J. Exp. Biol. (1973), 58, 785–796
With 6 text-figures
Printed in Great Britain

STUDIES ON THE ADAPTATION OF THE TOAD *BUFO VIRIDIS* TO HIGH SALINITIES: OXYGEN CONSUMPTION, PLASMA CONCENTRATION AND WATER CONTENT OF THE TISSUES*

BY URI KATZ

Department of Zoology, The Hebrew University of Jerusalem, Israel

(*Received* 14 *November* 1972)

INTRODUCTION

Several species of Anura are known to tolerate a saline environment of 0·7% NaCl (Bentley, 1971). However, only two species, the frog *Rana cancrivora* (Gordon, Schmidt-Nielsen & Kelly, 1961) and the toad *Bufo viridis* (Stocovici & Pora, 1951; Gordon, 1962; Tercafs & Schoffeniels, 1962) can tolerate salinities as high as 80 and 50% sea water respectively. This high salinity tolerance was associated with increased plasma osmotic concentration built up by NaCl and urea.

During the last few years we have been studying the adaptation of the toad *B. viridis* to high salinities, up to 800 mOsm NaCl solutions.

The wide range of sodium and osmotic concentration which is found in the plasma of this toad, when exposed to various environmental salinities, raised the question of their metabolic response to such changes (Potts & Parry, 1964; Whittam, 1964). We therefore measured the oxygen consumption of the animals under different environmental conditions, in an effort to correlate this with the concentration of the plasma and the state of hydration of the animals. Part of this study has been briefly reported elsewhere (Katz, 1972).

MATERIALS AND METHODS

The toad *B. viridis* is the common toad in Israel. About 250 animals of both sexes were collected throughout the year at the same location. Emphasis has been laid on season with respect to analysis or any measurements carried out. The average weight of the animals was 30 g. The animals were kept at 21 °C ± 2 °C in 2–3 cm deep tap water or various concentrations of NaCl solutions, in a sink; they were fed two or three times a week with maggots. Those animals that were used for the metabolic studies were kept in separate plastic boxes (10 × 5 × 5 cm) punctured with holes.

Weight was followed to the nearest 0·1 g after catheterization of the urine through the cloaca.

Oxygen consumption was measured using a compensated Perspex respirometer (Scholander, 1942), with a cup containing 20% KOH (w/v) to absorb CO_2 (Fig. 1); all measurements were taken at 25 °C ± 0·1 °C. The chambers were checked for leaks under pressure for over an hour.

* This work is part of a thesis to be submitted to the Hebrew University of Jerusalem for the Ph.D.

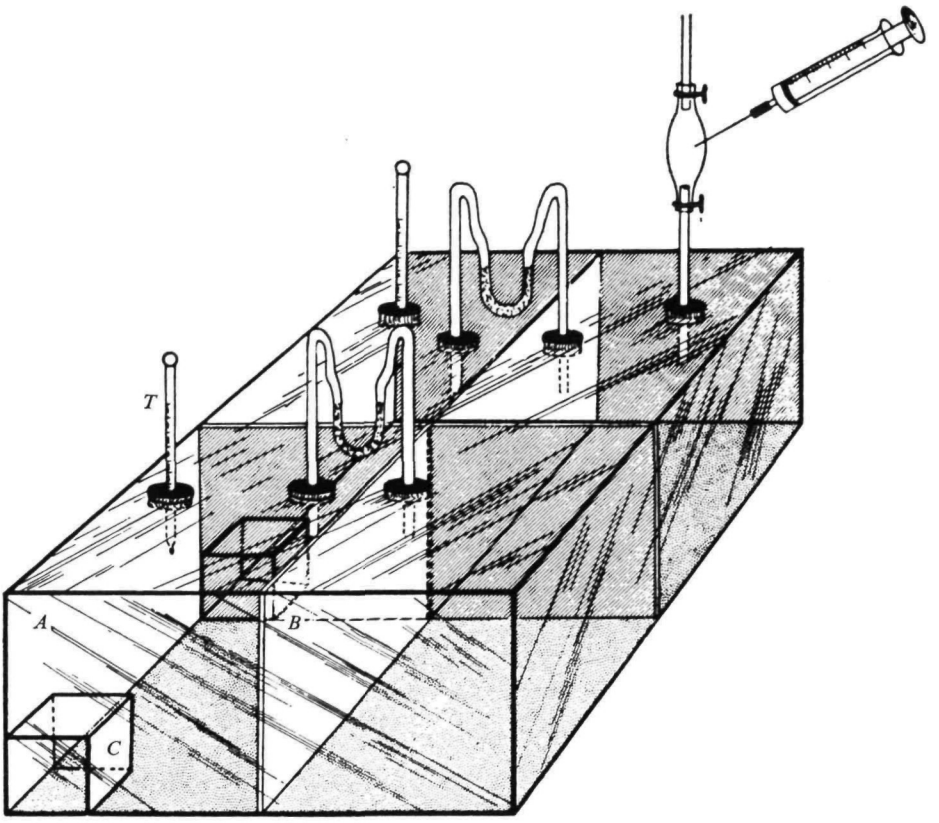

Fig. 1. The double Perspex chamber used to measure the oxygen consumption of two toads simultaneously. The size of each quarter of the chamber is 60 × 60 × 50 mm. All valves were made of glass sealed with silicon. (*A*), the animal chamber; (*B*), the compensating chamber; (*C*), KOH; (*T*), thermometer.

After temperature equilibration with the animals in the chamber for an hour, the respirometer was closed and measurements were taken at 10–15 min intervals for another hour. Oxygen was injected into the chamber, to the animal side, to compensate for the reduced pressure. Measurements were taken at the same time of day for each individual animal. Controls were run with each series, so that the effects of season and handling could be discounted.

Blood and urine analysis were performed on another series of animals. Urine was collected by glass capillaries from the bladder through the cloaca, after which the animal was pithed and blood was taken from the heart into heparinized (either sodium or ammonium heparin, 5000 u./ml) Pyrex capillaries. The blood was centrifuged, the cells were discarded and the plasma was used for analysis.

Osmotic concentration was measured immediately on the fresh plasma using a Knauer, Berlin 33, osmometer with a precision of ±3%. Urea was determined on fresh plasma by the microdiffusion technique of Conway (1957). Similar results were obtained with a calorimetric method (Sigma, *Technical Bull.* no. 14, 1969). Chloride was titrated according to Schales & Schales (1941) on stored plasma, whereas sodium and potassium were determined with a Beckman model DU flame-photometer.

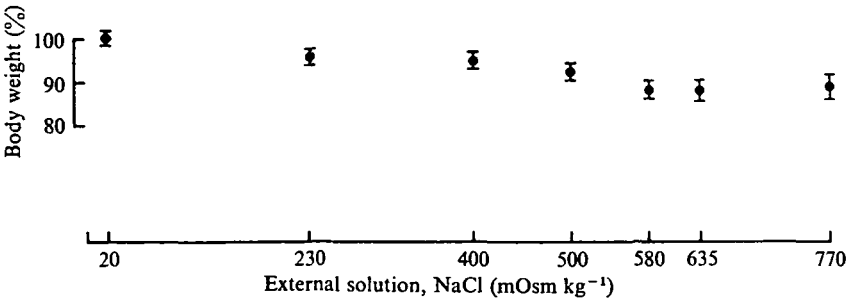

Fig. 2. Weight change of animals that were adapted gradually to high salinities. The animals spent 7–10 days in each salinity before they were transferred to higher salinity. Average weight of the animals was 34·2 ± 5 g. Mean ± S.D. (Each point represents the average of 9–15 animals.)

After collection of the blood the animals were dissected, whole tissues were taken, blotted on Whatman no. 2 filter paper and weighed to the nearest 0·1 mg. The tissues were dried to constant weight at 95 °C and the difference between fresh and dry weight gave the water content. For cation determination the dried tissues were boiled in distilled water and then were left for 24 h; analysis was made on the extracts.

The Student test was used for statistical analysis.

RESULTS

Slight mortality occurred among the animals during gradual adaptation to 600 mOsm NaCl; the animals could readily be adapted to high salinities, in which they survived for relatively long periods (4 weeks and more). Animals that survived for 10 days in a defined medium without any apparent ill effect were considered as 'adapted animals'.

Fig. 3 shows the weight change of animals that were transferred directly into high saline (500 mOsm) environment. The animals could not withstand such treatment and died within 2–4 days after losing about 25 % of their initial body weight. However, as can be seen from Fig. 2, animals that were transferred gradually from one salinity to another, and were left for 4–7 days in the new environment, survived well in solutions of relatively high salinity with almost no weight loss.

In spite of this it may be seen from Fig. 4 that the water content of the animals decreased with salinity following adaptation. Most labile among other organs in this respect were the skeletal muscles. The skeletal muscles make up more than 40 % of the whole body weight and could therefore make the greatest contribution to the total reduction in the body water content. It should be noticed that the organs responded differentially to saline adaptation: the water content of the heart remained almost constant throughout, whereas skeletal muscle was most variable in this respect. The same type of response was found also upon immediate transfer to 490 mOsm or upon dehydration in the air.

Table 1 gives the results of cation determinations made on liver and sartorius muscle dissected freshly from animals previously adapted to various salinities. Only in sodium concentration was there some increase; there was almost no change in potassium concentration of both tissues.

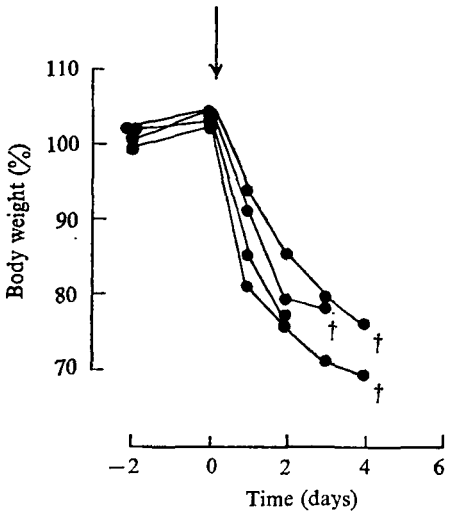

Fig. 3

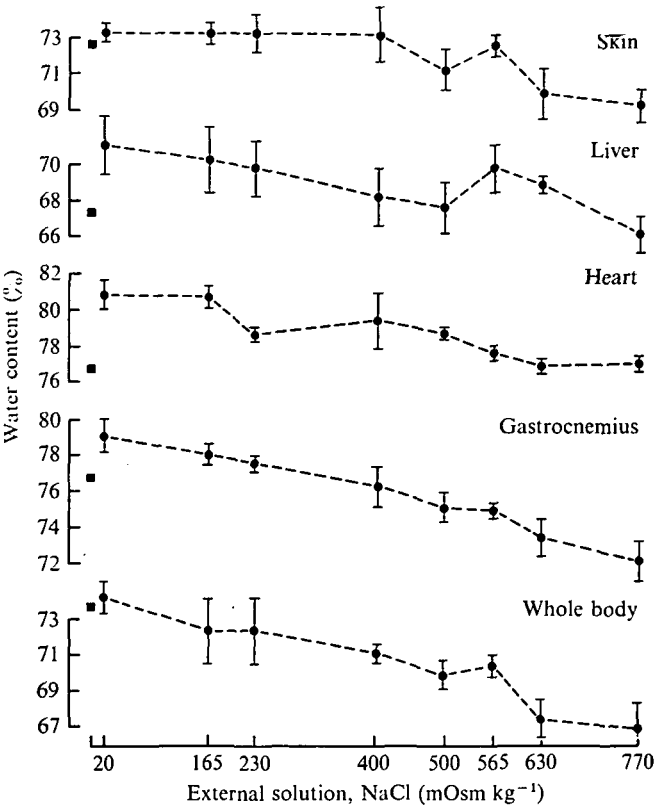

Fig. 4

Fig. 3. Weight change of animals transferred immediately into NaCl solution of 500 mOsm. Average weight of the animals was 34 g.

Fig. 4. Percentage of water content in the whole body and in various organs of the toad under adaptation to various salinities. The black square is the value of control animals that were kept in tap water. Mean ± S.D. (4 determinations on separate samples).

Fig. 5 shows the osmotic concentration of the plasma and its constituents for animals that were adapted to various salinities. The plasma was always hypertonic to the medium, a prerequisite for adaptation, since otherwise the animals would lose water and die. In Table 2 we compare the osmotic concentration and also the sodium and urea concentrations of the plasma in summer and winter toads – we also give the plasma composition of two different 'control' groups (i.e. animals that were kept in a terrarium or in the sink with free access to tap water). Note the essential hypertonicity of the toad's plasma compared with normal frog's Ringer (313 and 410 *vs.* 230 mOsm kg^{-1} H_2O) and that the variability of the osmotic concentration is due mostly to changes in urea concentration.

Table 3 gives the total osmotic concentrations and also the concentrations of sodium and urea in the urine; the urine was hypotonic to the blood in animals adapted to environments up to 400 mOsm and became isotonic at higher environmental salinities. The concentration of urea in the urine was less than in the plasma; in addition, there was a reduction in the urine flow, as measured by catheterization, from 0·74 ± 0·27 ml .

Table 1. *Sodium and potassium concentrations in the liver and sartorius muscle from toads previously adapted to various salinities*

Mean ± S.D. (4 determinations on samples from separate animals)

Environmental salinity (NaCl) (mOsm)	Liver (mM kg^{-1} fresh tissue)		Sartorius muscle (mM kg^{-1} fresh tissue)	
	Sodium	Potassium	Sodium	Potassium
Tap water	82·9 ± 18·4	199·3 ± 47·3	114·4 ± 4·6	318·9 ± 47·0
230	107·8 ± 24·6	195·9 ± 41·5	115·8 ± 51·6	355·9 ± 66·6
400	117·9 ± 22·3	209·6 ± 13·9	131·3 ± 12·2	317·1 ± 61·0
770	127·0 ± 16·0	190·2 ± 33·5	173·1 ± 149·6	287·6 ± 35·3

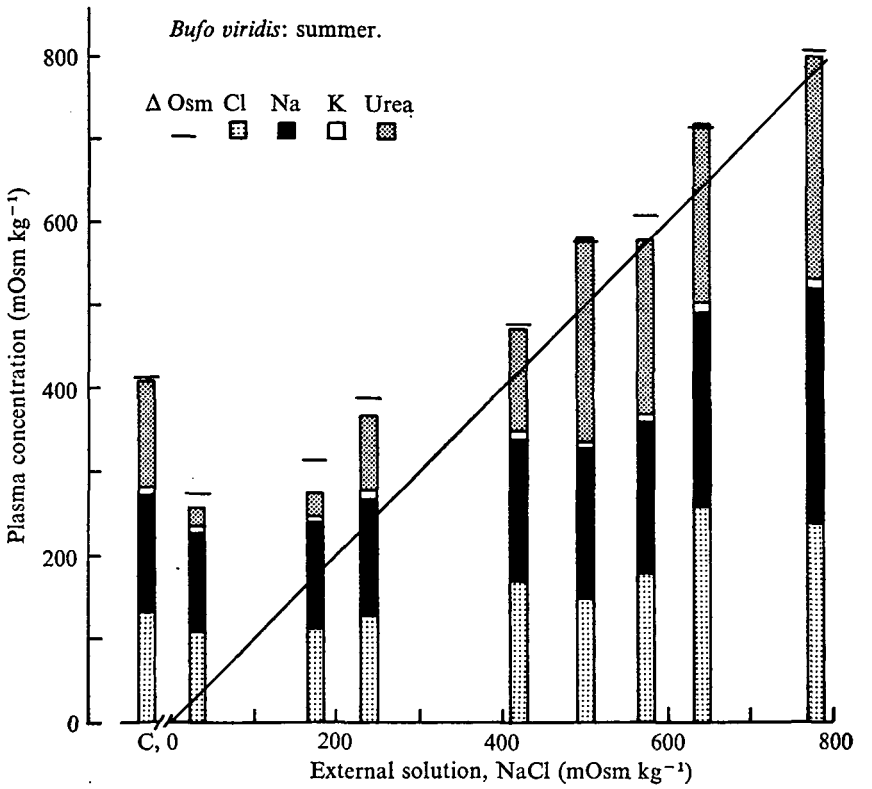

Fig. 5. Plasma osmotic concentration and its constituents in toads that were adapted to various salinities. The straight line shows the points of equality between the concentration of the plasma and external medium. *C*, control group that was kept in the sink with free access to tap water. Mean ± S.D. (4 animals in each group).

per animal per hour (11 animals) in tap water to 0·38 ± 0·19 (14 animals) in 230 mOsm NaCl solutions and 0·14 ± 0·09 ml per animal per hour (8 animals) in 400 mOsm NaCl. In toads adapted to high salinity the concentration of sodium in the urine equalled that in the plasma (Table 3).

Fig. 6 shows the oxygen consumption (calculated per gram body weight per hour) at various salinities, for animals that were gradually transferred from one salinity to

Table 2. *Seasonal effect on plasma osmotic concentration and urea concentration in toads* (Bufo viridis), *under various environmental salinities*

(Mean ± S.D. (4 animals in each group).)

Environmental salinity (NaCl) (mOsm)	Winter (February) ΔOsm (mOsm kg^{-1} H_2O)	Winter (February) Urea (mM $litre^{-1}$)	Summer (August) ΔOsm (mOsm $litre^{-1}$)	Summer (August) Urea (mM $litre^{-1}$)
Control (I)*	326·1 ± 9·0	23·5 ± 8·7	313·0 ± 21·3	44·6 ± 39·2
Control (II)†	—	—	410·7 ± 11·8	131·0 ± 12·7
Tap water	296·5 ± 17·0	22·2 ± 12·3	270·1 ± 6·1	22·1 ± 3·4
230	348·9 ± 14·5	60·3 ± 27·4	386·7 ± 17·5	90·2 ± 21·0
400	480·1 ± 3·3	71·3 ± 20·6	475·7 ± 17·4	124·5 ± 23·0
580	597·2 ± 10·9	158·5 ± 50·2	608·1 ± 16·5	214·0 ± 59·4

* Animals kept in the sink with free access to tap water.
† Another group of animals under the same conditions as in control (I), few days later.

Table 3. *Concentration of the urine in toads* (Bufo viridis) *previously adapted to various salinities*

(Mean ± S.D. (4 animals in each group).)

Environmental salinity (NaCl) (mOsm)	ΔOsm (mOsm kg^{-1} H_2O)	Urea (mM $litre^{-1}$)	Na^+ (mM $litre^{-1}$)	Plasma osmotic concentration (mOsm kg^{-1} H_2O)
Control*	153·2 ± 54·0	92·5 ± 60·0	10·0 ± 7·1	410·7 ± 11·8
Tap water	89·2 ± 34·2	15·0 ± 10·6	36·1 ± 9·9	270·1 ± 6·1
165	95·0 ± 7·0	31·7 ± 8·1	37·3 ± 17·9	312·1 ± 3·0
230	180·0 ± 52·0	40·1 ± 10·0	74·6 ± 61·3	386·7 ± 17·5
400	300·0 ± 56·5	85·0 ± 8·5	90·6 ± 37·0	475·7 ± 17·4
565	589·7 ± 14·4	56·0 ± 1·0	169·7 ± 38·0	608·1 ± 16·5
770	810·7 ± 16·6	157·0 ± 18·2	253·0 ± 52·0	807·7 ± 12·0

* Animals kept in the sink with free access to tap water.

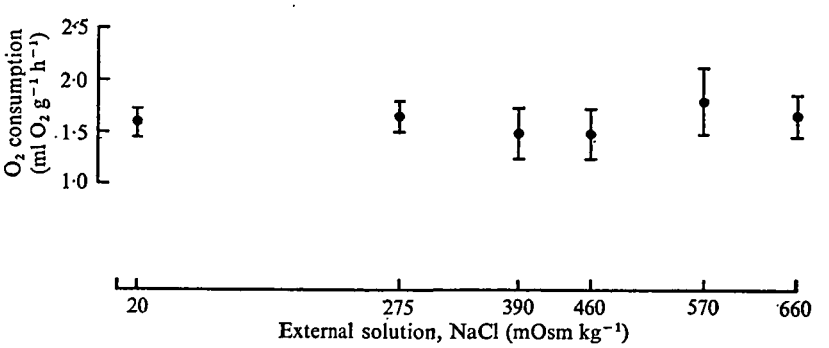

Fig. 6. Oxygen consumption of toads adapted to various salinities. Average weight of the toads was 38·2 ± 4·3 g. t = 25 °C. Mean ± S.D. (10 animals).

another. None of the differences was found to be statistically significant. Table 4*a* summarizes the results of the oxygen consumption measurements for animals that were transferred immediately to 500 mOsm NaCl. These animals lost weight and were dehydrated; there was a significant increase ($P < 0{\cdot}01$) in the oxygen consumption which returned to its control value after the animals were rehydrated in tap water.

Table 4. *Oxygen consumption (ml* $O_2\, g^{-1}\, h^{-1}$*) of the toad,* Bufo viridis, *upon dehydration by either (a) immediate transfer to* 500 *mOsm NaCl solution* or (b) by fast evaporation in air*‡

(Mean ± S.D. (number of animals in parentheses).)

	Immediate transfer to high salinity*		Gradual transfer to high salinity	
	Experimental group	Control group	Experimental group	Control group
(*a*)				
First period (control)‡	0·890 ± 0·229 (8)	0·952 ± 0·456 (2)	1·620 ± 0·195 (10)	1·631 ± 0·277 (6)
Second period	1·482 ± 0·455 (8)	0·945 ± 0·361 (2)	1·521 ± 0·244 (10)	1·718 ± 0·252 (6)
P	< 0·005	< 0·90	< 0·50	< 0·40
Third period (control)	0·937 ± 0·174 (8)	0·975 ± 0·460 (2)	1·539 ± 0·335 (10)	1·459 ± 0·156 (6)
P	< 0·001	< 0·70	< 0·80	< 0·005
Weight of the animals at the beginning	29·1 ± 2·0		38·3 ± 4·8	
	Fast evaporative dehydration (5 h)†		Slow evaporative dehydration (2 days)†	
	Experimental group	Control group	Experimental group	Control group
(*b*)				
First period (control)‡	0·932 ± 0·217 (8)	0·900 ± 0·265 (3)	0·845 ± 0·151 (4)	1·150 ± 0·071 (2)
Second period	1·667 ± 0·357 (8)	0·833 ± 0·106 (3)	0·825 ± 0·150 (4)	1·150 ± 0·071 (2)
P	< 0·001	< 0·50	< 0·10	0·0
Third period (control)	0·960 ± 0·175 (8)	0·787 ± 0·110 (3)	0·883 ± 0·126	0·975 ± 0·177 (2)
P	< 0·001	< 0·01	< 0·70	< 0·10
Weight of the animals at the beginning	34·1 ± 5·5		36·5 ± 5·0	

* The animals that were transferred immediately into 500 mOsm solution lost 17·5% of their body weight in 15 h.

† Both groups of animals that were dehydrated by evaporation in the air lost 25% of their initial body weight.

‡ Animals were dehydrated according to the method indicated above, between the first and second periods. The third period is another control after recovery of the animals.

The same results were obtained with animals that were rapidly dehydrated in air (Table 4*b*). Such treatment caused a significant increase in their oxygen consumption. No significant increase was observed with control animals that were placed in water in front of a fan, nor with animals that were slowly dehydrated during 2–3 days in air without water.

DISCUSSION

In spite of several studies that have already been made on the adaptation of the toad *B. viridis* to high salinities (Gordon *et al.* 1961; Tercafs & Schoffeniels, 1962; Eilath, 1964) there is no agreement about the role of urea in this process. Our results (Fig. 5 and Table 2) show that discrepancies may be due to seasonal effects. According to our experience toads can be adapted to the highest salinities only during the summer, while in winter they die as soon as they are transferred from 500 mOsm NaCl to higher salinities. This observation may be correlated with the higher urea concentration which was measured in the plasma of summer toads. In fact Gordon

Table 5. *Comparison of plasma osmotic concentration and concentrations of sodium and urea in selected species of anurans, adapted for at least 7 days to the salinity of the environment*

Species	ΔOsm (mOsm kg^{-1} H_2O)	Na^+ (mM $litre^{-1}$)	Urea (mM $litre^{-1}$)	Reference
Xenopus laevis				
Tap water	—	100	5	McBean & Goldstein (1970)
300 mOsm NaCl	—	130	100	
Bufo bufo				
Tap water	222	106·3	13·6	Ferreira & Smith (1968)
0·7% NaCl	305	126·7	22	Ackrill *et al.* (1970)
Scaphiopus couchi				
Tap water	305	159	39	McClanahan (1967)
Emerging in summer (from aestivation)	630	286	228	
Rana cancrivora				
Tap water	290	125	40	Gordon *et al.* (1961)
25% s.w.	340	161	110	
80% s.w.	830	252	350	
Rana esculenta				
Tap water	212	104	3	Ackrill *et al.* (1969)
0·7% NaCl	300	144	14	
R. temporaria				
Tap water	218	105	—	Ackrill *et al.* (1969)
0·7% NaCl	262	138	—	
R. ridibunda				
Tap water	247	115	11	Katz (unpublished)
230 mOsm NaCl	300	121	44	
300 mOsm NaCl	337	131	53	

(1962) who found only low concentrations of urea in the plasma of saline-adapted toads, reported high mortality among his animals with only few left for analysis. Seasonal effects on electrolyte and urea concentrations have been described in amphibians both in the field for the spadefoot toad (Shoemaker, McClanahan & Ruibal, 1969) and in the laboratory for the frog *R. pipiens* (Jungreis, 1971).

Two mechanisms may contribute to building up urea concentration in the plasma: (1) increased urea synthesis by specific increase of ornithine-urea cycle enzymes (McBean & Goldstein, 1967); and (2) increased urea reabsorption in the kidney. We have some evidence (unpublished results) that urea reabsorption was increased in animals adapted to high salinity, but as there was only small difference between the concentrations of urea in the urine and in the plasma a considerable increase of urea synthesis is to be expected. An induction of the enzyme system related to urea synthesis may be dependent on season, as has been suggested by Jungreis (1971).

Of the anuran species studied only a few can be adapted to saline environment (Table 5). Of these, only two species, the frog *R. cancrivora* and the toad *B. viridis* can tolerate salinities that are as high as 800 mOsm kg^{-1} H_2O of NaCl. A third species, the spadefoot toad, *Scaphiopus couchi*, aestivates during the summer (McClanahan, 1967) when it digs deep in the sand of the Californian desert. It would be interesting to know whether this species could also be adapted to highly saline environments.

rom Table 5 it seems that it is the blood hypertonicity, built up by both electrolytes and urea, which enables those few species of amphibians to tolerate highly saline environments. It would be interesting to see to what extent this adaptability is connected with inducible enzyme systems related to urea synthesis (McBean & Goldstein, 1967).

The adaptability to high salinity is a time-consuming process. In Fig. 3 we can see that the animals did not die immediately as a result of the water loss upon their transfer to hypertonic solution, but only 2–4 days later. Examination of the gut of animals that died in hypertonic solutions containing the neutral dye Congo Red did not show any sign of drinking; comparable observation on the frog *R. ridibunda* (unpublished) revealed that they drank upon transfer to hypertonic solution. Recently Bentley & Schmidt-Nielsen (1971) pointed to the role of accumulated sodium in the cause of death of *R. pipiens* upon transfer to sea water. It would be interesting to know whether some species of amphibians are essentially more resistant to high sodium concentrations, and if it is so, how this resistance is developed.

The range of osmotic concentration of the plasma displayed by the toad *B. viridis* is one of the widest reported among vertebrates. It has already been stressed (Katz, 1973) that in studies of adaptation to environmental changes two major alternative lines should be considered: one which is predominant in most animals, depends on mechanisms that are concerned with the constancy of the internal millieu (Cannon, 1929); in few species, however, it is their ability to tolerate great changes in their internal environment which is most important for their adaptation to environmental changes. The toads *B. viridis* and *S. couchi* and the frog *R. cancrivora* are certainly representatives of the latter group of species. It is remarkable that among other amphibians so far studied neither essential hypertonicity of the blood under normal environmental conditions (tap water) nor a progressive increase in their plasma osmotic concentration in saline environment was found (Table 5).

The water content of the whole body as well as that of various organs varied considerably under adaptation to saline environments (Fig. 4). However, under normal conditions (in tap water) the water content of the whole body (75%) and that of skeletal muscles (79%) was rather high compared with other vertebrates. This high proportion of water gives a wide margin for variations in the water content of skeletal muscles without any apparent damage or disturbance to their normal function. Hypertonic treatment of frog skeletal muscle is accompanied by uncoupling of excitation from contraction, very probably because of increase in its viscosity (Howarth, 1958). Adaptation of skeletal muscles and other tissues to highly saline conditions should therefore include accumulation of osmolytes to restore the water loss; urea is a favourable candidate for this function because it easily passes through biological membranes and it is present in high concentrations in the plasma under saline conditions (Table 2). In toad erythrocytes under high saline conditions we have actually found high intracellular urea concentrations (unpublished results). Eilath (1964) could not protect the gastrocnemius muscle of the toad in hypertonic solutions with urea; it seems therefore that some process which is time-dependent is needed in order to adjust the necessary fluxes for osmotic balance.

The function of the bladder as a water reservoir (Overton, 1904) need not be considered here, under conditions of long-term adaptation to high saline environment.

The kidneys, which continue their filtration function, produce urine which is almo isotonic to the plasma and there is almost no osmotic gradient across the bladder. At the same time, the concentration of circulating hydroosmotic activity in the blood under these conditions (Katz & Weissberg, 1971) was not different from that of the blood of animals in tap water. Without these two factors the bladder cannot contribute much as a water reservoir.

The essential high water content of toads is therefore of high adaptive value because they can endure a great deal of variation in this parameter without any apparent difficulty.

The wide range of osmotic and sodium concentrations (from 270 mOsm kg^{-1} H_2O to 820 mOsm kg^{-1} H_2O and from 120 mM $litre^{-1}$ to 280 mM $litre^{-1}$ respectively) that were found in the plasma of *B. viridis* under various environmental conditions raise the question of their metabolic response to such variations. Studies on ion transport in different tissues (Whittam, 1964; and many others) have demonstrated the dependency of the metabolism of isolated tissues on the active transport of ions. Muller (1962) found a close correlation between sodium concentration in the medium and the lactic acid production of isolated sartorius muscle taken from the toad *B. marinus*; apparently the Mg-dependent, $Na^+ + K^+$ stimulated ATPase (Skou, 1964) had been stimulated under these conditions so that glycolysis and oxygen consumption were increased (Whittam & Ager, 1964). An increase of the oxygen consumption was found also in the whole animal with turtles (Bentley, Bretz & Schmidt-Nielsen, 1967) and similar explanation was given by Katz *et al.* (1973) for their results with living rats.

From the energetic point of view the adaptation to high salinity meets with no apparent change in the overall energy demand of the intact animal as measured by its oxygen consumption (Fig. 6). Under conditions of acute dehydration, however, similar changes in the concentrations of body fluids caused the expected increase in the oxygen consumption of the intact animal (Table 4). Gordon & Tucker (1968) also reported that the oxygen consumption of *R. cancrivora* was independent of salinity. Although most of the increase in intracellular osmotic concentration of the frog under highly saline conditions is accounted for by urea (Gordon & Tucker, 1968), we have also measured a substantial increase in active cation transport in the toad's erythrocytes under adaptation to high salinity (unpublished results). The osmoregulatory organs, on the other hand, do not have to increase their energy demand considerably since urine concentration is very close to that of the plasma and active sodium transport across the skin is greatly reduced (Katz *et al.* 1972).

Adaptation of the toad *B. viridis* as well as that of the other two species mentioned (*R. cancrivora* and *S. couchi*) to live in highly saline environments, is therefore dependent on the mechanisms which increase the osmolality of their body fluids and maintain it hypertonic to the medium. It also depends very heavily on the ability of various organs to function adequately under variable internal environmental conditions and changes in their composition (Table 1).

From the ecological point of view this kind of adaptation to a variable external environment might have stemmed from the habitat: these animals are exposed to a variable and changing environment while having only a poor integumental protection from it. Only few vertebrates of those so far studied show such large variations in the composition of their body fluids as has been observed in the toad. The changes in the

various physiological mechanisms under such variations have already proved most interesting (Eilath, 1964; Katz & Weissberg, 1972; Katz *et al.* 1972) and should be carefully studied.

SUMMARY

1. The toad *Bufo viridis* was adapted to various salinities up to 800 mOsm NaCl solutions.

2. The blood was always hypertonic to the external solutions and had a high urea concentration. Urea concentration in the plasma was higher in summer than in winter. Urine was hypotonic to the plasma in low salinities and became isotonic with the plasma at 400 mOsm and higher salinities.

3. Water content of the whole body and the skeletal muscles decreased somewhat with salinity.

4. Oxygen consumption was unaffected by salinity when animals were gradually adapted to high salinities. It increased, however, upon acute dehydration whether this was caused by immediate transfer to high salinity or by rapid evaporative dehydration in air.

The adaptation of the toad to high salinities is discussed from the point of view of the effect of changes of the internal environment on various organs other than those concerned with osmoregulation.

The technical assistance of Miss Judith Weissberg is gratefully acknowledged.

REFERENCES

ACKRILL, P., HORNBY, R. & THOMAS, S. (1969). Responses of *Rana temporaria* and *Rana esculenta* to prolonged exposure to a saline environment. *Comp. Biochem. Physiol.* **28**, 1317–29.

ACKRILL, P., DIXON, J. S., GREEN, F. & THOMAS, S. (1970). Effects of prolonged saline exposure on water, sodium and urea transport and on electronmicroscopical characteristics of the isolated urinary bladder of the toad *Bufo bufo*. *J. Physiol.* **210**, 73–85.

BENTLEY, P. J. (1971). *Endocrines and Osmoregulation*. Berlin, Heidelberg, New York: Springer-Verlag.

BENTLEY, P. J., BRETZ, W. L. & SCHMIDT-NIELSEN, K. (1967). Osmoregulation in the diamondback terrapin, *Malaclemys terrapin centrata*. *J. exp. Biol.* **46**, 161–7.

BENTLEY, P. J. & SCHMIDT-NIELSEN, K. (1971). Acute effects of sea water on frogs (*Rana pipiens*). *Comp. Biochem. Physiol.* **40A**, 547–8.

CANNON, W. B. (1929). Organization for physiological homeostasis. *Physiol. Rev.* **9**, 399–431.

CONWAY, E. J. (1957). *Microdifusion Analysis and Volumetric Error*. London: Lockwood and Son.

EILATH, U. (1964). Adaptation of the gastrocnemius muscle from the toad *Bufo viridis* to hypertonic solutions. M.Sc. Thesis, The Hebrew University, Jerusalem. (In Hebrew.)

FERREIRA, H. G. & SMITH, M. W. (1968). Effect of a saline environment on Na transport by the toad colon. *J. Physiol.* **198**, 329–43.

GORDON, M. S. (1962). Osmotic regulation in the green toad (*Bufo viridis*). *J. exp. Biol.* **39**, 261–70.

GORDON, M. S. & TUCKER, V. A. (1968). Further observations on the physiology of salinity adaptation in the crab-eating frog (*Rana cancrivora*). *J. exp. Biol.* **49**, 183–93.

GORDON, M. S., SCHMIDT-NIELSEN, K. & KELLY, H. M. (1961). Osmotic regulation in the crab-eating frog (*Rana cancrivora*). *J. exp. Biol.* **38**, 659–78.

HOWARTH, J. V. (1958). The behaviour of frog muscle in hypertonic solutions. *J. Physiol.* **144**, 167–75.

JUNGREIS, A. M. (1971). Seasonal effects of hyper-osmotic sodium chloride on urea production in the frog, *Rana pipiens*. *J. exp. Zool.* **178**, 403–14.

KATZ, U. (1972). Adaptation of the toad *Bufo viridis* to high salinities. 4th Ann. Symp. Environmental Physiology, Beer-Sheva, 1971. *Israel J. med. Sci.* **8**, 1008.

KATZ, U. (1973). The importance of the constancy of plasma osmotic concentration in physiological adaptation of rodents (Mammalia) and Anurans (Amphibia) to environmental changes. Submitted for publication.

KATZ, U., SMITH, M. W. & ERLIJ, D. (1972). Adaptation of the sodium transport system in the skl of the toad *Bufo viridis* adapted to high salinities. Abstract, IV, *Biophys. Congress Moscow*, **3**, 109.

KATZ, U., BORUT, A. & SARNE, Y. (1973). The effect of water deprivation and hypertonic salt injection on the oxygen consumption and plasma NaCl concentration in the albino rat. *Pflügers Arch. ges. Physiol.* (In the press.)

KATZ, U. & WEISSBERG, J. (1971). Role of skin and neurohypophyseal hormone in the adaptation of the toad *Bufo viridis* to high salinities. *Nature, Lond.* **232**, 344–5.

MCBEAN, R. L. & GOLDSTEIN, L. (1967). Ornithin urea cycle activity in Xenopus laevis: adaptation in saline. *Science, N.Y.* **157**, 931–2.

MCCLANAHAN, L. (1967). Adaptation of the spadefoot toad, *Scaphiopus couchi*, to desert environment. *Comp. Biochem. Physiol.* **20**, 73–99.

MULLER, M. H. (1962). Metabolic aspects of ionic shifts in toad muscle. *Biochim. biophys. Acta* **57**, 475–94.

OVERTON, E. (1904). Neununddreissig Thesen über die Wasserökonomie der Amphibien und die osmotischen Eigenschaften der Amphibenhaut. *Verh. phys.-med. Ges. Würzb.* **36**, 277–95.

POTTS, W. T. W. & PARRY, G. (1966). *Osmotic and Ionic Regulation in Animals*, p. 330. Oxford: Pergamon Press.

SCHALES, O. & SCHALES, S. S. (1941). A simple and accurate method for determination of chloride in biological fluids. *J. biol. Chem.* **140**, 879–84.

SCHOLANDER, P. F. (1942). Volumetric microrespirometer. *Rev. scient. Instrum.* **13**, 32–3.

SHOEMAKER, V. H., MCCLANAHAN, L. & RUIBAL, R. (1969). Seasonal changes in a field population of the spadefut toads. *Copeia* no. 3, 585–91.

SKOU, J. C. (1964). Enzymatic aspects of active linked transport of Na^+ and k^+ through the cell membrane. *Prog. Biophys.* **14**, 131–66.

STOICOVICI, F. & PORA, E. A. (1951). Comportarea la variatinui de salinitate. Nota XXX: Influenta variatiunilor de salinitate si a factorului ecologic asupra supravietuirii si mediului interior la Bufo viridis in diferitele periode ale anului. Studii si cercetari stiintifice. *Acad. Rep. Pop. Romane, Fil. Cluj.* **2**, 159–219.

TERCAFS, R. R. & SCHOFFENILES, E. (1962). Adaptation of amphibians to salt water. *Life Sci.* **1**, 19–23.

WHITTAM, R. (1964). The interdependence of metabolism and active transport. In *Cellular Function of Membrane Transport* (ed. J. F. Hoffman), pp. 139–54. Prentice Hall Inc.

WHITTAM, R. & AGER, M. E. (1964). Vectorial aspects of ATPase activity in erythrocyte membranes. *Biochem. J.* **93**, 337–48.