of higher water potential than that of the blood (Baldwin, 1974; Christensen, 1974; Fair, 1970; McClanahan and Baldwin, 1969; Walker and Whitford, 1970).

Toads and other terrestrial anurans tolerate large losses of body water, up to about 50 % or more. On reestablished contact with water this is absorbed at rates that may be one to two orders of magnitude higher than the rates in the fully hydrated state (de Almeida, 1926; Claussen 1969; Overton, 1904; Smith and Jackson, 1931; Thorson, 1955).

Brunn's (1921) demonstration that injection of neurohypophysial extracts caused frogs in water to increase dramatically in weight initiated research into effects of neurohypophysial principles on osmotic regulation in amphibians, and it became generally accepted that water balance is regulated by the water balance hormone, eventually identified as arginine vasotocin. The hormone was assumed to be released in response to dehydration, causing antidiuresis and increased permeability of the urinary bladder wall and the skin. On land, the hormone would enable amphibians to utilize urinary depots of water by increasing resorption through the bladder wall; on contact with water to rapidly rehydrate.

Amphibian water balance research has followed two main streams, one endocrinological concerned with effects of neurohypophysial hormones on amphibian water balance, and one adaptational, or ecological, particularly focusing on how xeric amphibians cope with arid environments. Both main streams have been repeatedly reviewed, often dealt with together (Alvarado, 1979; Deyrup, 1964; Bentley, 1971, 1974; Heller, 1945, 1950; Koefoed-

Johnsen, 1979; Sawyer, 1956, 1987; Scheer *et al.*, 1974; Shoemaker, 1987; Shoemaker and Nagy, 1977).

The general interpretation of effects of arginine vasotocin and its analogues in terms of osmoregulatory control is correlated with a paucity of studies concerned with testing of the correctness of assumptions and inferences. Many basic parameters in amphibian water balance remain to be elucidated. Questions that may be asked include: (1) Is the permeability of skin and bladder wall, as well as rate of urine production closely correlated with the hydration of the organism, particularly the size of a water deficit? (2) Do terrestrial anurans primarily maintain hydration by reestablishing contact with water in response to a deficit in body water or is the hydration behaviour secondary, i.e., independent of the actual water balance? (3) Is the water content of the organism larger in animals in water than on land, where bladder urine constitutes the only water resource, i.e., does utilization of bladder water depend upon dehydration?

In the following study, attempts have been made to answer these and other questions. Experiments were made on toads that were acclimated to a simple simulation of a terrestrial habitat. The animals were minimally interfered with in order to obtain responses that were representative of behaviour and function in the natural habitat. The studies aimed particularly at establishing the individual variation in span and pattern of water balance, as assessed through many observations and recordings in a small group of toads.

Material and Methods

Experiments were made on five male toads, *Bufo bufo*, kept at 16°C and a 12L/12D lightening scheme in a plastic box with a bottom area of 35×55 cm and 32 cm high walls, to keep the toads from escaping. The floor was covered by filter paper. The box was furnished with a 17×22 cm plastic tray with water, about 1 cm deep. Moreover, a shelter made by halving a black plastic flower pot was present most of the time. The length of the shelter was 11 cm and the height of the entrance was 6 cm. The volume was about 600 cm^3, to be compared with the total body mass of the toads of about 175 g.

The toads were fed meal worms *ad libitum*, except during the period when standard body hydration was established (see below).

Records were kept of location chosen by the toads, whether inside or outside the shelter, or in water. The location was correlated with amounts of urine stored in the bladder, measured by emptying of the bladder through a catheter introduced into the cloaca. When urine was absent from the bladder any negative water balance (= dehydration) could be measured by placing the toads in water and recording the body mass when rate of urine formation equalled the rate of water uptake through the skin, indicating actual water balance. A water deficit or surplus could also be estimated as the difference between the actual body mass and a standard body mass (see below).

The role of dehydration in water balance was estimated from the frequency and degree of spontaneous water deficits, as recorded in the individual toad in the presence or absence of the shelter. Moreover, responses to provoked dehydration were investigated by removing the water tray.

Definitions. The organism is in water balance when water intake equals water loss. In terrestrial anu-

rans on land, water intake is from the reservoir stored in the bladder. Catheterization therefore interferes with the normal water balance by initiating dehydration, which progresses until the next drinking event. Water balance was therefore defined as undisturbed only when recorded after the effects of emptying of the bladder had vanished, usually after 2 to 5 days (see below). Merely placing a toad on the balance to read its weight before replacing it in the terrarium did not seem to affect water balance behaviour or physiology.

The water balance, i.e., steady state hydration of the body, was found to vary substantially. In order to assess this variation with time, and to elucidate factors that might affect the water balance, references were established by recording body mass of the fully hydrated starving toads for periods long enough to determine decreases in body mass caused by metabolic losses of mass. The regression of body mass on time served as a reference for a mean hydration or a mean body mass, defined as the standard hydration or body mass.

Dehydration, whether spontaneous or imposed, could affect steady state hydration up to 3 days after rehydration. Behaviour and water balance was therefore defined as undisturbed only when recorded ≥ 3 days after a dehydration-rehydration event.

Water uptake and urine production. The rates at which toads took up water, reflecting cutaneous water permeability, and produced urine were determined by weighings on a Sartorius balance with a maximum loading capacity of 160 g. Steady state levels in rates were mostly established over periods of 4-5 hours in toads kept in pails with about one cm of water. At the beginning of each period the bladder was emptied, the animal dried in a soft cloth and weighed before returning to the pail with water. Water uptake was the increase in weight after periods of 1 hour, and urine production the loss in weight after catheterization. Usually, the toads remained resting during the determinations, under which conditions urine produced is not voided during the experimental period but transferred to the bladder (Jørgensen, 1949).

In order to estimate the accuracy of determinations a series of 25 consecutive weighings were made, using the standard procedure of drying the toad before weighing, followed by replacement in water before the next drying and weighing, each sequence lasting 1 min. The relationship between weights and time could be described by the equation $y(g) = 31.16 + 0.0101x(min)$, $r^2 = 0.99$. The mean difference ± S.D. between successive weighings was 9.6 ± 7.3 µl, indicating that the mean influx was 9.6 µl min^{-1}, determined with a standard deviation of 7.3 µl. The absolute water influx varied from 100 µl to more than 500 µl. Rates of water influx were therefore determined with good to high accuracy.

Results

Behaviour

In the simulated terrestrial habitat the toads had the choice of staying in or out of water and outside or inside the shelter when this was present. The various settings affected the behaviour of the toads.

Thus, in the presence of the shelter the undisturbed toads stayed in the dry compartment in 76 % of 135 observations made on 36 days over a period of about 4 months. However, the frequencies varied indi-

Table 1. Frequency of occupation of dry compartment by toads acclimated to a simulated terrestrial habitat, in presence or absence of shelter, and $\geqq 3$ days after rehydration from severe dehydration; figures are percentages of observations.

Toads	#1	#2	#4	#5	#6
Shelter present	56	94	92	63	53
Percentage within shelter	85	100	88	80	96
Shelter absent	38	77	62	-	54

Based on 36 observation days with shelter present (24 in toad # 5), and 13 observations with shelter absent.

vidually from about 50 % to more than 90 % (Table 1). The toads fell into two groups, three occupying the dry compartment in 53 to 63 % of the observations, against 92 and 94 % in the remaining two toads. The physiological consequences of these differences in behaviour are dealt with below. Moreover, in the dry compartment the toads spent most of the time within the shelter, usually crowding at the bottom (Table 1).

The procedure of determining the size of the water depots by emptying of the bladder, and thus depriving the toads in the dry compartment of the resource for the maintenance of water balance, resulted in dehydration at rates that varied with the rates of cutaneous evaporation. To see whether the deprivation of bladder urine affected behaviour, the location of the toads were recorded as a function of time elapsed after emptying of the bladder. Behaviour remained unaffected. During the 2 days after catheterization toads 1, 5 and 6 were in water in 43 % of the records against 40 % after 3 days or more. In toads 2 and 4 the percentages were zero and 9, respectively.

By contrast, removal of the shelter affected preference; in 3 out of the 4 toads (one had died) the number of observations in water markedly increased. One toad even frequented water more often than it did the dry compartment. Notably, the relative frequencies of occupancy of the dry compartment remained the same among the toads (Table 1).

The crowding of toads at the bottom of the shelter suggests that a hiding site is selected in prefer-

ence to the open areas of the dry compartment. The active nature of the crowding behaviour out of water was confirmed when the shelter was removed. To be sure, the frequency with which the toads appeared single in the dry compartment increased, from an overall percentage of eight to 36 (Table 2). But most of the toads established body contacts, typically in one corner of the box. With increasing numbers in the crowd, the toads tended to sit on top of each other.

Table 2. Frequencies of single appearances among toads occupying the dry compartment of a simulated terrestrial habitat, in presence or absence of shelter, as percentages of observations.

Toads	#1	#2	#4	#6	Overall
Shelter present	15	0	12	4	8
Shelter absent	45	38	27	38	36

The increase in toads appearing single may be correlated with the increase in visits to the water bath, which may again be related to the removal of the shelter, increasing the rate af water turnover.

It was thus indicated that partitioning of time spent in the wet and dry compartments varied with the "aridity" of the simulated terrestrial habitat.

Dehydration and rehydration
Behavioural responses to an arid environment were studied further when the water bath was removed from the model habitat. Fig. 1 shows an example, illustrating the behaviour of the undisturbed toads. The behaviour after removal of the external water resource is indicated by symbols: ($\cdot$), present in the water bath when this was removed; resting in crouching posture inside (●) or outside (○) the shelter; (+), restless behaviour, alternating between crouching and crawling around in the enclosure, including attempts to climb the walls and short visits to the shelter. With progressing dehydration, extreme crouching predominated, combined with digging movements of the hindlegs, alternating with escape attempts. The magnitude of the water depots in the urinary bladders at removal of
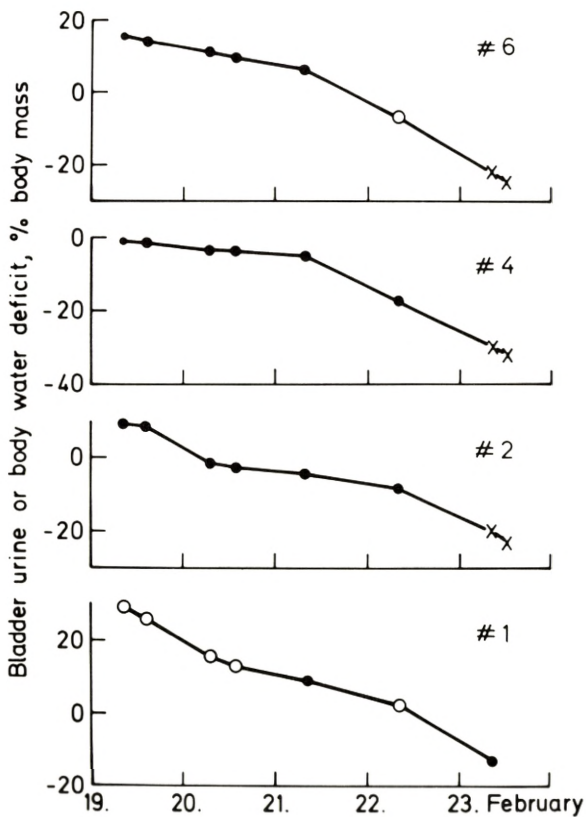
Fig. 1. Temporal relationships between progressive water loss by evaporation and behaviour in toads acclimated in a simulated terrestrial habitat after removal of the external water resource. The symbols indicate behaviour of the toads: resting in water bath at its removal (·); resting inside (●) and outside (○) shelter; escape behaviour (+). The ordinate indicates actual water balance, reflected in initial presence of bladder urine (values above zero) followed by increasing body water deficits.

the external resource was estimated as the difference between the body mass at the start of the experiment and body mass with empty bladder after 3 to 4 days of rehydration.

It may be seen in Fig. 1 that size of the bladder depots varied among the toads from corresponding to about 30 % of the body mass (#1) to insignificant (#4). During the first 3 days of water loss by evaporation the toads, except #1, mostly rested inside the shelter adopting the typical crowding behaviour, piling on top of each other. Resting be-

haviour also predominated after the bladder depots were emptied until a dehydration corresponding to more than 10 % of the body mass. However, at a body water deficit corresponding to about 20 % of the body mass, restlessness predominated.

Upon return of the water bath, toad #1, which was only moderately dehydrated and rested within the shelter, had entered the water within 45 minutes; toad #4 was observed to enter the bath 4 hours after its return, when the toad was dehydrated to 32 %. After 8 hours, toads #2 and #6 were recorded in the water. They presumably entered the water at deficits corresponding to about 26-27 % of the body mass.

When the toads voluntarily visited the water bath they mostly adopted a characteristic posture with flattened body and stretched legs. They remained in this posture also after repletion of the bladder depot. By contrast, when terrestrial-acclimated toads were placed in water they typically remained seated, or they left the water.

Water balance

Standard hydration
As mentioned, the range of spontaneous variation in body water was assessed by plotting body mass after emptying of the bladder against time in fully hydrated, starving toads. Steady state water balance did not vary with the environment, whether wet or dry, i.e., whether standard hydration depended upon urine production balancing cutaneous influx + resorption of water from the urinary bladder or only upon resorption of bladder water. Data obtained from toads in steady state water balance, staying in or out of water, were therefore pooled. Two examples are shown in Fig. 2, and the regression parameters of all the toads are listed in Table 3, along with spontaneous deviations from the standard values, varying individually from 4 to 9.5 % of the body mass.

The squared correlation coefficients indicated that loss of body mass correlated with starvation
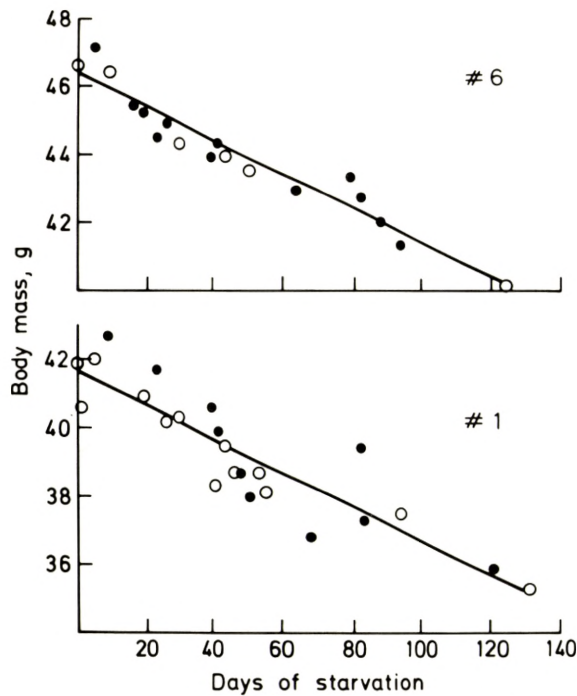
Fig. 2. Steady state water balance in starving toads in (●) or out of (○) water in a simulated terrestrial habitat.

Table 3. Relationship between body mass of fully hydrated, starving toads and duration of starvation, described by $y(g) = a+bx(days)$. Toads acclimated to a simulated terrestrial habitat.

Speci-man no	Regression parameters				"Spontaneous" devations from standard water balance, as percentages of b.m.	
	n	a	b	r^2	Range	Total
#1	23	41.7	−0.050	0.81	−3.9-4.8	8.7
#2	14	37.0	−0.028	0.76	−1.6-3.2	4.8
#4	20	36.9	−0.031	0.60	−2.6-4.3	6.9
#5	17	28.1	−0.049	0.70	−3.3-6.2	9.5
#6	18	46.4	−0.051	0.93	−1.8-2.2	4.0

accounted for from 93 to only 60 % of the variation of body mass with time.

Water balance and environment
Out of water the toads may become dehydrated. The frequency with which the undisturbed toads incurred water deficits was determined from the water balance, as recorded four or more days after the last catheterization of the bladder. Presence of bladder urine signified water balance, whereas absence of urine indicated dehydration. A water deficit was estimated as the difference between standard body mass, read from the regressions listed in Table 3, and actual body mass. The frequency with which the toads became dehydrated before drinking varied with the experimental conditions. In the presence of the shelter water balance was negative in only two out of 34 observations of undisturbed toads, both recorded 4 days after the previous emptying of the bladder (see below). This is in contrast to the situation in the absence of the shelter, where the toads were in negative water balance in seven out of 31 observations, or 19 % (Table 4). It is thus indicated that the increased frequency of staying in water did not balance the effect of removing the shelter on the evaporative water loss. The bladder content in water tended to increase in the absence of the shelter, correlated with more frequent and/or longer stays in water (Table 4) (see also below).

Table 4. Water balance in undisturbed terrestrial-acclimated toads, assessed from bladder contents of urine or water deficits when dehydrated, in the presence and absence of shelter. Means and ranges (in g), and number of observations are indicated.

Specimen no	Shelter present			Shelter absent		
	In water Urine	In dry compartment Urine	Deficit	In water Urine	In dry compartment Urine	Deficit
#1	4.1(2.4-6.6)(5)	3.7(0.9-7.6)(10)	2.0(1)	6.9(3.8-9.0)(7)	5.0(1.9-8.5)(5)	-
#2	1.4(1)	2.9(1.0-5.8)(3)	-	4.5(2.5-6.7)(3)	4.3(1.6-8.0)(6)	1.7(0.2-4.0)(4)
#4	0.9(0.0,1.8)(2)	2.6(0.4-6.3)(7)	2.5(1)	5.4(3.0-8.2)(4)	3.4(0.8-6.0)(8)	1.1(1)
#5	4.2(0.5-9.6)(6)	4.0(2.1-5.9)(9)	-			
#6	4.2(1.4-9.7)(7)	3.6(1.3-4.9)(3)	-	6.3(2.5-9.7)(6)	2.0(0.0-4.6)(5)	0.8(0.8,0.8)(2)

Factors affecting hydration

Size of bladder depots. When toads out of water resorb urine the bladder water depot must ultimately drop to levels that affect hydration of the body. This level was determined in toads with access to the shelter by plotting change in body mass against amounts of urine in the bladder when the animals were placed in water (Fig. 3). The change in body mass (= water) was recorded over a period of about 4 hours, which suffices to restore water balance in dehydrated toads (see below). At bladder contents be-
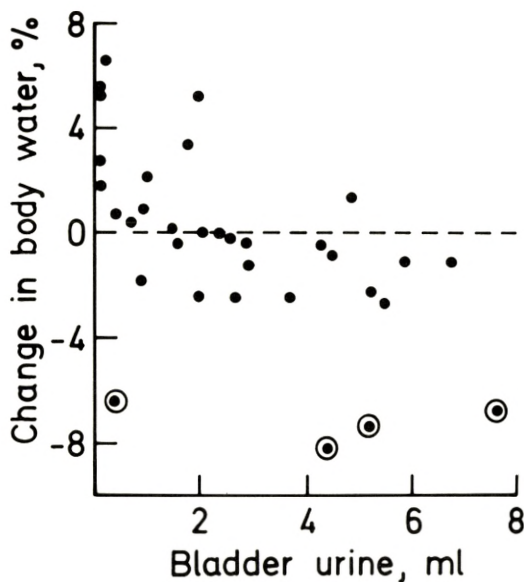

Fig. 3. Relationship between amount of bladder urine in toads occupying dry compartment of a simulated terrestrial habitat and change in body mass during 3-5 h periods of measurements of water influx and urine production. A circle around symbols indicate that the toad attempted to escape from the pail with water during the period of measurement.

low about 1 ml, body mass mostly increased, indicating that the organism reacted on a water deficit, i.e., was in negative water balance.

"Stress". Fig. 3 moreover shows that at larger bladder depots body water tended to decrease slightly or moderately during the period of measurements, suggesting that handling of the toads, including emptying of the bladder, drying and weighing, acted diuretically. A "stress" effect was clearly demonstrated in occasional losses of >6% of the body mass in about 4 hours. These losses were consistently correlated with escape behaviour, the toad trying to climb the walls of the pail throughout the period of measurement. The remaining data were all obtained from resting animals (Fig. 3).

"Spontaneous" deviations. Fig. 3 finally shows that even in the presence of amble amounts of bladder urine the toads might retain water on exposure as if they were dehydrated. Such "spontaneous" deviations from the general trend are consistent with the large scatter of actual steady states around the standard hydrations (Fig. 2 and Table 3).

Moulting. Water balance was followed in a toad observed in the shelter immediately after a moult, when the slough had just been eaten and the body was still slimy (Bendsen, 1956). The body mass was 9.5% above standard mass, but the bladder was empty indicating negative water balance. When the toad was placed in water, the rate of water uptake was several times influx in the toad in its normally hydrated state. Also urine production was relatively high (Fig. 4). During the subsequent hours water influx decreased and urine production increased, approaching a steady state hydration. However, at the end of the 4 hours period of measurements both rates of water uptake and urine flow were still above the rates at a true steady state in the terrestrial-adapted toad (Table 8). Moreover, the quasi steady state water balance corresponded to a body water content of 6 ml above standard hydration, or about 16% of the standard body mass. Despite the surplus water, the moulting toad thus reacted as if the organism was in a substantial negative water balance.
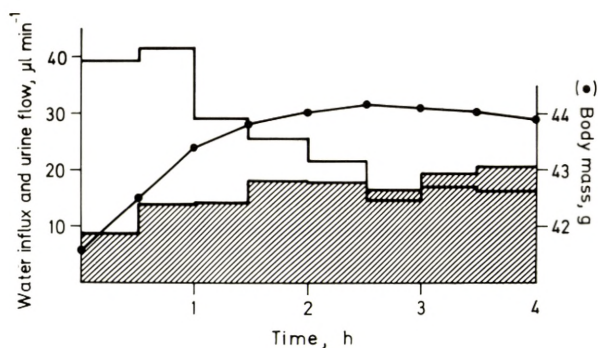
Fig. 4. Water balance in a toad immediately after moulting, reflected in initially high rates of water influx, decreasing with time concurrently with increasing rates of urine flow, as indicated by hatching.

Effect of emptying of the urinary bladder

When toads on land are deprived of their internal water resource they immediately starts to dehydrate. The rate of dehydration is determined by the rate of evaporation. As mentioned, emptying of the urinary bladder did not induce drinking in the toads. The records of toads with empty bladder must therefore increase on days following a catheterization. Table 5 shows that the day after emptying of the bladder the toads were in negative water balance in from 15 to 69 % of observations, varying individually. Also the duration of the effect varied among the toads, correlated with the frequency of recorded occupations of the water bath. The severity of the water deficits, expressed as percentages of the standard body mass, increased from the first to subsequent days, consistent with a longer period of dehydration. Thus, imposed draining of the water depot severely interfered with the water balance, implying that the toads could not be considered undisturbed until from 2 to about 5 days after a catheterization (Table 5).

Table 5. Effect of emptying of the urinary bladder on frequency and degree of dehydration in toads acclimated to a simulated terrestrial habitat, in the presence of the shelter (means ± S.D., number of observations in parentheses).

Speci- men no	Days									
	1		2		3		4		≧6	
	% dehydrated	Dehydration % b. m.	% dehydrated	Dehydration % b. m.	% dehydrated	Dehydration % b. m.	% dehydrated	Dehydration % b. m.	% dehydrated	
#1	15(13)	3.9(3.4,4.3)	14(7)	8.8	0(6)		17(6)	5.2	0(4)	
#2	77(13)	4.2±2.4	100(7)	8.1±3.3	83(6)	6.4±4.6	67(6)	8.7±4.3	0(4)	
#4	69(13)	3.5±2.1	57(7)	4.9±3.6	33(6)	6.3(4.3,8.2)	17(6)	7.5	0(4)	
#5	33(9)	2.1(1.4-3.1)	0(4)		0(6)		0(3)		0(2)	
#6	69(13)	3.8±1.3	43(7)	7.1(5.3-9.7)	50(6)	5.0(0.9-7.3)	0(6)		0(4)	

Water loss and depot size

In order to estimate the functional significance of the urinary bladder depot in the water balance of the toads in dry surroundings its size should be compared with the rate at which water is lost.

Water may be lost by voiding of urine and by cutaneous evaporation. However, undisturbed toads out of water presumably only void urine exceptionally. Only in one out of 103 observations on daily losses in body mass was this loss so large that it could not be accounted for by evaporation, imply-

ing that urine had been voided since the previous weighing. Thus, emptying of the urinary bladder in toads out of water was practically exclusively by resorption through the bladder wall.

The rate of evaporation varied markedly with the presence or absence of the shelter, increasing by a factor of about 3 upon its removal, with no difference in mean content of water in the urinary bladder (Table 6). In the presence of the shelter the mean amount of urine stored in the bladder corresponded to from 1.5 to 2.4 times the daily mean

Table 6. Relationships between daily dehydration losses by evaporation and mean amounts of urine stored in the bladder in toads occupying dry compartment of a simulated terrestrial habitat in the presence (+) or absence (−) of shelter (means ± S.D., numbers of observations in parentheses).

Specimen no	#1		#2		#4		#5		#6	
	+	−	+	−	+	−	+	−	+	−
Evaporation,	2.0±0.43	5.5±2.4	1.2±0.39	3.9±1.1	1.3±0.61	3.9±1.9	1.7±1.1	-	1.7±0.56	5.3±1.7
ml d^{-1}	(5)	(6)	(22)	(12)	(16)	(12)	(4)		(13)	(13)
Bladder urine, ml	3.3	5.0	2.9	1.9	2.0	2.9	4.0		3.6	1.2
Urine/evaporation	1.7	0.9	2.4	0.5	1.5	0.7	2.4		2.1	0.2

evaporative losses, against less than 1 in the absence of the shelter (Table 6).

In the absence of the shelter the crowding behaviour significantly reduced the rate of evaporatory water losses, to about 3 ml d^{-1} when the heap included all 4 toads, against about 6 ml d^{-1} in toads that were single when recorded (Table 7). The

Table 7. Relationship between number of toads crowding and rate of evaporation (ml d^{-1}) (means and ranges, numbers of observations in parentheses) in toads acclimated to a simulated terrestrial habitat in the absence of shelter.

Toads	Number of toads in a crowd		
single	2	3	4
5.7(3.4-8.8)(16)	5.0(3.3-7.2)(11)	3.6(0.8-6.6)(7)	2.9(0.8-3.8)(8)

One-tailed Wilcoxon test for statistical significance of differences (Siegel and Castellan 1988). Single vs 3, P<0.013; Single vs 4, P<0.0002; 2 vs 4, P<0.001.

toads that occupied the position at the bottom of a heap exhibited the smallest losses in body mass, down to 0.8 g d^{-1}. But also the top position seemed to offer some protection against water loss, as compared to single toads. This is indicated by maximum losses of 3.8 ml d^{-1} in crowding toads, against almost 9 ml d^{-1} in single toads. Behaviourally determined differences in evaporative water losses among the toads out of water thus amounted to an order of magnitude.

Dehydration-rehydration: quasi and actual water balance
In order to assess water deficits in dehydrated toads a reference is needed. This reference may be the standard body mass, as defined above. The experi-

ments, however, revealed other possibilities. Thus, when dehydrated toads returned to water, a steady state was established within about 4-5 hours, when urine production balanced water uptake (Fig. 5).
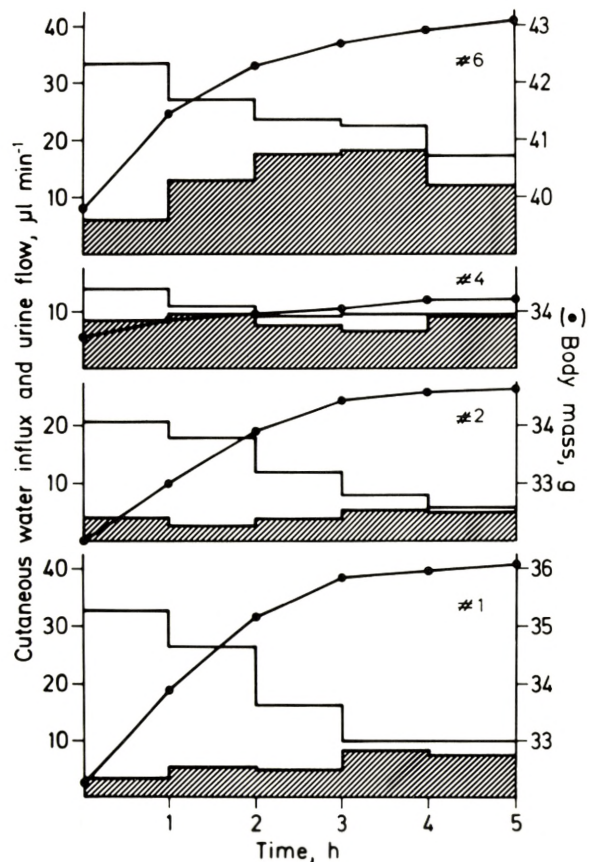

Fig. 5. Examples of pattern of rehydration in dehydrated toads. The diagrams showing rates of urine flow are hatched. Dehydration in per cent of body mass: # 1, 11 %; # 2, 8 %; # 4, 2 %; # 6, 8 %.

This steady state body mass tended to be below that prior to dehydration, varying with the degree of dehydration. Figure 6 shows the relationship bet-
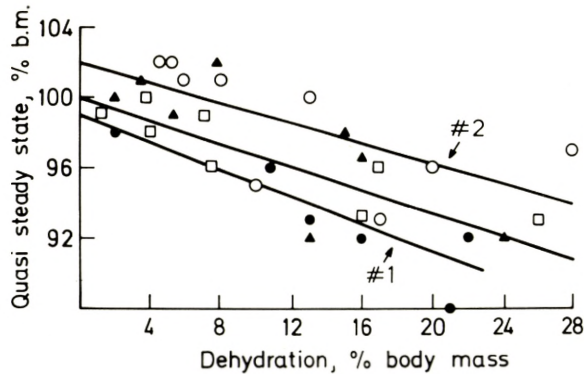

Fig. 6. Relationship between water deficit in dehydrated toads and quasi steady state water balance at rehydration. Regression lines are indicated for two individual toads as well as for all toads. Symbols indicate: ●, toad # 1; ○, toad # 2; □, toad # 4; ▲, toad # 6. See text for further explanation.

ween percentage loss of body mass (= body water), spontaneous or imposed in the absence of an external water resource, and steady state body mass after 4-5 hours rehydration. Scatter and individual variation were large, but a linear regression showed a significant negative slope, described by the equation $y = 100 - 0.34x$, $r^2 = 0.52$, $n = 34$, where y is the steady state body mass, as a percentage of body mass prior to dehydration, and x is percentage dehydration.

The water balance reached after 4-5 hours of rehydration was not final, at least not after moderate to severe dehydration, and during the following 2-3 days a higher level was approached asymptotically (Fig. 7). Physiologically, the acute level reached within some hours of rehydration thus reflected a quasi steady state balance. Behaviourally, however, the organism reacted as if still dehydrated: the toads remained in rehydration position, and if removed from the water they would return.

After 2-3 days the rehydrated toads resumed their normal behaviour, but the water balance might still differ substantially from the standard. It
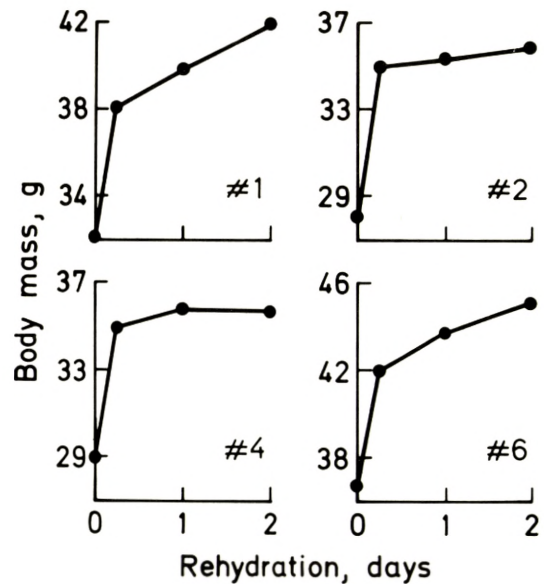

Fig. 7. Examples of temporal transition from quasi steady state to actual water balance in rehydrating toads initially dehydrated to about 75 % of body mass at quasi steady state.

may be defined as the actual water balance. The difference from the standard body mass in undisturbed, fully hydrated toads reflected the spontaneous deviations in water balance, mentioned above.

Cutaneous water permeability

Ultimately, water balance in the toad depends upon uptake of water through the skin, or more specifically through the pelvic patch. Cutaneous water permeability is thus a basic parameter in the toad water balance. The permeability varies with a number of factors, both environmental and internal, including the hydration of the organism.

Fully hydrated toads

The cutaneous water permeability, determined as the rate at which toads take up water when placed in pails with about 1 cm deep water, is labile and highly variable even in the fully hydrated state. However, significant trends could be discerned in the toads acclimated to the simulated terrestrial habitat. Thus, toads taken from the dry compart-

Table 8. Cutaneous water uptake (μl min^{-1}) in toads kept in water or in a simulated terrestrial habitat (means ± S.D., figures in parentheses are numbers of consecutive hourly (4 or 5) or half hourly (7-11) measuring periods).

Environment	Days in water	#1	#2	#4	#6
Pail with water	≧30	5.0±0.93(5)	4.2±0.25(5)	4.1±0.36(5)	3.8±0.57(5)
	2-5	5.9±2.9 (4)	5.7±0.30(5)	7.4±0.55(5)	7.7±1.0 (5)
		6.7±0.19(5)	6.0±0.49(5)	9.7±0.88(5)	10.6±0.93(5)
		7.2±0.91(5)	6.8±0.47(5)		
	~1	12.5±0.31(4)			8.5±0.58(5)
Terrestrial, in water		5.7±1.8 (5)		7.7±0.89(8)	6.4±1.5 (5)
		6.2±0.76(4)			7.3±1.5 (5)
		10.3±2.2 (5)			8.8±1.5 (8)
					10.2±1.8 (4)
in dry compartment		12.6±1.5 (5)	7.6±0.29(5)	10.7±0.84(4)	10-7(11)
		15-8(7)[a]	20-7(7)[a]	11-7(7)[a]	6[b]
		16-5(11)[a]	14[b]	16-9(11)[a]	
		25[b]	15[b]	12[b]	

[a] Initial and final level of influx; [b] mean influx over measuring period.

ment tended to absorb water faster than when they were taken from the water bath (Table 8). Rates of water influx in toads from the dry compartment might remain high throughout the 4-5 hours period of observation, or they might decline (Table 8).

The time course in the transition from terrestrial-acclimated water permeabilities to aquatic-acclimated varied, but the low influx rates measured in the long-term, ≥ 30 days, aquatic-acclimated state were not reached after 2-5 days in water (Table 8). By contrast, the transition from wet to dry environment levels of water permeability proceeded fast. Thus, leaving the long-term water-acclimated toads dry in their pails for about 1 hour might increase the water permeability by a factor of 3.

Dehydrated toads
The effect of dehydration on the cutaneous water permeability may be characterized by the relationship between degree of dehydration and initial rates of water uptake, when contact with water is re-established, and by the temporal pattern of return to permeabilities typical of the fully hydrated state.

To assess degrees of dehydration water deficits were expressed as percentages of the body mass at the quasi water balance established within the first hours of rehydration. This reference was chosen be-

cause it represented the immediate physiological state towards which the organism adjusted.

Dehydration and initial water influx. When the dehydrated toads returned to water their initial rates of water influx varied with the degree of dehydration. The relationships could be described by linear regressions of water influx, in μl min^{-1}, on percentage dehydration, y = a + bx. Table 9 indicates a close correlation: squared correlations coefficients mostly exceeded 0.80.

Table 9. Relationship between degree of dehydration in toads, acclimated to a simulated terrestrial habitat, and initial rate of water uptake at rehydration, expressed by the linear regression y(μl min^{-1}) = a+bx(% dehydration).

Specimen no		Regression parameters		
	n	a	b	r^2
#1	9	16.7	1.31	0.84
#2	17	13.2	1.80	0.83
#4	14	12.3	1.93	0.83
#5	6	15.8	0.65	0.93
#6	13	21.6	1.60	0.61

Halftimes of declining water influx. The decline in water influx in rehydrating toads could be described as logarithmic functions of time, lny = a + bt. Mostly,

the decline in influx closely approximated this relationship, shown by the high correlation coefficients (Table 10).

Table 10. Relationship between decline in cutaneous water influx in rehydrating dehydrated toads with time, expressed by the regression equation $\ln y$ (μl min^{-1}) $= a + bx(h)$.

Specimen no	Dehydration %	Regression parameters		
		a	b	r^2
#1	16	3.45	−0.099	0.89
	21	3.75	−0.184	0.98
	22	4.08	−0.259	0.98
	13	3.34	−0.115	0.94
#2	13[a]	3.91	−0.157	0.93
	20	4.03	−0.192	0.93
	28	4.14	−0.167	0.98
	17	3.57	−0.158	0.99
	10	3.05	−0.112	0.98
#4	17	4.08	−0.223	0.90
	26	3.88	−0.175	0.95
	16	4.10	(−0.479)	0.90
	7.2	3.19	−0.359	0.997
	7.6[a]	3.06	−0.242	0.98
#5	19	3.44	−0.152	0.96
#6	13	3.89	−0.072	0.99
	24	3.90	−0.118	0.91
	15	4.01	−0.181	0.99
	16	3.89	−0.129	0.98

[a] Spontaneous dehydrations

The exponential decline in water permeability with time in rehydrating toads prevailed until the quasi-equilibrium water-balance had been reached, when the rate of deceleration in water influx tended to increase. Fig. 8 shows some examples.

The rate and pattern of decline in water influx in the rehydrating dehydrated toads further elucidated the dynamics of the cutaneous water permeability. Thus, the rate of decline varied with the dehydration. The rate of decline, expressed by the halftime ($t_{1/2}$) of the exponential phase of decline, was linearly correlated with the initial dehydration, $y(t_{1/2}) = a + bx$ (% dehydration). Fig. 9 shows examples based on 8 or 12 recordings of the relationship. The slopes of the regressions were statistically significant from zero. However, the scatter was
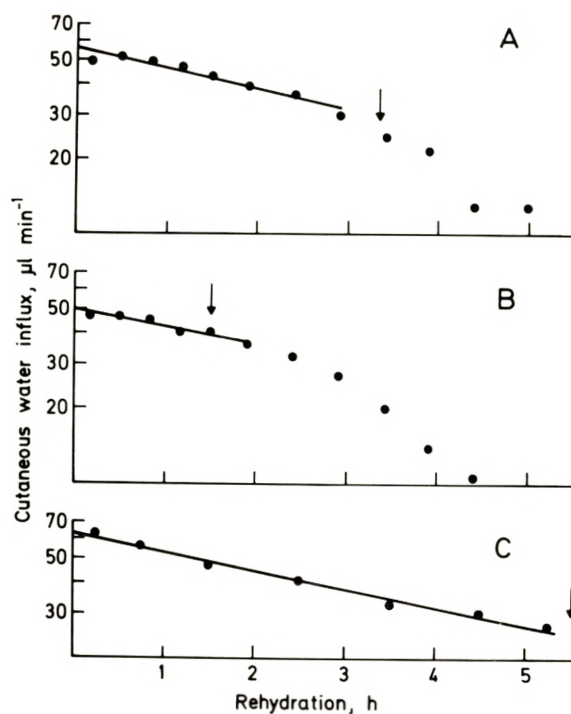

Fig. 8. Examples of pattern of rehydration in dehydrated toads, as reflected in declining cutaneous water influx with time in the same toad (# 2). The arrow indicates establishment of quasi steady state water balance. Dehydration in per cent of body mass at quasi steady state: A, 24 %; B, 11 %; C, 28 %.

large. The squared correlation coefficients indicated that the relationship accounted for from 40 to 70 % of the variation.

Finally, it is notable that extrapolation of the relationships to zero dehydration corresponded to halftimes of about 2 hours. This is consistent with the large increase in the cutaneous water permeability when the toads leave the water in the absence of any dehydration. Fig. 9 shows that this immediate response to a dry environment equalled water deficits corresponding to 16-18 % loss of body mass by evaporation.
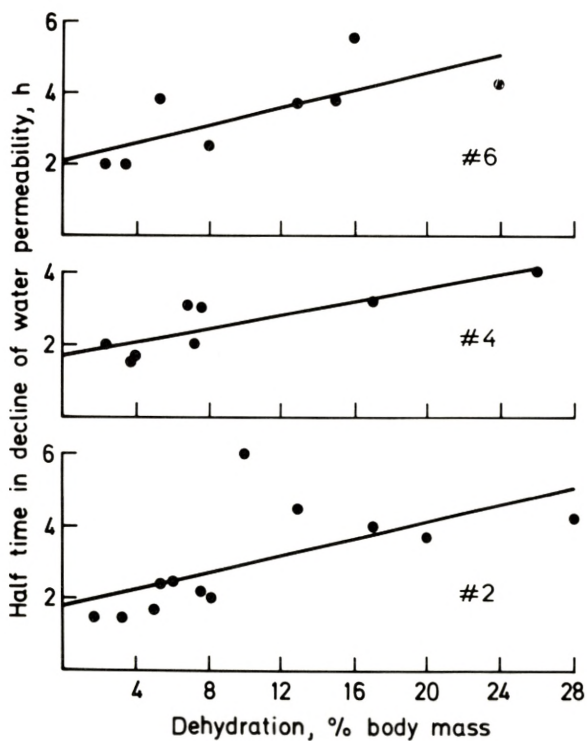
Fig. 9. Relationships between dehydration and halftimes in decline of cutaneous water permeability in rehydrating toads. The regression lines are inserted. The regression equations and statistical significances of slopes differing from zero were the following: # 2, y = 0.119x + 2.14, r^2 = 0.53, P = 97.5 %; # 4, y = 0.092x + 1.70, r^2 = 0.72, P = 99.5 %; # 6, y = 0.117x + 1.81, r^2 = 0.41, P = 97.5 %.

Kidney-bladder function

Excretion and depot function

The toad kidney-bladder complex acts both in excretion and water storage. It is therefore of interest to ascertain the relative importance of the complex in serving these two functions. This implies proportioning of bladder urine voided, i.e. excreted, and urine resorbed, thus serving as a water depot. As mentioned, undisturbed toads seldomly voided urine out of water in the simulated terrestrial habitat. Excretory function was thus predominantly reserved for the stays in water. The extent to which water taken up during visits to the water bath was used in excretion and in replenishing urinary bladder depots could be estimated by comparing time needed for such replenishment and time actually spent in water by the individual toad. The estimates were based on means for daily evaporative water losses, cutaneous water permeability in terrestrial-acclimated toads, and time spent in water, calculated as the percentage of observations of toads in the water bath, expressed in hours. Estimates were made both in the presence and absence of the shelter. The ratio between time spent in water and time needed for replenishment of evaporative losses expresses the significance of excretory function of urine production in relation to depot repletion during visits to the water resource.

Table 11. Comparison of time spent in water with time needed to replenish urinary bladder water lost by evaporation in toads acclimated to a simulated terrestrial habitat, in presence and absence of shelter.

Specimen no	Daily evaporative loss Shelter		Cutaneous water influx[a]	Time for replenishment Shelter		Daily time spent in water Shelter		Ratio: Time spent/ Replenishment time Shelter	
	present ml	absent ml	ml h^{-1}	present h	absent h	present h	absent h	present	absent
#1	2.0	5.5	0.9	2.2	6.1	11	15	5.0	2.4
#2	1.2	3.9	0.9	1.3	4.3	1.4	5.5	1.1	1.3
#4	1.3	3.9	0.7	1.9	5.6	1.9	6.7	1.0	1.2
#5	1.7	-	0.7	2.4	-	9	-	3.8	-
#6	1.7	5.3	0.6	2.8	8.8	11	11	3.9	1.3

[a] Typical of terrestrial-acclimated toads, presumably too high for long-term stays in water (cmp. Table 8).

Table 11 shows pronounced individual variation, as well as an effect of presence or absence of the shelter. Thus, in the presence of the shelter the ratio varied from about 1 to 5, i.e., from predominance of depot replenishment behaviour to the voiding of about 4/5 of water absorbed. In the absence of the shelter most of the water was used for refilling of the bladder depots, and only little was used for excretion before the toads returned to the dry compartments. Only in toad #1 most of the water absorbed was still voided during the stay in water.

Exploitation and pattern of use of urinary bladder capacity
The absolute, anatomical, capacity of the toad bladder in storing urine is not known, but we may define a physiological capacity as the maximum amount of urine found in a large number of recordings from undisturbed toads, e.g., expressed as a percentage of the body mass. This maximum amounted to 26 % in one out of a total of 106 recordings in the toads observed in or out of water. This high percentage was an exception. In the remaining 105 recordings the maximum amounts of urine varied individually from 17 to 22 % in the presence of the shelter, and from 22 to 23 % in the absence of the shelter.

The pattern of use of this physiological water storage capacity in undisturbed terrestrial-acclimated toads with access to water might theoretically range between two extremes. One extreme would be to establish contact with the water resource only when the depot was depleted, perhaps even postponing contact until the water balance had become negative, and then to stay in water at least until bladder urine had accumulated in amounts corresponding to the physiological capacity. The other end of the range would imply intermediate, but rather constant fillings of the bladder, maintained by short but frequent visits to the water bath. Between these extremes intermediary patterns might prevail. Moreover, the pattern might vary individually or with the environment, e.g., with the presence or absence of a shelter.

The actual pattern adopted may be evaluated

from the frequency distributions of amounts of urine and water deficits recorded in the undisturbed toads. Values were pooled for toads #1, 5 and 6, which exhibited high ratios between time actually spent in water to time needed for replenishment of water lost by evaporation and for the toads #2 and 4 with the low ratios (Table 11). It seemed reasonable to suspect that if toads differed significantly in patterns of use of bladder capacity, such difference should correlate with this ratio. Moreover, the data were grouped according to location of the toad, in or out of water, as well as to presence or absence of shelter.

If toads habitually resorbed bladder urine to depletion before repleting to physiological capacity all degrees of bladder filling should tend to be equally represented, whereas the other end of the range
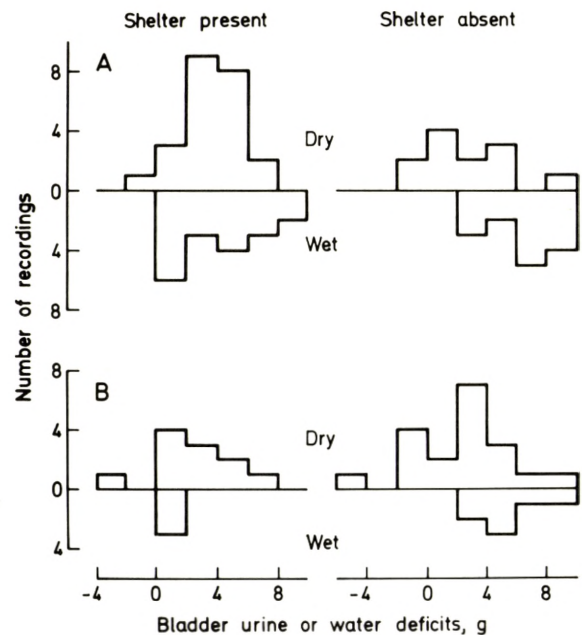

Fig. 10. Frequency distribution of amounts of urine stored in bladder or body water deficits in undisturbed toads taken out of or in water in a simulated terrestrial habitat in the presence or absence of a shelter. (A) presents data from 3 toads that visited the bath frequently, (B) 2 toads that were infrequent visitors. See text for further explanation.

Table 12. Amounts of urine in bladder of undisturbed toads, acclimated to a simulated terrestrial habitat, in presence or absence of shelter. Means and ranges(g) of recordings form 2 toads (#2 and 4) visiting water resource primarily for replenishment of water lost by evaporation, and 2 toads (#1 and 6) tending to stay longer (see Table 11). (Figures in parentheses are numbers of recordings).

Specimen no	Shelter present		Shelter absent	
	In water	In dry compartment	In water	In dry compartment
#2 and 4	1.1(0.0-1.8)(3)	2.2(0.4-6.3)(11)	5.0(2.5-8.2)(7)	2.4(0.8-8.0)(19)
#1 and 6	4.2(1.4-9.7)(12)	3.6(1.3-7.6)(13)	6.6(2.5-9.7)(13)	2.8(0.0-8.5)(12)

Wilcoxon test for statistical significances of differences in bladder urine in water: presence vs absence of shelter; #2 and 4, P = 0.0083; #1 and 6, P = 0.0078.

should tend towards a modal distribution. Fig. 10 shows that in toads #1, 5 and 6, when occeupying the dry compartments of the simulated, terrestrial habitat in the presence of the shelter, data were modally distributed with most recordings of bladder urine falling between 2 and 6 ml. The mode was 3-4 ml. It is thus indicated that water lost by evaporation was usually replenished before the depot in the bladder was depleted. It is moreover noteworthy that the amounts of bladder urine in water were about the same as out of water in these toads that stayed 3-4 times longer than needed to replenish water lost by evaporation. It thus seems that also during the prolonged contacts with water the bladder capacities were not fully exploited but urine was mostly voided at intermediate degrees of filling. Finally, relatively frequent replenishments of evaporative water losses, combined with moderate exploitation of bladder capacities, would minimize differences between mean contents of bladder urine in and out of water, as actually found (Table 12).

The number of observations made on toads #2 and 4 are too few to be conclusive. The data are, however, compatible with a predominantly moderate exploitation of the bladder capacity also in these toads.

Removal of the shelter had no obvious effect on storage of urine in the toads taken from the dry compartment. But removal significantly increased mean bladder content in water, both in the toads that mainly visited the water bath for replenishment of evaporative losses and in the toads that spent most of the time in water, using urine production for excretion (Table 12).

Urine production in absence or presence of water

The rate at which urine is produced varies with the state of hydration of the body, but the environment, whether wet or dry, may also be of importance. The course of urine production out of water was therefore followed in fully hydrated toads upon transfer from water to dry pails with moist filter paper under the lid to minimize evaporative water losses.
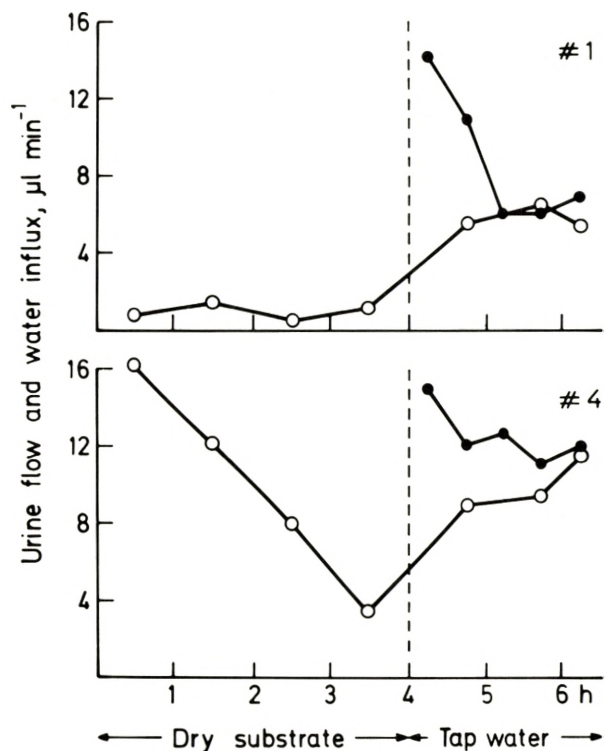

Fig. 11. Examples of effects of transition from a dry environment to tap water on urine flow (○) and cutaneous water influx (●) in fully hydrated toads.

Urine production was measured by hourly emptying of the bladder. Fig. 11 shows that the rate of urine production began to decline immediately, and after some hours the rates had dropped to a level of about 2 µl min⁻¹, or one third or less the rate at steady state in water (comp. Table 8). The body water deficit amounted to 1-2 % of the body mass, i.e., within the range of spontaneous variation (Table 3).
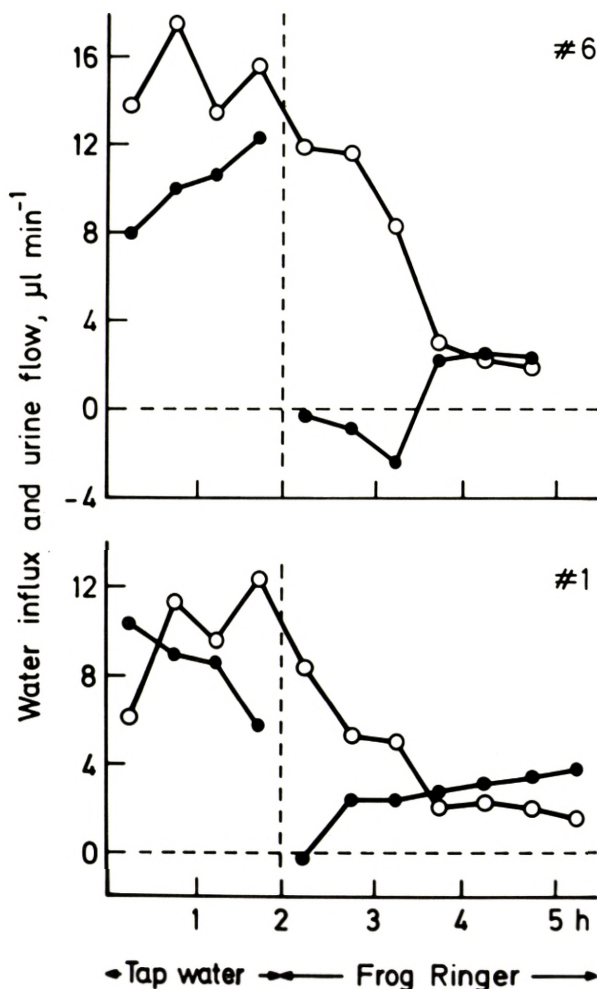
Upon return to water, rates of urine production

Fig. 12. Examples of effects of transition from tap water to frog Ringer or isoosmotic NaCl solution on cutaneous water influx (●) and urine flow (○) in toads.

immediately rose, approaching levels in toads in steady state in water (Fig. 11). In a typical experiment, the mean diuresis rose from 2.3 ± 1.0 (S.D.) µl min⁻¹, measured in the 4 toads after a 4 hours stay in the dry pails, to 6.1 ± 1.9 and 7.3 ± 2.3 µl min⁻¹ during two consecutive hours following addition of water to the pails. Influxes of water concurrently decreased from 11.8 ± 3.6 to 8.4 ± 2.3 µl min⁻¹.

Urine production in isotonic saline

It was thus indicated that other factors than loss of body water are involved in the control of urine production upon transition from water to a terrestrial environment and *vice versa*. To disclose such factors, toads were transferred from tap water to an isotonic medium, frog Ringer or 130 mM NaCl solution, and rates of water influx and urine production were measured half-hourly to follow the time course of the two parameters in greater detail.

As expected, cutaneous water influx dropped immediately to values about zero, but tended to increase moderately during the 4 or 4½ hour periods of recordings, perhaps correlated with uptake of NaCl. Rates of urine production declined according to the same pattern as in a dry surrounding, to approach about 2 µl min⁻¹. Some examples are shown in Fig. 12. Thus, the kidney function was indifferent to the nature of the environment to which the skin was exposed. But the rate of urine production varied with the cutaneous water influx.

Urine production during rehydration

The quasi steady state of water balance that developed in rehydrating dehydrated toads arose from an enhanced kidney function. The rate of urine production approached or exceeded the rate of cutaneous water uptake when this was still much higher than in toads in final water balance. Fig. 5 shows some examples of the relationships between cutaneous influx and urine production, which determine the pattern of rehydration.

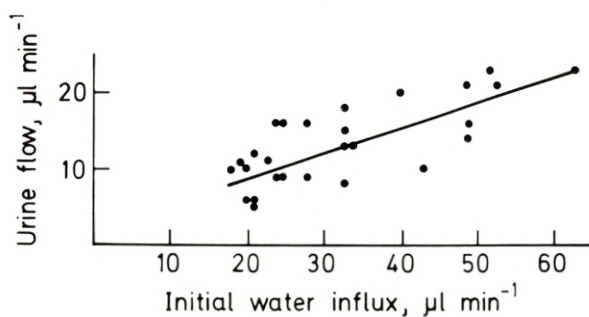The actual stimulus for the increased diuresis during rehydration is not known. But it is notewor-

Fig. 13. Relationship between initial water influx and urine flow at quasi steady state water balance in rehydrating dehydrated toads. The inserted line is the linear regression: y(maximum urine flow) = 0.31x (water influx) + 3.29, r^2 = 0.57, n = 27. The statistical significance of the slope differing from zero was P = 97.5 %.

thy that the level of urine flow at establishment of the quasi steady state was highly correlated with the initial rate of water influx (Fig. 13). During re-

hydration polyuria may thus develop at remaining water deficits that result in anuria during dehydration from the fully hydrated state.

Discussion

Anticipatory drinking

In agreement with their nature of terrestrial anurans, the toads in the simulated terrestrial habitat spent most of their time in the dry compartments, visiting the water only from time to time. These occasional visits to the water bath to compensate for water lost by evaporation mostly sufficed to prevent deficits in body water from arising. In the presence of the shelter, water balance was negative in only 6 % of the observations, against 19 % in the absence of the shelter. The higher rate of water loss was thus correlated with a higher incidence of deficits in body water in the toads out of water. Mostly, however, water contact was reestablished before depletion of the bladder depots. Katz *et al.* (1986) obtained similar results with the toad *Bufo viridis* kept in a simulated terrestrial environment, including soil and a water bath. The toads maintained plasma and urine osmolarities at the same levels as in nature and urine was consistently stored in the bladder in amounts ranging from 1.4 to 5.5 % of the body mass. The toads maintained normal hydration and osmolarity of the body fluid by visiting the water bath a few times a day. Between visits they

reduced evaporative losses of water by burrowing in the soil (Katz, 1989). These observations on toads kept under terrestrial conditions with access to water raises the question about the nature of the physiological mechanisms that control water uptake behaviour.

Mammalian physiologists distinguish between primary and secondary drinking. Primary drinking is drinking that can be attributed to a water deficit, whereas in secondary drinking there is no immediate need for water, so that drinking does not appear to be related to the actual fluid economy of the body. Primary drinking is thus an emergency behaviour, whereas secondary drinking is geared to anticipated water requirements (Fitzsimons, 1979).

These concepts may be adopted also in amphibian water economy where cutaneous uptake of water is functionally equivalent to oral intake of water in other vertebrate groups. We may define primary or emergency water uptake behaviour as the establishment of contact with water in response to a deficit in body water, and secondary or anticipatory water uptake behaviour as a pattern of "drinking"

events, of frequency and duration that secure normal levels of body water, based on water depots in the urinary bladder.

The question arises how to distinguish primary from secondary drinking behaviour. The postponement of drinking until water deficits develop need not imply emergency drinking, i.e., drinking in response to the deficit. Occasional water deficits are compatible with a pattern of habitual visits to water, i.e., anticipatory drinking. It may be a characteristic of emergency drinking behaviour that it is correlated with restlessness and exploratory, water seeking behaviour (see below).

Presumably, anticipatory drinking behaviour predominated in the toads in the simulated terrestrial habitat. The existence of behavioural patterns of visits to the water bath geared to an anticipated demand was strongly supported by the finding that deficits in body water typically developed if the bladder depots were emptied by catheterization.

The literature on amphibian water balance does not discuss whether drinking is primary or secondary, presumably because the drinking behaviour is generally believed to be determined by immediate needs for water. Studies of amphibian water balance have focused on aspects related to dehydration, such as tolerance towards desiccation and rates of rehydration, correlated with habitat (Claussen, 1969, 1973; Farrell and MacMahon, 1969; Katz and Graham, 1980; Krakauer, 1970; Schmid, 1965; Thorson, 1955). These studies show that terrestrial amphibians tend to be more resistant to desiccation and to rehydrate faster than aquatic amphibians. But the results do not inform about the role that dehydration and rehydration plays in the amphibian water economy.

The relative importance of emergency and anticipatory drinking in the life of amphibians can be ascertained from the actual water balance in freshly caught animals. The scattered data available are consistent with a predominant role of anticipatory drinking in maintaining normal hydration. Sinsch (1987) compared body hydration in frogs caught in the field during rain and after one week of dry weather. The frogs were consistently in water balance, both the two terrestrial species, *Rana temporaria* and *Rana lessonae*, and the more aquatic *Rana ridibunda*. In field studies in Israel, Katz *et al.* (1986) found that the terrestrial toad *Bufo viridis* remained normally hydrated, also in the dry season of the year. The plasma osmolarity was consistently normal and the bladder contained urine ranging from 2.5 to 8.8 % of the body mass.

Normal hydration of the body can be maintained by means of water depots in the bladder even in xeric habitats. Thus, Lee (1968) found that the burrowing frog, *Heleioporus eyrei*, might store urine in amounts that prevented dehydration while the frogs foraged at night in the hot arid summers of southwestern Australia. In only 5 out of 15 animals was the bladder depleted of urine at the end of the foraging period. Moreover, the data presented in Fig. 1 of the article indicate that there was no clear correlation between urine remaining in the bladder and plasma Na concentrations.

There seems to be striking similarities between water balance behaviour in anurans and mammals. Also mammals predominantly satisfy their needs for water by anticipatory drinking not motivated by true thirst (Fitzsimons, 1979; Toates, 1979). In man and other mammals, osmoregulatory thirst has been regarded "as an emergency mechanism that intervenes only when the water in food and anticipatory or habitual drinking, in combination with fully utilized effect of ADH, are insufficient to maintain Na concentrations below a certain level" (Andersson *et al.*, 1982). Also in toads and frogs osmoregulatory "thirst" may be regarded as an emergency mechanism, which, however, differ from that applying in mammals by being independent of neurohypophysial function (Jørgensen et al., 1969; Jørgensen, in manuscript). Anuran water balance may therefore reflect vertebrate water balance behaviour and physiology at a less evolved level.

Urinary bladder: water depot function

A large literature deals with the urinary bladder as

a water depot in terrestrial environments. Most studies have been concerned with the storage capacity of the bladder, which tends to be correlated with habitat, larger in the more terrestrial species (Bentley, 1966; Espina and Rojas, 1972; Monge, 1963; Ruibal, 1962; Shoemaker, 1987; Shoemaker *et al.*, 1969). Less is known about the actual utilization of the storage capacity. According to Claussen (1974) "the situation is analogous to having a good knowledge of the total lung capacity of an organism while knowing little about tidal volume."

The anatomical capacity of the bladder in *Bufo bufo* is not known, but the highest bladder fillings measured in the simulated terrestrial habitat amounted to 20-30 % of the body mass. This corresponds to the maximum volume voluntarily retained in *Bufo fowleri* (Claussen, 1974) and in *Bufo marinus* (Shoemaker, 1964), but is less than the 40 % observed in *Bufo cognatus* from arid regions in North America (Ruibal, 1962). In *B. bufo*, the utilization of the capacity of the bladder both varied individually and with the "aridity" of the environment, the mean bladder contents in water varying from <50 % of the physiological capacity in the presence of the shelter to about 70 % in the absence of the shelter. This tendency to increased utilization of the bladder capacity under more arid conditions was also observed in the toad *Bufo arenarum* (Schmajuk and Segura, 1982) and in frogs *Rana* spp (Sinsch, 1987). Thus in *R. temporaria* and *R. lessonae* mean urine volume in animals caught in the field during rainy weather was about 2 % of the body mass, against 11-12 % in dry weather. As mentioned, *Bufo viridis* only accumulated moderate amounts of urine even under terrestrial conditions (Katz *et al.*, 1986), despite its nature of steppe animal and preference for warm and dry microhabitats (Freisling, 1948; Jungfer, 1954; Gislén and Kauri, 1959).

Dehydration-rehydration behaviour

Absence of water, resulting in dehydration, affected the behaviour of the toads, which altered between crowding with minimized surface area and escape behaviour. The latter was practically absent when water and shelter were available in the simulated terrestrial habitat. The escape behaviour was attempts to climb the walls, and it could be interpreted as water seeking.

It is notable that the toads did not adopt such water seeking behaviour until the losses in body water approximated 20 % of the body mass. Losses in the presence of the water resource seldomly exceeded about 10 % of the body mass, consistent with the concept of predominantly anticipatory drinking among the toads (see above).

Similar behavioural patterns in a desiccating environment are known from other amphibians. Thus crowding, reducing loss of body water by evaporation, has been described both in urodeles (Gehlbach *et al.*, 1969) and anurans (de Almeida, 1926; Heatwole *et al.*; 1971, Johnson, 1969b). Progressing dehydration typically seems to induce escape activity alternating with crowding and water conserving postures (Heatwole and Newby, 1972; Putnam and Hillman, 1977.).

When the toads in the present study voluntarily visited the water bath they adopted the posture that has been interpreted as a behavioural adaptation for rehydration in dehydrated frogs and toads (Dole, 1967; Freisling, 1948; Hillyard *et al.*, 1987; Johnson, 1969a; Stille, 1952, 1958). This interpretation is based on the finding that dehydrated frogs and toads, when they establish contact with water or a wet substratum such as moist soil, adopt a flattened posture, pressing the pelvic patch and inside of the thighs against the substratum. The observation that also fully hydrated toads adopt this posture, indicates that it is not only a behavioural response in dehydrated toads. It rather seems to be part of the habitual behaviour that maintains the water depots in the fully hydrated state of the organism. This interpretation is also consistent with observations made on toads and frogs in the field. Thus, Carpenter and Gillingham (1987) observed a population of *Bufo marinus* in a subtropical forest in Puerto Rico to hydrate in water holes or on wet soil

or a large decaying log with such frequency that their water uptake behaviour may well have been anticipatory. The same seems to apply to Stille's (1952) observations on behaviour of toads and frogs on the wet sandy beaches of Lake Michigan, although Stille himself interpreted the water uptake behaviour as rehydration in dehydrated animals.

Water balance-water turnover relationships

Water balance in the undisturbed toads, as reflected in standard body mass, varied, but not with the compartment occupied, whether wet or dry (Fig. 2). Water economy was thus independent of whether the resources for maintenance of the balance included both external water and bladder urine or only urine. This environmental independence of water balance should be appreciated on the background of a drastic change in rate of water turnover with change of compartment. In the experiments, toads in steady state in water absorbed about 6-10 μl min^{-1}, amounting to a daily turnover of 9-13 ml of water. In the presence of the shelter, the net water turnover in the fully hydrated toads out of water was about 1.5 ml d^{-1}, corresponding to the evaporative water loss. Thus, in these toads the net absorption of urine from the bladder was about 1 μl min^{-1} independently of the volume of urine within the range from about 1 ml to 10 ml, the maximum approached (Table 4). If we assume the bladder to be approximately spherical, the areas of the bladder wall at the two fillings would be 1.2 and 5.6 cm^2, respectively, and area specific net water absorption would thus amount to 0.83 and 0.18 μl cm^{-2} min^{-1} over the range of areas.

Net absorption of water from the bladder is the difference between water flux through the bladder wall and concurrent production of urine. Maintenance of water balance with decreasing stores of urine in the bladder must therefore depend upon decreasing rates of urine production and/or increasing permeability of the bladder wall to water. The relationship between these two components in the

kidney-bladder function in terrestrial-acclimated fully hydrated toads is unknown.

Irrespective of the mechanisms applying, the area specific flux of water from bladder lumen to the body must exceed 0.83 μl cm^{-2} min^{-1}, at least when the bladder content approaches 1 ml. This permeability may be compared with bladder permeabilities measured in isolated bladders. In such *in vitro* preparations from frogs and toads in water, fluxes amounted to about 0.1-0.2 μl cm^{-2} min^{-1}, increasing to 1-2 μl cm^{-2} min^{-1} in bladders from dehydrated animals, or in bladders stimulated by neurohypophysial preparations (Bentley, 1966; Sawyer, 1960; Sawyer and Schigall, 1956). *In vivo* permeabilities of the bladder in fully hydrated terrestrial-acclimated toads thus approached the upper end of the range measured *in vitro*, at least with declining stores of urine.

Maintenance of normal hydration based on bladder urine is also indicated from findings that plasma osmolarities in dehydrating animals did not increase until the bladder reserve of water was depleted, e.g., in the toad *Bufo marinus* (Shoemaker, 1964) and the spade foot toad *Scaphiopus couchi* (McClanahan, 1967).

Independence of water balance on environment has furthermore been observed in the terrestial toads *Pelobates syriacus* and *Bufo viridis*. Thus, *P. syriacus* maintained constant body mass whether in water or in soil at 60 % soil moisture for 3 months, the duration of the experiment (Degani *et al.*, 1983). The independence is even more strikingly illustrated in *Bufo viridis*. Thus, Katz and coworkers found that in the simulated terrestrial habitat, toads remained normally hydrated when burrowing in soil at a water content of about 10 % (Hoffman and Katz, 1990; Katz and Gabbay, 1986). The maintenance of hydration of the body was at the expense of using water for excretion. As a result, the concentration of urea increased in the body fluids, within the 3 months of observation to reach concentrations several times those prevailing in the presence of free water. *B. viridis* thus tolerate urea concentrations in the body fluids far above those

maintained by means of anticipatory behaviour, in a simulated terrestrial habitat as well as in nature (Degani *et al.*, 1984; Katz and Hoffman, 1990).

Cutaneous water permeability and pattern of decline in rehydrating toads

Transfer of water-acclimated toads to a terrestrial environment increased the cutaneous water permeability by a factor of two or more with no change in hydration of the body. Little is known about such effects of transition from water acclimation to terrestrial conditions in other anurans, but effects may vary from absent or insignificant, e.g., in *Bufo viridis* (Katz and Graham, 1980; Katz and Ben-Sasson, 1984) to extreme, e.g., the spadefoot toad *Scaphiopus couchi* (Hillyard, 1975, 1979). Thus, *S. couchi* collected in the breeding ponds absorbed water at a rate of 0.9 ml 100 g^{-1} h^{-1}. After 3 days in moist soil the rate had increased to about 2 ml 100 g^{-1} h^{-1} to reach 4-5 ml in long-term acclimated toads. Notably, the terrestrial-acclimated toads did not respond to salt loads or vasotocin with increased rates of water uptake, as did the water-acclimated toads. It is thus suggested that a terrestrial acclimation by itself exploited the potential of the epidermis for increasing water permeability, leaving no "scope" for additional actions of a salt load or water balance hormone.

When in the present experiment dehydrated toads returned to water, the initial high rate of water uptake typically declined exponentially with time (Fig. 8). The rate of decline increased linearly with dehydration. The halftimes of the exponential declines in water permeability, reflected in the rates of cutaneous water uptake, ranged from about 4-5 hours at massive dehydration (corresponding to about 25 % loss of body mass) to about 2 hours, extrapolated to zero dehydration. The rate of decline in water influx tended, however, to accelerate when full hydration at the quasi steady state was reached (Fig. 8).

The patterns of the amphibian water balance response to dehydration and the determining factors

have not been systematically studied, but relevant parameters can be estimated from scattered data in the literature. Bakker and Bradshaw (1977) found that *Bufo marinus*, dehydrated to about 80 % of their standard body mass, rehydrated fast. From Fig. 1 in Bakker and Bradshaw's paper the initial halftime in the exponential decline in water influx could be estimated to be about 30 min. Also, after 30 min full hydration was reached, without any obvious change in the rate of exponential decline in water influx. Influx was followed for 2 hours, and the Figure shows that the rate of decline eventually decreased, corresponding to a $t_{1/2}$ value of about 1 hour during the second hour in water. At the end of the measuring period the cutaneous water permeability was still about 3 times the permeability in water-acclimated toads.

The faster decline in cutaneous water permeability in *B. marinus* than in *B. bufo* may be related to the difference in temperature, which was 23°C in the *B. marinus* experiment against 16°C in *B. bufo*. However, temperature alone does not seem to account for the differences. The increase in water permeability in response to dehydration was much stronger in *B. marinus*, where the initial water influx at 20 % dehydration was about 17 times the influx in water-acclimated toads. The corresponding increases in *B. bufo* amounted to only about 11-12 times or less, also at high temperatures of 21-25°C (Jørgensen and Rosenkilde, 1956; Jørgensen *et al.*, 1956).

Katz and Graham (1980) compared water relations in the toad *Bufo viridis* and the frog *Rana ridibunda*. From Fig. 7 in their paper it can be estimated that in animals that were dehydrated to 80 % of their initial body mass the halftimes of rates of decline in water uptake during rehydration at 20°C was about 50 min in *B. viridis* and 45 min in *R. ridibunda*. Initial water influxes were about an order of magnitude higher than prior to dehydration in both species. The rates of water uptake were higher in *B. viridis* than in *R. temporaria*, consistent with full rehydration within about 1 hour in the toads against about 2.5 hours in the frogs.

Shoemaker and McClanahan (1980) measured

rates of water uptake in 12 species of Bornean anurans from a variety of habitats and representing three genera. The animals were dehydrated to 85-80 % of their original body mass. The data are mostly too erratic for accurate estimates of rates and pattern of decline in water intake, but halftimes seemed primarily to be determined by taxonomic relationship rather than by habitat, ranging from aquatic to terrestrial. Thus, halftimes were typically short in *Bufo* spp and *Rhacophorus* spp and long in *Rana* spp, correlated with high initial rates of water uptake in the bufonids and, particularly, the rhacophorids and low rates in the ranids. Normal hydration was restored within about half an hour or less in the bufonids and rhacophorids against 1 to 3 hours in the ranids. As might be expected, rates of water uptake, expressed as percentages of the body mass, as well as rates of rehydration tended to increase with decreasing body mass.

Other studies have been concerned with the relationship between dehydration and rehydration in amphibians, generally in an adaptational context (e.g., Bentley *et al.*, 1958; Claussen, 1969; Main, 1968; Thorson, 1955), but the published data do not permit reconstruction of the patterns of rehydration.

The mechanisms that control the cutaneous water permeability in response to dehydration and rehydration are unknown.

Dynamic aspects of water balance

Water balance in the toad depends upon the integration of water influx through the skin and/or bladder wall and urine production. In the present study there were several indications of couplings between these parameters. Thus, urine flow began to decline shortly after transition from water to a dry or isoosmotic environment before any significant reduction in body water. Also, urine flow increased to above steady state values upon return to water, even at marginal changes in body water. Moreover, during dehydration, oliguria or even anuria developed already at moderate water deficits, whereas substan-

tial rates of urine production returned during rehydration of the toads despite large remaining water deficits. The integration of cutaneous and kidney-bladder activities during rehydration thus acts as a cybernetic mechanism that asymptotically restores normal hydration of the body despite persisting high influxes of water.

Scattered data from the literature are consistent with a functional coupling between skin and kidney in anuran water balance. Adolph (1936) thus states, without comments, that frogs, *Rana pipiens*, developed oliguria without change in glomerular function within a few minutes of exposure of the skin to 0.5 M NaCl or to a drying atmosphere. Also other anurans have been observed to respond acutely with oliguria when exposed to hyperosmotic or isoosmotic solutions without change in osmotic pressure of the body fluids (*Rana esculenta*, Mayer, 1969; *Bufo marinus*, Tufts and Toews, 1986; *Bufo arenarum*, Reboreda *et al.*, 1987; Petriella *et al.*, 1989). Adolph (1943) found that the same water load given to a frog out of water was eliminated by the kidney at less than half the rate in frogs in water, and he comments that "paradoxically, water entrance appears to promote net water elimination."

Rey (1937) made the interesting observation that the rate of urine production in frogs, *Rana esculenta*, varied inversely with the rate of evaporation, measured over a period of about 14 hours. As a result, the total loss of body water decreased from 15 % at zero evaporation in a saturated atmosphere, to 12 % at an evaporatory loss of 0.30 ± 0.04 ml 100 g^{-1} h^{-1}, to 8.8 % at an evaporatory loss of 0.53 ± 0.07 ml 100 g^{-1} h^{-1}, (calculated from data in Table VIII, Rey, 1937). Rey himself concluded that: "Il y a un mécanisme régulateur qui restreint la perte par les reins lorsque la perte par la peau tend à augmenter."

When toads in the simulated terrestrial habitat changed from the wet to the dry compartment they maintained normal hydration of the body by increasing the rate of resorption of water from the depot stored in the urinary bladder. Equilibrium

between loss of water by evaporation and resorption from the bladder was thus established without change in body water. This finding is contrary to established beliefs, according to which "toads and frogs reabsorb water from the urinary bladders when they are dehydrated" (Bentley, 1971, p. 176).

More subtle mechanisms must therefore exist for the utilization of urinary bladder water to maintain water balance in toads that discontinue contact with an external hydration resource. This may apply also to other anurans. Sinsch (1987) thus found that the terrestrial frog, *Rana temporaria*, remained normally hydrated when in contact with soil down to low water potentials, corresponding to about 5 % water content, or − 5.5 bar. With decreasing water potentials, cutaneous water influx and diuresis also decreased, but simultaneously resorption from the bladder increased from insignificant levels to complete resorption of urine produced (Figs. 2 and 5 in Sinsch, 1987).

The phenomenological analysis of the relationships between water influx through the skin and/or bladder wall and rate of urine production in anurans thus indicates that integrative mechanisms operate to maintain water balance in the absence of deviations from normal hydration. The nature of these mechanisms is not well understood, and their existence hardly appreciated. Adolph (1936) found that also frogs with crushed brains responded with oliguria to drying of the skin or exposure to hyperosmotic saline. Petriella *et al.* (1989) found that in toads hypophysectomy and hypothalamic transection only moderately reduced the response to exposure to saline, whereas the response was drastically reduced after elimination of sympathetic function by 10 days of administration of the sympatoplexic guanethidine. It may therefore be profitable to look for nervous mechanisms at levels lower than the brain in order to unravel the mechanisms that integrate effector functions in anuran water balance. However, even though the mechanisms that integrate water flux and urine production are unknown, it may be hypothesized that influx, whether through the skin or from the bladder, determines urine flow rate rather than the reverse, as suggested by Adolph (1927).

Acknowledgements

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