J Comp Physiol B (1988) 158:537–546

Journal of Comparative Physiology B Biochemical, Systemic, and Environmental Physiology

Adaptations of the reed frog *Hyperolius viridiflavus* (Amphibia, Anura, Hyperoliidae) to its arid environment

V. Iridophores and nitrogen metabolism

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Accepted August 29, 1988

Summary. Of all amphibians living in arid habitats, reed frogs (belonging to the super species *Hyperolius viridiflavus*) are the most peculiar. Froglets are able to tolerate dry periods of up to 35 days or longer immediately after metamorphosis, in climatically exposed positions. They face similar problems to estivating juveniles, i.e. endurance of long periods of high temperature and low RH with rather limited energy and water reserves. In addition, they must have had to develop mechanisms to prevent poisoning by nitrogenous wastes that rapidly accumulate during dry periods as a metabolic consequence of maintaining a non-torpid state.

During dry periods, plasma osmolarity of *H. v. taeniatus* froglets strongly increased, mainly through urea accumulation. Urea accumulation was also observed during metamorphic climax.

During postmetamorphic growth, chromatophores develop with the density and morphology typical of the adult pigmentary pattern. The dermal iridophore layer, which is still incomplete at this time, is fully developed within 4–8 days after metamorphosis, irrespective of maintenance conditions. These iridophores mainly contain the purines guanine and hypoxanthine. The ability of these purines to reflect light provides an excellent basis for the role of iridophores in temperature regulation. In individuals experiencing dehydration stress, the initial rate of purine synthesis is doubled in comparison to specimens continuously maintained under wet season conditions. This increase in synthesis rate leads to a rapid increase in the thickness of the iridophore layer, thereby effectively reducing radiation absorption. Thus, the danger of overheating is diminished during periods of water shortage when evaporative cooling must be avoided. After the development of an iridophore layer of sufficient thickness for effective radiation reflectance, synthesis of iridophore pigments does not cease. Rather, this pathway is further used during the remaining dry season for solving osmotic problems caused by accumulation of nitrogenous wastes. During prolonged water deprivation, in spite of reduced metabolic rates, purine pigments are produced at the same rate as in wet season conditions. This leads to a higher relative proportion of nitrogen end products being stored in skin pigments under dry season conditions. At the end of an experimental dry season lasting 35 days, up to 38% of the accrued nitrogen is stored in the form of osmotically inactive purines in the skin. Thus the osmotic problems caused by evaporative water loss and urea production are greatly reduced.

Introduction

Members of the super species *Hyperolius viridiflavus* (Schiøtz 1971) inhabit seasonally very hot, dry African savannas. The climatic conditions at metamorphosis are unpredictable especially towards the end of the rainy season when froglets leave their breeding site, and during the transitional period between wet and proper dry seasons. Immediately after metamorphosis, the froglets, weighing between 200 and 300 mg, must be able to survive intermediate dry periods of several days and, only a few weeks later (weighing between 300 and 700 mg), must be prepared for proper estivation. Neither newly metamorphosed froglets nor estivating juveniles try to avoid the harsh climate prevailing above ground during dry periods by withdrawing into microclimatically favorable crevices in the ground. Rather, they endure the extremely unfa-

vorable conditions in exposed positions, where they must contend with high solar radiation load (SRL) (Kobelt and Linsenmair 1986) and steep water vapor gradients (Geise and Linsenmair 1986). Water reserves are very limited (Schmuck and Linsenmair 1988); therefore, evaporative cooling is not an appropriate means of avoiding critically high body temperatures. Temperature regulation must therefore be achieved by other means. Reducing SRL by improved skin reflectance could be one essential device for diminishing the danger of overheating. This could be achieved by increasing the number of skin iridophores filled with purine platelets which very effectively reflect incoming radiation in the visible and the infrared range (Withers et al. 1982a; Kobelt and Linsenmair 1986). Froglets also have to deal with the problem of accumulating nitrogenous wastes. In contrast to fossorial amphibians, since reed frogs do not assume a torpor-like state, reduction in metabolism is rather limited. Consequently, they accumulate more nitrogen end products per unit of time than fossorial amphibians.

Investigations of those anurans which remain above ground during dry periods show that evaporative water loss (EWL) is considerably higher than in amphibians which withdraw into the ground (Bentley 1966; McClanahan 1967; Mayhew 1968; Ruibal et al. 1969; Warburg 1972), even though these anurans possess highly effective skin mechanisms to reduce EWL. This is due to the much lower average humidity of the surrounding air above ground compared to the more favorable microclimates in burrows. Both a higher rate of nitrogen accumulation as well as a higher water loss severely aggravate the osmotic problems that all amphibians face during prolonged periods of water shortage. Therefore, red frogs must have developed especially effective mechanisms to solve the problems of dealing with catabolic nitrogen end products in a water-conserving and osmotically harmless way. In many terrestrial amphibians, urea is the main end product of nitrogen metabolism and is accumulated during dehydration stress. However, its high solubility strongly increases the osmolarity of the body fluids and therefore causes osmotic problems when critical concentrations are reached.

Only a few anurans of the genera *Chiromantis* and *Phyllomedusa* have convergently evolved the mechanism of excreting nitrogen end products as osmotically inactive urate, which is also found in many reptiles and in birds (Loveridge 1970; Shoemaker et al. 1972; Shoemaker 1975; Balinsky et al. 1976; Drewes et al. 1977; Shoemaker and McClanahan 1982). Additional advantages of uricotelism in anurans are: (1) such species can feed during dry seasons, and (2) they might be able to selectively bind cations to insoluble urate-salts at high electrolyte concentrations (Shoemaker and McClanahan 1975). Because the economical detoxification of nitrogen end products by converting them to urea engenders several problems, uricotelism at first glance would seem to be the optimal solution for *H. viridiflavus spp.* These reed frogs, however, are ureotelic (Withers et al. 1982b; Geise and Linsenmair 1986; Schmuck and Linsenmair 1988). Is this due to a poor degree of adaptation to a xeric environment, or did *H. viridiflavus spp.* find another solution of how to circumvent the problems caused by accumulation of nitrogen end products?

During dry season conditions, the number of chromatophores with light-reflecting platelets (iridophores) in the skin of *H. v. taeniatus* greatly increases. These platelets mainly consist of the purines guanine (85–92%) and hypoxanthine (8–15%). As already stressed, iridophores act as radiation reflectors that can considerably reduce high SRL (Kobelt and Linsenmair 1986). In the initial phase of iridophore synthesis, skin reflectance strongly increases. However, after the synthesis of 2–3 layers which may already be present in juvenile frogs prior to the onset of the dry period, further production of iridophores, which always takes place under dry season conditions, only marginally increases reflectance. This finding suggests that iridophores have additional functions. Being a store for nitrogen end products, besides allowing color changes and reducing SRL, might be a third function of the iridophores in *H.v.spp.* (Schmuck and Linsenmair 1988) and was further investigated in this study.

In freshly transformed froglets, only one incomplete layer of iridophores is present in the dorsal skin and only a few iridophores are found in the ventral skin. In addition, there are only small differences among individuals in the amount of skin purines at the time of metamorphosis. In juvenile frogs living for only a few weeks in wet season terraria, however, amounts of skin purines show considerable variation between individual frogs in regard to their quantity (Schmuck and Linsenmair 1988). This variation is probably caused by microclimatic heterogeneity within the terraria. In some places conditions prevail that induce changes to dry season physiology (just below the hot 125 W lamp). Therefore, freshly transformed froglets seem to be more suitable for the study of (1) the differences in rates of skin purine synthesis under

different climatic conditions and (2) the correlation between purine synthesis and skin reflectance.

Material and methods

Treatment of frogs. Specimens of *H.v. taeniatus* were kept and bred in the laboratory at 28 °C/24 °C (day/night) and about 70%/100% RH. Freshly transformed froglets (stages 64–66 of the normal table of Nieuwkoop and Faber (1956), or stages 45 and 46 according to Gosner (1960)) weighing 200–300 mg were kept in plastic tubes for 2–3 days on wet filter paper at 20–23 °C, 90–100% RH and 13.5/10.5 h day/night cycle for acclimation.

After full hydration, 80 froglets were divided into two groups and kept separately in small plastic tubes for 35 days without feeding at 30 °/20 °C and 25–35%/55–65% RH (day/night cycle). Frogs were not fed during this time since under dry season conditions they never try to catch prey. Foraging was observed only when RH exceeded 90%.

Group 1, the wet-adapted controls, were watered daily for 60 min to the full hydration level by placing them in 500 µl double-distilled water.

Group 2 frogs were placed in 500 µl double distilled water (15 min) only when water losses exceeded 30–35% of body mass; they were considered as dry-adapted. All froglets were rehydrated to the full hydration level at day 4. Afterwards, they needed a first water supply between day 4 and day 12, and a second between day 12 and day 24. The times at which a third supply was needed showed considerable individual variation; in 90% of the froglets it was necessary between day 20 and day 35, and only about 10% received a third supply before day 20.

Reflectance measurements. Skin reflectance was measured with a pyroelectric radiometer (Molectron PR 200, spectral sensitivity 0.3–40.0 µm) with an integrating sphere (Earling). To simulate a natural spectrum, a 30 W tungsten quartz halogen lamp (color temperature 3150 K) was run at about 37 W (Willmer and Unwin 1981). The light was focused to a small spot and broadband filtered by quartz lenses reducing the spectrum to 300–3500 nm.

To determine the maximal reflectance, froglets sitting on black metal plates were placed on a dish heated with a thermostat to 40 °C. At this body temperature most froglets became white (displaying high reflectance values) and remained almost motionless. Thus, when handled carefully, anaesthesia was not necessary. After 45 min of temperature and color adaptation, a white frog on its metal plate was transferred to a small movable dish also heated to 40 °C. The focused beam of white light was directed onto the back of the frog and the dish was then placed close to the measuring hole of the sphere. Skin remittances were compared to that of a white standard, a frog-shaped plaster-cast covered with a 2 mm thick layer of Eastman 6080 paint.

Frog preparations. To take blood and/or lymph samples, froglets were first precooled and double pithed. The fluids were then drawn into heparinized micro glass tubes (Fa. Serva-Prax). The dorsal and ventral areas of the skin were removed and prepared for further analysis. The remaining carcasses were dried at 60 °C to constant weight.

Light microscopy. Small pieces of skin (2–3 mm^2) were fixed in 6.25% glutaraldehyde in 0.1 *M* phosphate buffer (pH 7.2) for 30 min, then washed briefly in phosphate buffer and post-fixed for 1 h in 1% osmium tetroxide/potassium dichromate (pH 7.3) (Wohlfahrt 1957). The tissue was dehydrated in a graded series of acetone and flat-embedded in Durcupan (Fluka) plastic resin. For light microscopy, sections were cut with a ultramicrotome (Reichert-Jung Ultracut) at 2 µm and stained with methylene blue/azure II (Richardson et al. 1960).

Purine analysis. The remaining parts of the removed dorsal and ventral skin were dried at 60 °C, homogenized with 10% phosphoric acid (2 washings) and kept at room temperature for 24 h. Samples were then exposed to ultrasound for 60 min and centrifuged for 10 min at 10000 RPM. The supernatant was buffered with NaH_2PO_4 to pH_2. Analysis was performed with a HPLC-analyser (Kontron). A sulfopropyl-daltosil 100 column (Serva) served as the stationary phase, and a triethylamine buffer (pH 7.5) as the mobile phase. The flow rate was 1.8 ml/min. Synthetic standards were used as identification markers.

Urea analysis. Plasma was obtained by centrifugation of blood and/or lymph samples. The amount of urea in these samples was photometrically determined with diagnostic kits (Sigma No. 535, colorimetric determination). Since the amount of ammonia in the urine of all the tested samples ($n=20$) was only 2% of the total amount of nitrogen, it was not calculated separately.

To measure urea excretion, frogs sitting in their tubes received 500 µl double distilled water for 15 min (group 2) or 60 min (group 1). The remaining water (i.e. not absorbed) was removed with a pipette and analyzed for its urea content.

Total body protein analysis. Total body protein was measured by the method of van Beurden (1980), using the dried carcasses. The initial body protein content was determined using a calibration curve representing protein content in relation to body size in 30 freshly metamorphosed froglets of the same stock. At each measurement interval, protein content of test froglets was determined and subtracted from the initial values in order to obtain protein consumption. Since the liver and skin were used for other analyses, the data do not give a quantitative measurement of the total protein catabolism rate. We assumed, however, that these changes reliably reflect the decrease of total body protein.

Fat-pad analysis. The fat-pads of the dissected animals were removed and dried for 2–3 days at 50 °C. The weight of the fat pads was expressed as a percentage of the body weight of the animal at the full hydration level.

Nitrogen balance. In order to analyse the compartmentalisation of the metabolized nitrogen, a nitrogen balance was determined. Three compartments were considered: body fluids, excreted urine, and the iridophores. The amount of nitrogen stored in the body fluids was calculated from the urea concentration of the plasma sample. It was assumed that, as in the case of juvenile *H. v. taeniatus* (Schmuck and Linsenmair 1988), urea is evenly distributed in the body fluids. The percent values shown in Fig. 6 were calculated from the nitrogen difference between two measurements; the nitrogen content of the various compartments are presented in relative amounts.

Results

Light microscopy

At the end of metamorphosis the dermal iridophore layer in the skin of the froglets is still incomplete. Iridophores are still absent in approximately 20–50% of the dorsal skin. In both groups the

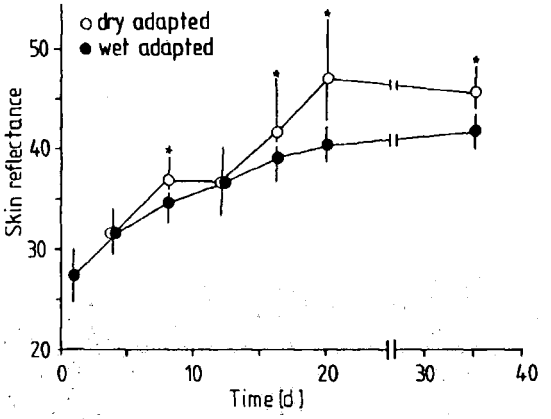

Fig. 1. Increase in dorsal skin reflectance during the postmetamorphic stage in *Hyperolius viridiflavus taeniatus*. The skin reflectance of wet-adapted froglets increases in a logarithmic pattern. Dry-adapted froglets show a faster increase. Values represent means of 10 specimens; vertical lines represent standard deviations. Stars indicate significant differences between wet- and dry-adapted frogs ($P<0.05$; t-test)

layer becomes completed within 4–8 days after metamorphosis. In group 1 the synthesis of a second layer was not completed within the 35 days of the experiment. The thickness of the layer was 20 ± 6 µm ($n=10$). Group 2 had synthesized a second layer after 20 days. At day 35 the iridophore layer was 31 ± 5 µm (significantly thicker than that of group 1, $P \leq 0.01$, t-test; $n=10$).

Reflectance measurements

Just after transformation, the average dorsal skin reflectance of froglets is about 28%, which increases to 32% by the first day of measurement under acclimation conditions (Fig. 1). The reflectance of wet-adapted froglets increases from 32% to 41% within 28 days in a logarithmic pattern. The reflectance of dry-adapted froglets increases more rapidly from 32% to 45%, and reaches a distinctly higher level than in group 1. The curve shows a conspicuous plateau between the 8th and the 12th day. No comparable plateau could be detected when investigating the increase in total purine content of the skin (see below). After day 20 further increase in skin purine content apparently affects skin reflectance to a lesser degree than before. The equations best describing the relationship between the skin reflectance (R) and the experimental time (t) in days throughout the first 20 days following metamorphosis were:

Group 1: $R=4.139 \ln t+27.165$
$r=0.891$; $n=63$; $P<0.001$

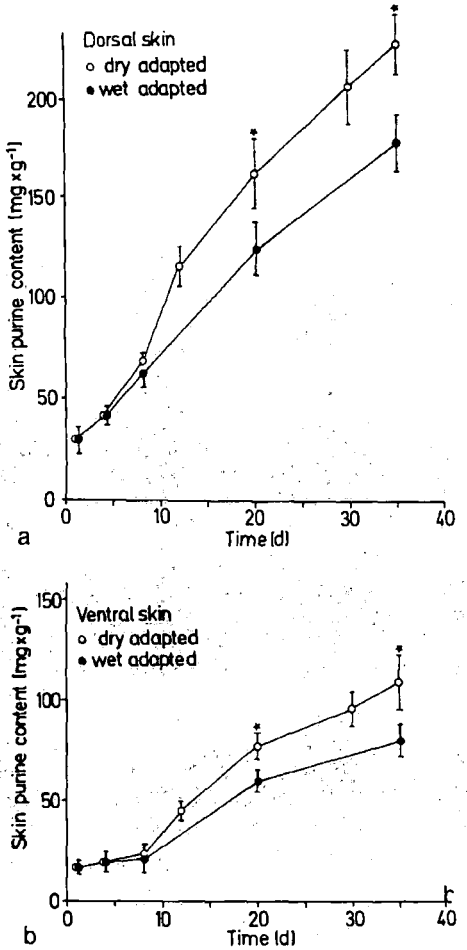

Fig. 2. Increase in the amount of purines (mg/g skin) in dorsal (**a**) and ventral (**b**) skin of *Hyperolius viridiflavus taeniatus* froglets during the course of their postmetamorphic development. Froglets were either watered daily (wet-adapted) or when water losses exceeded 30–35% of body mass, on average three times within 35 days (dry-adapted). Values represent means of 3 specimens; vertical lines represent standard deviations. Stars indicate significant differences between wet- and dry-adapted froglets ($P<0.05$; confidence interval)

Group 2: $R=5.55 \ln t+26.023$
$r=0.77$; $n=77$; $P<0.001$

The slopes of the relationships for group 1 and 2 differ significantly ($t=3.641$, $P \leq 0.01$; Sachs 1978, p. 341).

Changes in the total amount of skin purines

The iridophores of *H. v. taeniatus* froglets contain mainly the purines guanine (85–92%) and hypoxanthine (8–15%). Traces of adenine were found in only a few individuals. The curves showing the

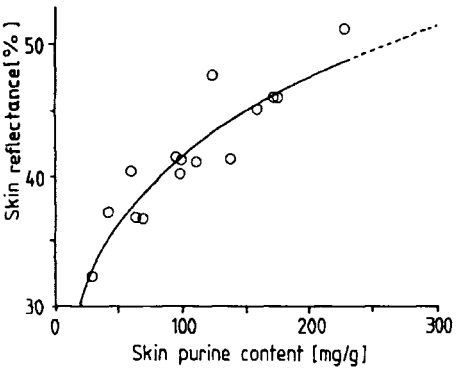

Fig. 3. Relationship between skin purine content and skin reflectance in dry-adapted *Hyperolius viridiflavus taeniatus* froglets

increasing amount of purines in the dorsal skin are similar in both groups (Fig. 2a). A phase of increased purine synthesis in group 2, however, can be recognized between day 8 and day 16. In this phase the amount of purine increases in group 2 at approximately twice the rate of group 1. Afterwards, purine synthesis continues at about the same rate in both groups for the remainder of the test time of 35 days.

Comparable events are found in the ventral skin (Fig. 2b). A phase of increased synthesis in group 2 also starts at day 8, but the rise amounts to only approximately half the highest level in the dorsal skin. This phase lasts about 8 days then the rate of synthesis decreases. The increase of purines (P) in the dorsal skin of the dry-adapted froglets affects skin remittance (R) according to the following exponential function:

$$R = 0.171\ P^{0.192}\ (\text{mg/g})\ \text{(see Fig. 3)}$$

Additional purine storage sites during long lasting dry periods

H. v. spp. also use other connective tissues for storing large quantities of purine crystals. In 2–4 month old *H. v. taeniatus*, which were maintained under dry season conditions for periods of 2–3 months and supplied on average 3 times with water, the heart and liver epithelia served as deposition sites. The liver epithelium of about 5–8 month old *H. v. nitidulus*, taken from the field 4 weeks after the first rainfall, was tightly packed with iridophores. The thickness of the layer was about 60 µm approaching that of the iridophore layer in the stratum spongiosum of ventral skin (about 75 µm).

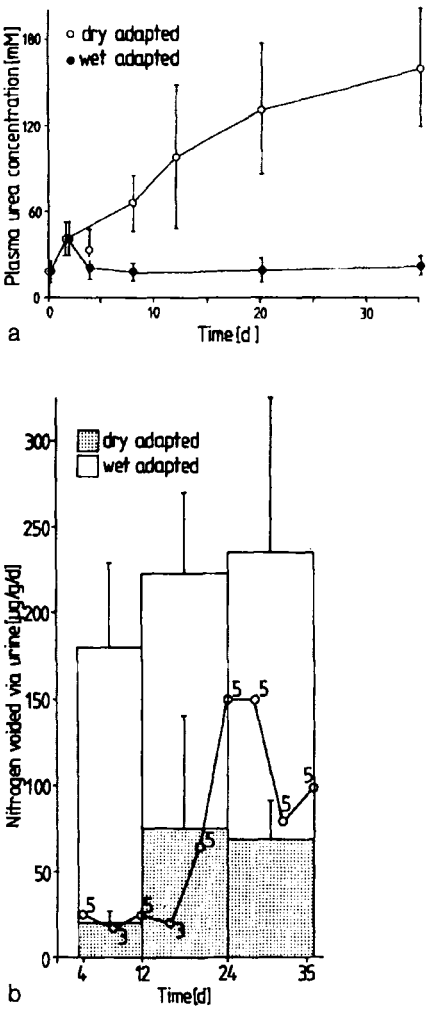

Fig. 4. Changes in plasma urea concentration (**a**) and urine excretion (**b**) in *Hyperolius viridiflavus taeniatus* froglets during their postmetamorphic development, dependent upon water availability. Wet-adapted froglets were watered daily for 60 min to the full hydration level; dry-adapted specimens received water for 15 min, only if water losses exceeded 30–35% of initial body weight. Columns indicate the average rate of nitrogen release in the considered time interval. Since the time of water supply in group 2 strongly varied between individuals, the mean values of sub-groups watered at a specific time were also plotted. Numerals indicate the number of individuals per measurement

Changes in the amount of blood urea nitrogen

Blood urea nitrogen (BUN) increases in late metamorphic climax (day 0–2). It then decreases to a constant level in group 1 (Fig. 4a), whereas in group 2 only a slight decrease, caused by the first water supply, could be observed. The BUN then rises until day 12 at about the same rate as in metamorphic climax (166 µg urea-N/g/d). This is fol-

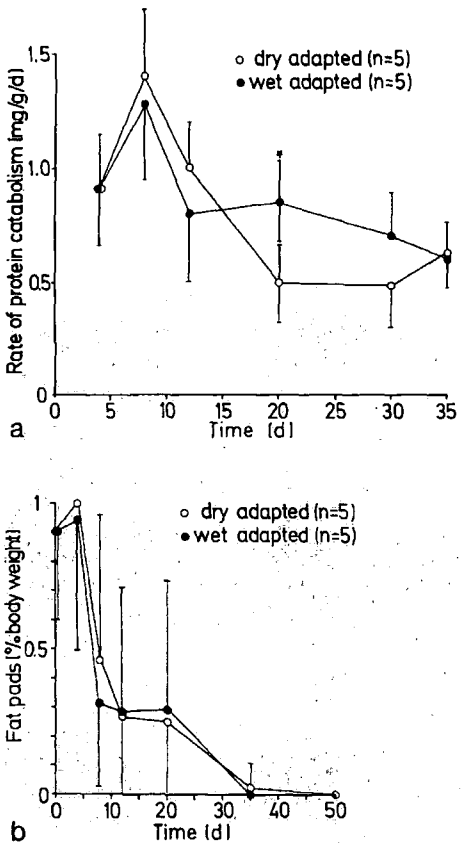

Fig. 5. Catabolism rate of protein (a) and fat reserves (b) during postmetamorphic development without energy input, dependent upon water availability. The protein catabolism rate represents the amount of protein in the carcasses, without skin and liver. Although only a part of the total catabolism rate could be measured, this rate reliably reflects the decrease in total protein. Stars indicate significant differences between wet- and dry-adapted froglets ($P<0.01$; confidence interval)

lowed by an increase at a somewhat reduced rate (of about 100 µg urea-N/g/d) to 122 m*M* at day 20. Thereafter, the rate of urea accumulation is greatly reduced, mainly due to urea excretion following water supply between day 20 and day 35, but also because of a reduction in protein catabolism (Fig. 5a).

Changes in the amount of urea excretion

Froglets of group 1 excrete 125–360 µg urea-N/g/d during each water uptake ($\bar{x}=212.5$ µg/g/d). If froglets of group 2 are given water (according to the schedule described earlier), they excrete only about 20–25 µg urea-N/g/d between day 4 and day 16. Afterwards, the urea excretion of group 2 froglets sharply increases up to 150 µg urea-N/g/d. After the third supply of water, between day 30 and 35, the excretion rate decreases to about 80 µg urea-N/g/d.

In order to obtain the mean value of N excreted per individual at the time of each measurement, it was necessary to combine single values over specific time intervals (Fig. 4b). The intervals were chosen so that they reflected the time frame in which water ($n=3$) was administered. The mean values given are calculated from the amount of urea excreted at the time of the watering, relative to the length of the respective time interval. Figure 4b summarizes these results. Urination was detected exclusively during watering. However, if a frog had urinated between any of the water sample intervals, its voided urea would have been collected and measured with the next water sample. Therefore, the total amount of urea excreted by both groups could be quantitatively measured by analyzing these water aliquots.

Changes in the amount of body protein and fat-pads

Until day 12 both groups metabolize protein at almost the same rate. After day 12 a considerable reduction of protein catabolism is seen in group 2 (Fig. 5a). In contrast, in group 1 the rate of protein catabolism does not decrease after day 12 and, except between day 30 and 35, never reaches such low values as in group 2. Therefore, protein reserves are consumed faster in group 1 than in group 2. The higher protein consumption in group 1 is also reflected in higher rates of nitrogen release.

The rate of fat consumption is similar in both groups (Fig. 5b). Maximum fat catabolism can be observed between day 4 and day 8.

Nitrogen balance

During the total time span of the experiment, an almost constant fraction of the released nitrogen in group 1 is used for purine synthesis. Only a very small portion of the nitrogen is found in the plasma of the froglets. Nearly all nitrogen is temporarily stored in and excreted via the bladder (Fig. 6). In group 2, until day 20 the nitrogen used for purine synthesis is not more than about 12% of the accrued nitrogen. Afterwards, up to 38% is used for purine synthesis. The portion of nitrogen flowing into the body fluids is markedly reduced between day 20 and day 35 due to urea excretion.

Discussion

Urea accumulation

During dehydration, many anurans are able to tolerate high urea contents in their body fluids (e.g. McClanahan 1967; McClanahan 1975; Shoemaker

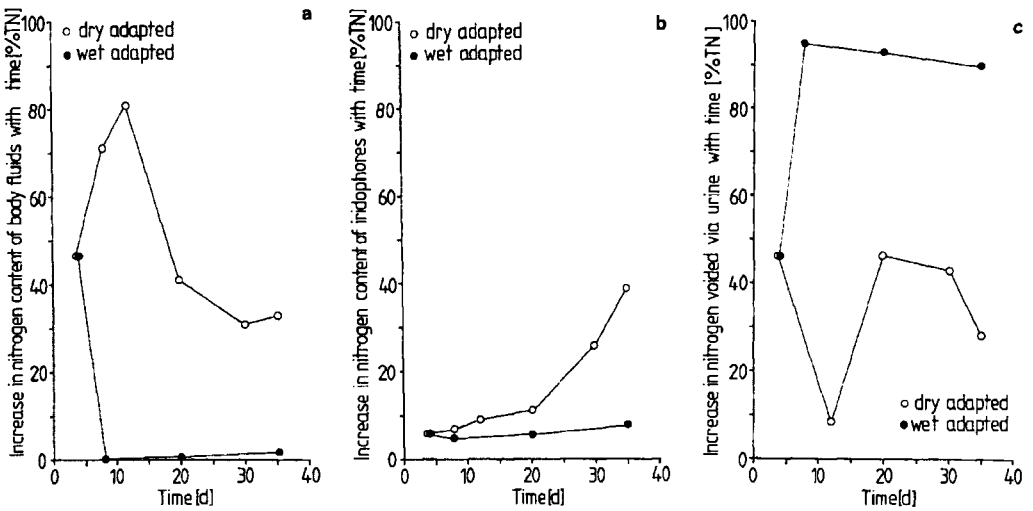

Fig. 6. Compartmentalisation of nitrogenous end products in *Hyperolius viridiflavus taeniatus* froglets, dependent upon water availability. Nitrogen stored in body fluids (**a**) and iridophores (**b**) or voided via urine (**c**) is expressed as percentage of the increase in total nitrogen (TN) between the corresponding measurement and the one preceeding, during 35 days following metamorphosis

1975). Accumulation of urea, however, is not only seen during water-deprivation but also in late metamorphic climax. After the transition from ammonotelism to ureotelism during metamorphosis, the activity of the ornithine-urea cycle enzymes is markedly increased (e.g., Dolphin and Frieden 1955; Dodd and Dodd 1976; Fox 1984). In metamorphic climax of anurans, the larval mouthparts degenerate and the adult jaws develop. During this period, amphibians are unable to feed. By this time, larval growth has ceased and all the energy, as well as the metabolites needed for differentiation and nutrition, are provided mainly by the resorption of the tail and by stored fat. This process is associated with protein catabolism and anabolism leading to a high output of urea (Dolphin and Frieden 1955). After transition to terrestrial habitats, the continuous water influx ceases although metamorphosis is still incomplete. When water reserves are limited urine production should be reduced. This may have resulted in a primary selection pressure towards developing tolerance for high urea concentrations in the body fluids during this ontogenetic phase.

Under dehydration stress, the amount of urea in the body fluids rises strongly to levels more than three times as high as in metamorphic climax, probably requiring a high degree of tolerance which might have developed as mentioned above. Thus, a tolerance to urea accumulation, primarily developed to avoid urea toxicity during metamorphic climax, may represent the evolutionary basis for the selection of a secondary tolerance to high urea concentrations occurring later in life. This tolerance is vital for ureotelic anurans if they are to successfully inhabit seasonally arid habitats.

Differentiation of pigmentation pattern in postmetamorphic stages

The morphological changes in pigmentation pattern during metamorphosis include, among others, the differentiation and the numerical increase of the chromatophores (Smith-Gill and Carver 1981), as well as the production of new pigments and/or the development of new organelles (Bagnara 1976; Bagnara et al. 1978). The metamorphic and postmetamorphic morphological color changes in wet-adapted *H. v. taeniatus* are caused by a rapid increase in the number of iridophores in the dorsal dermis. This increase almost doubles under dehydration stress, leading to the synthesis of an iridophore layer within 10 to 12 days, which in group 2 is about twice as thick (+12.1 μm) as in wet-adapted froglets (+6.7 μm). The increase in the thickness of the iridophore layer is accompanied by a rapid increase in skin reflectance. Between the 8th and the 12th day, however, no increase in skin reflectance of dry-adapted froglets could be detected. This possibly results from processes connected to the changeover to dry season physiology that may also affect the hormonal state of the frogs, and therewith their ability to physiologically change color. Such an assumption seems justified, since no comparable plateau could be detected by investigating the increase in total purine content of the skin. After the initial increase in purine synthesis, the rate of production during the

following three weeks in dehydrated frogs is reduced to values similar to wet-adapted animals. This latter rate seems to be a basic level at which guanine synthesis is maintained before and after the transition phase.

Under dehydration stress, the initially increased purine synthesis greatly reduces the high SRL prevailing in tropical arid habitats. During the wet season, this type of protection from SRL is not needed to such a great degree as in dry season, because sufficient water is available and EWL can be used for cooling. During this time, the main function of the iridophores might be seen in the context of color change (Bagnara 1976) and possibly UV radiation absorption.

Changes in protein catabolism during dry season state

At day 12, after a water restriction lasting 6–8 days, protein catabolism is remarkably reduced. Thus the output of nitrogen end products is minimized. This reduction in protein catabolism most probably marks the point at which the frog has completed the transition to dry season physiology. Starvation alone does not exert such an effect.

Nitrogen balance during the dry season state

During the dry season state, nitrogenous wastes, unavoidably resulting from protein catabolism and protein turnover, are predominantly stored as urea in the body fluids. Although water is occasionally present during the dry season for a limited period, urea excretion is nevertheless suppressed during the first phase of adaptation to dry season physiology; the stored urea most probably serves to accelerate water uptake along a steep osmotic gradient (Schmuck and Linsenmair 1988). Most frogs can survive a long-lasting dry season only by using every chance to replenish lost water (Geise and Linsenmair 1986). Water is available during the transition from wet to dry season mainly as dew, and during the dry season water is only occasionally available as poor rains that are very short and which evaporate very quickly. Therefore, dry season frogs must minimize EWL and maximize uptake of available water. However, as soon as the disadvantages of nitrogen accumulation exceed the advantages of increasing rehydration rates, urea excretion via the urine should be used to avoid dangerously high osmotic concentrations. Before the frog fully changes to dry season physiology, only about 12% of the released nitrogen is stored in iridophores. In dry-adapted frogs, the relative portion of nitrogen (up to 38%) that is stored as non-toxic osmotically inactive purines is strongly increased by reducing protein catabolism and maintaining a high rate of purine synthesis. Thus, guanine (and to a far lesser extent hypoxanthine) now serves as an outlet, that helps to regulate the urea level in the body. Our conclusion that guanine, besides its role in physiological color change and in reduction of SRL, also serves as a store for nitrogen end products, is supported by the fact that the endothelia of the heart and the liver of frogs may become heavily filled with guanine crystals during estivation. This phenomenon is especially apparent in animals that have survived several months under dry season conditions in the field. The storage of guanine in these tissues does not reduce high SRL. The only plausible explanation we can see is that of nitrogen storage. The climate space diagram of *H. v. nitidulus* indicates that in West African habitats skin reflectance has to be above 0.6 to avoid overheating during the hottest times (Kobelt, unpublished). Given that such a high skin reflectance is a vital need for survival during the dry season, then guanine is, because of its higher refractive index and better crystalline qualities, more effective in raising skin reflectance than uric acid. Also due to its better N:C ratio, guanine is more effective than uric acid in eliminating nitrogen end products in an osmotically inactive form. Thus, guanine probably has a higher adaptive value for *H. viridiflavus* than uric acid, which is produced by other anurans which endure dry periods above ground (Shoemaker et al. 1972; Balinsky et al. 1976).

The storage of nitrogen end products in the form of skin pigments does not require the acquisition of new enzymatic pathways or qualitatively new storage sites. Iridophores are supposed to have been primarily developed for color change (Bagnara 1966). Their ability to reflect light provides an excellent basis for their use in temperature regulation (Withers et al. 1982a; Kobelt and Linsenmair 1986). Because the onset of a dry period can never be accurately predicted, it is highly advantageous to build up and maintain an effective antiradiation safeguard, and this requires synthesis of purines at a rate sufficient to counteract the decrease in the thickness of the iridophore layer caused by body growth. Therefore, it comes as no surprise that a continuous synthesis of purines is also found in wet-adapted froglets. Within certain limits, however, purine synthesis rate can be considerably raised when early onset of dry season conditions necessitates a rapid increase in skin reflectance. Besides its function in color change and

temperature regulation, the iridophore system has gained an additional and very essential importance in reducing osmotic problems by storage of nitrogen end products during times of prolonged water deprivation. The stratum spongiosum of the dorsal and ventral skin of *H. v. taeniatus*, which were exposed to dry season conditions over a long period of time (>3 month), is completely filled with iridophores.

Geise (unpublished) demonstrated that survival time during dry periods is highly correlated with body size and stored fat reserves. Only frogs reaching a body length of more than 1.6 cm and storing about 14% of body dry weight as fat (subcutaneous fat deposits, fat pads and body lipids combined) can successfully survive the following dry season. Because adult *H. viridiflavus spp.* spawn throughout the wet season some froglets transform at the end of the rainy season, or even later during the transition period to true dry season (Linsenmair, unpublished field observations). Therefore, freshly transformed froglets also have to be able to survive intermediate dry periods that frequently and unpredictably occur during the transition period. Our results indicate that froglets are able to tolerate such intermediate periods even immediately after metamorphosis by increasing their skin reflectance and, subsequently, by reducing their energetic demands. On average, however, these froglets are too small to survive a prolonged dry season and must therefore use every opportunity for energy accumulation and growth during the transition period between rainy and dry season. After rainfall or dew, froglets are able to feed immediately, thereby rapidly accumulating energy reserves through a high net production rate (Schmuck, unpublished). The rise in skin reflectance by purine deposition and the use of purine as a store for nitrogen end products during intermediate dry periods provides an important adaptation for survival under fluctuating and temporarily extreme environmental conditions.

Acknowledgements. This study was supported by the Deutsche Forschungsgemeinschaft Research Grant Li 150/11-1/2. We thank two anonymous referees for valuable criticism and Mrs. L. Rott for checking our English.

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