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SEASONAL OXYGEN METABOLISM AND CUTANEOUS OSMOREGULATION IN THE CALIFORNIA NEWT, *TARICHA TOROSA*¹

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While breathing air at rest, aquatic *Taricha torosa* consumed $0.054 \text{ cm}^3 \text{ O}_2 \text{ g}^{-1} \text{ h}^{-1}$, which represent oxygen metabolism twice that of the terrestrial phase. There was no significant difference in the rate of oxygen consumption between phases upon submergence. Aquatic newts accumulated a significantly higher lactate debt than the terrestrial phase while underwater. Aquatic newts had a higher emerged heart rate and exhibited a significantly greater bradycardial response after 40 min of dive. Although initiation of the response was relatively slow in both phases, lowest heart rates were realized after 60–120 min. The reduced oxygen uptake and increased blood lactate levels during a dive by aquatic newts suggest that skin permeability may be a limiting factor for oxygen uptake. Data from this study do not indicate cutaneous changes to have respiratory consequence. Aquatic *T. torosa* skin exhibited significantly lower in vitro rates of water transfer in an osmotic gradient of 210 mOsm/liter and a significantly greater rate of sodium transport when compared to the terrestrial phase. The results of these experiments imply an osmoregulatory function for seasonal morphological skin changes displayed by *T. torosa*.

INTRODUCTION

The life cycle of the California Newt, *Taricha torosa*, is interesting because a complete change in habitat takes place on a seasonal basis. Male newts, during the aquatic breeding phase, undergo obvious morphological skin changes from granular to smooth appearance (Twitty 1966). Movement away from the water in late spring is accompanied by morphological skin changes back to the terrestrial state. These transitional changes are not evident in females who remain in the water only long enough to breed and deposit egg packets (Smith

1941; Twitty 1942). The anatomical changes associated with newt "water drive" have been described by previous workers (Miller and Robins 1954; Grant and Grant 1958; Pimentel 1959), but the physiological consequences of such changes are still largely obscure. Transition to an aquatic environment places both respiratory and osmotic demands upon this basically terrestrial animal. Gas exchange in lunged urodeles involves both pulmonary and cutaneous surfaces (Whitford and Hutchinson 1965; Guimond and Hutchinson 1968). Bannikov (1948) showed that the breeding newt, *Diemictylus viridescens*, placed in water deprived of atmospheric oxygen survived longer than did nonbreeding specimens under the same conditions. This suggested that the aquatic phase was characterized by lower metabolic requirements and/or greater anaerobic capacities.

Two factors which are important in

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determining the duration of a dive by salamanders are the oxygen requirements of the animal and its ability to obtain oxygen from the water. The fresh water habitat poses two additional stresses; water gain by osmosis and electrolyte loss by diffusion (Bentley 1971). Cutaneous compensation mechanisms for maintaining homeostasis under such conditions are: reduction in permeability to water (Kirschner et al. 1971) and increased active transport of sodium (Ussing and Zerahn 1951; Bricker et al. 1962; Aceves, Erhij, and Edwards 1968). In this study respiratory and osmotic characteristics of the aquatic and terrestrial newt skin were investigated in relationship to seasonal morphological changes.

METHODS

Collection and maintenance of animals.—Male *Taricha torosa* were collected in Trabuco, Holy Jim, and Harding Canyons, in Orange County, California, during the spring and autumn of 1972. Individual specimens, with an average body weight of 9.7 g, were kept in the lab for approximately 3 weeks before being replaced by new ones. Aquatic newts were maintained in artificial pond water (APW) as described by Alvarado and Johnson (1965). Terrestrial newts, on the other hand, were maintained in a terrarium with moss and leaf litter. Both groups were force-fed every 3 days on *Tenebrio* larvae. Metabolic studies were confined to mid-morning and early afternoon hours to minimize the influence of diurnal rate fluctuations. Test animals taken during the aquatic and terrestrial stages were acclimated at 15 C for 72 h without food and light prior to experimentation.

Aerial respiration.—Standard resting rates of oxygen consumption were measured at 15 C in a darkened 250-cm³ chamber. The animals were allowed to

settle down in the chamber for 3 h. The chamber was then flushed with air and the system closed for a 20-min period. At the end of 20 min a 90-cm³ sample of air was removed through a serum cap port by a syringe fitted with a two-way stop cock. Oxygen in the sample was determined with a Beckman E-2 Paramagnetic Oxygen Analyzer.

Aquatic respiration.—Animals were restrained in a 4 × 12-cm perforated cylindrical tube which was inserted into a 1-liter container filled with APW previously equilibrated at ambient pO₂. The system was closed with a rubber stopper housing an O₂ electrode. With this electrode, changes in oxygen content were monitored on a Beckman Model 160 Physiological Gas Analyzer which was calibrated by bubbling nitrogen through water previously equilibrated in air. The entire container was submerged for 30 min in a water bath maintained at 15 C.

Heart rate.—Heart rate was determined with two EEG disc electrodes fitted in a harness secured to the pectoral region of the salamander. Continuous electrocardiograms (EKGs) were recorded on a physiograph (Narco Biosystems, Inc.). The salamander, with attached dermal leads, was placed in a 4 × 12-cm perforated cylindrical container to restrict his movement. The container housing the salamander was suspended, by means of attached string, inside a 5,000-cm³ chamber half-filled with APW. The chamber was, in turn, submerged in a 15 C water bath, thereby maintaining both the air and water at a constant temperature. A 2-h adjustment period was allowed in which the animal was suspended above the water in the perforated container. A 1-h forced dive was initiated by lowering the animal within the perforated chamber into the water. A 1-h recovery period was initiated by raising the animal back into the air phase of the submerged chamber.

Blood lactic acid.—Blood samples (50 μ l) were taken by decapitation 10 min after emergence from a 3-h forced dive. Preliminary experiments indicated that the blood lactate was maximal at this time. Blood lactic acid was analyzed using the Boehringer Mannheim LDH (lactate to pyruvate) test kit.

Cutaneous sodium and water transport.—In vitro sodium transport was measured on skin excised from the ventral region of terrestrial and aquatic phase newts. The skin was mounted between two Lucite chambers of a design similar to that employed by Ussing and Zerahn (1951). The electrical potential difference across 3.6 cm² of skin was measured with a pair of Beckman 39170 Calomel electrodes connected to a Sargent Model SRLG 10-inch recorder. Current was passed through the salamander skin from an external source via Ag-AgCl electrodes and measured with a microammeter. Both sides of the skin were bathed in Ringer solution as described by Packer (1967). Potential difference (PD) was continually recorded while short circuit current (SCC) was determined every 15 min. Each microampere of SCC is equivalent to 10.5×10^{-6} μ mol of Na⁺ ions transported per second (see Packer 1967).

In vitro cutaneous water exchange was measured on excised ventral skin which was tied with nylon thread to the end of

a glass cylinder so that 0.39 cm² of the epidermal surface faced inward. This was then attached with rubber tubing to the end of a needleless Hamilton 0.05-ml syringe barrel, which was calibrated in 1- μ l divisions. The skin was immersed in 20 ml of aeriated Ringer solution at 15 C while the pipette tip and syringe barrel were filled with 10% Ringer solution. The osmotic gradient from mucosal to serosal side was 210 mOsm/liter. The rate of water transfer from the pipette to the beaker of Ringer was read directly from the syringe barrel (Baldwin 1973; Hillman 1974).

RESULTS

Oxygen consumption and heart rate before and during a dive.—The average aerial oxygen consumption was significantly higher ($P < .05$) for newts in the aquatic phase (table 1). A value of 0.054 cm³O₂ g⁻¹h⁻¹ at rest for the aquatic phase represents an oxygen consumption rate twice that of the terrestrial phase. A significant reduction in oxygen consumption was observed in aquatic ($P < .005$), but not terrestrial newts ($P > .10$) upon submergence.

Both aquatic and terrestrial *Taricha torosa* exhibited a bradycardial response upon complete submergence at 15 C (fig. 1). Reduction in heart rate was not immediate, and the full extent of decline took from 60 to 120 min. Aquatic newts

TABLE 1
MEAN RATES OF OXYGEN CONSUMPTION AND BLOOD LACTIC ACID^a
OF AQUATIC AND TERRESTRIAL "TARICHA TOROSA"^b

	Aquatic	Terrestrial
Aerial respiration (cm ³ O ₂ g ⁻¹ h ⁻¹).....	0.054 ± 0.004 (12)	0.025 ± 0.008 (12)
Aquatic respiration (cm ³ O ₂ g ⁻¹ h ⁻¹).....	0.019 ± 0.008 (7)	0.022 ± 0.011 (10)
Lactic acid (mg %):		
Pre-dive Rest.....	12.4 ± 1.6 (8)	10.3 ± 1.6 (8)
Post-dive.....	109.5 ± 6.7 (7)	84.9 ± 4.57 (8)

^a Pre-dive rest and after 3 h of submergence with 10 min of recovery.

^b Mean and 2 SE of the mean. No. of experiments in parentheses.

have a significantly higher presubmergence heart rate ($P < .05$), indicating seasonal variations in agreement with oxygen consumption data. At 50 min the submersed aquatic newts exhibited a greater reduction in heart rate than did terrestrial newts ($P < .005$). This continued until the time of emergence. Recovery after emergence to presubmergence rate was the same for both aquatic and terrestrial newts ($P > .10$).

Blood lactic acid.—Resting, predive lactic acid levels were not significantly different between phases (table 1). Aquatic newts, however, accumulated a significantly higher concentration of lactic acid ($P < .025$) than terrestrial newts,

indicating greater anaerobiosis for the aquatic stage animals while under water (table 1).

Water and sodium transport across excised skin.—Aquatic excised skin showed a significantly reduced ($P < .05$) rate of water transfer and higher rate ($P < .005$) of sodium transport in comparison to the terrestrial excised skin (table 2).

DISCUSSION

The results of the present study indicate that the resting metabolism of terrestrial newts after estivation is less than newts in the aquatic phase. This is in agreement with the work of Noble (1931) and Vernberg (1952), who found that the

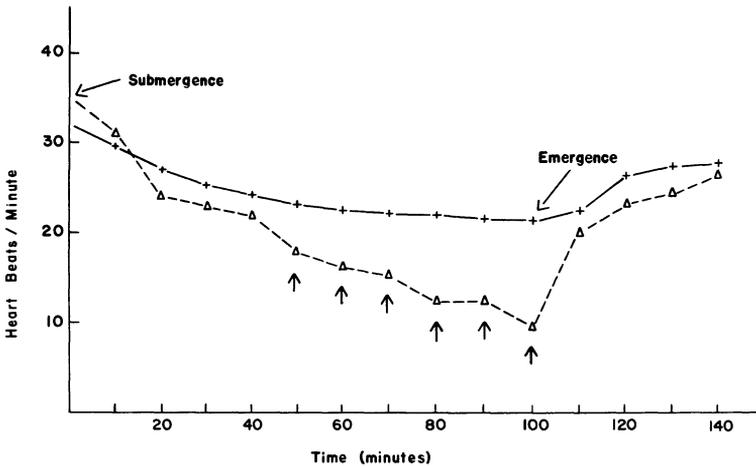


FIG. 1.—The effect of submersion on heart rate of *Taricha torosa*. Triangles represent heart rates of aquatic newts; crosses represent heart rates of terrestrial newts; vertical arrows depict significantly different rates; diagonal arrows indicate heart rate prior to submergence and emergence. All points represent mean values ($N = 8$).

TABLE 2
IN VITRO WATER AND SODIUM TRANSPORT ACROSS AQUATIC AND TERRESTRIAL "TARICHA TOROSA" SKIN^a

Life Stage	Water Transfer ($\mu\text{l}/\text{cm}^2/\text{h}$)	SCC ($\mu\text{A}/\text{cm}^2$)	PD (mV)	Sodium Transport ($\mu\text{eq}/\text{cm}^2/\text{h}$) ^b
Aquatic	15.7 ± 4.6 (10)	21.1 ± 2.3	37.09 ± 5.2	0.79 ± .008
Terrestrial	29.0 ± 10.2 (10)	12.8 ± 3.1	21.5 ± 5.1	0.48 ± .011

^a Mean and 2 SE of the mean. No. of experiments in parentheses.
^b Obtained by multiplying: (SCC) (10.5×10^{-6} $\mu\text{moles of Na}^+$ ions transported per second) (3.6×10^8 sec).

greatest resting metabolic rates for salamanders were during the months of May–June and the lowest rates were in October–November. Fromm and Johnson (1955) also showed a higher metabolic rate associated with the spring breeding season in three species of frogs. Higher metabolic rates of *Taricha torosa* in the breeding state may therefore reflect greater energy requirements associated with aquatic life.

Oxygen availability to an aquatic newt would be greatly influenced by several obvious factors, such as: (1) basic circulatory parameters including peripheral circulation, blood flow, hemoglobin concentration, plasma volume, and blood oxygen carrying capacity, and (2) general physical restraints such as skin permeability and surface to volume ratio.

Czopek (1959) observed an increase in the number of skin capillaries in *Triton cristatus* during the breeding state and suggested that this functioned to increase cutaneous blood flow and enhance O₂ uptake. In addition, Whitford and Hutchinson (1965) have shown that there is less diffusion of O₂ through the skin of *Ambystoma tigrinum* in water than in air at 15 C. This reduction could be related to a lower O₂ gradient between the environment and the dermal capillaries. For these reasons, an increase in peripheral capillarity would increase oxygen availability for an aquatic organism. Aquatic *Taricha torosa* however, when submerged, did not show a higher oxygen consumption per unit body weight over that of submerged terrestrial phase newts. In addition, they exhibited greater activity while underwater, thus incurring higher

energy requirements which were reflected by a deeper bradycardial response (fig. 1) and higher blood lactate levels (table 1). These data suggest that changes in newt skin did not increase oxygen availability to the animal but may serve an alternate function associated with re-entry into an aquatic habitat.

Schmid (1965) found skin of aquatic anurans to be less permeable to water than the more terrestrial species. A reduction in cutaneous water permeability, as demonstrated in this study on aquatic phase *Taricha torosa*, would significantly decrease the problem of excess water influx while in an aquatic environment.

The solute efflux from urinary sources in turn must be replenished by transport across the skin and by feeding. This was demonstrated by Crabbe (1961) who showed that the rate of sodium transport across in vitro anuran skin increased when the animal had been previously maintained for several days in distilled water. Similarly, in this study, the in vitro sodium transport across the skin of newts in the aquatic phase was 64% greater than the skin of terrestrial phase newts, thereby compensating for electrolyte losses imposed by the aquatic environment.

It is suggested, therefore, that the described cutaneous characteristics of breeding *Taricha torosa*, while not affording a respiratory advantage, do show adaptive osmoregulatory benefits over the nonbreeding newts for survival in a fresh water habitat by limiting osmotic flooding and increasing active sodium uptake.

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