981) 144:241–246

Journal of Comparative Physiology · B

Respiration and Acid-Base Balance in the Salamander, *Ambystoma tigrinum*: Influence of Temperature Acclimation and Metamorphosis

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Accepted May 24, 1981

Summary. Effects of temperature acclimation (5 or 25 °C for 2–4 weeks) and metamorphosis on oxygen uptake, acid-base balance and blood-O_2 affinity have been investigated in *Ambystoma tigrinum*. The results differ from previous studies in three ways. (1) The transition from gilled to gill-less adults had no effect on the O_2 affinity of blood. (2) Cold acclimation increased blood O_2 affinity in neotenes and had no effect in adults. (3) O_2 uptake increased, rather than decreased, after acclimation to a higher temperature. The results resemble previous studies also in three ways. (1) O_2 uptake increased with the transition from gilled-neotenes to gill-less adults as did the dependence on air-breathing. (2) Metamorphosis resulted in CO_2 retention and a fall in arterial pH. (3) The temperature coefficient of blood pH was about -0.014 $d\text{pH}/dT$ in vivo and in vitro. The physiological significance of the results is discussed with respect to the natural history, modes of breathing, and dependance on aerial respiration of *Ambystoma tigrinum*.

Introduction

Amphibians must contend with two environmental variables important for gas exchange. These are temperature, which affects oxygen demand, and the respiratory medium (water vs air), which affects oxygen availability. Previous studies have shown that metabolic compensation often occurs with temperature acclimation (cf. Prosser 1973). It is also known that changes in hemoglobin-oxygen affinity may accompany acclimation to temperature and O_2 levels of the respiratory medium (cf. Wood 1980).

A further complexity in amphibian gas exchange involves the changing roles of the gas exchange and transport mechanisms during metamorphosis from larval to adult forms. Of course, metamorphosis per se may be influenced by ambient temperature and oxygen availability. The study of amphibian metamorphosis and its relation to gas exchange and to temperature is of interest not only in terms of ontogeny, but may also provide insights into possible adaptive changes occurring with the evolution of terrestrial air breathing vertebrates.

Metamorphic events wich lead to a more terrestrial existence are highly complex in the Urodeles, as in Anuran amphibians. Typical of the Urodeles in many respects is the genus *Ambystoma*, which includes the Mexican axolotl and the tiger salamander. Larval *Ambystoma* generally possess external gills, thin vascularized skin and, in the later developmental stages of the larva, a simple pair of lungs ventilated with air by a buccal pump. Depending on ambient temperature, larval stages of *Ambystoma tigrinum* may achieve from 10%–50% of their oxygen uptake from the air (Whitford and Sherman 1968). In many Ambystomid species, further external development may be arrested at this stage, although the larvae, then called neotenes, become sexually mature.

Ambystoma tigrinum is a particularly suitable amphibian species in which to investigate the respiratory physiology of metamorphosis. Depending on genotype, location, seasonal temperature, altitude, and other factors, individual populations may contain small larvae, large neotenic larvae and fully metamorphosed adults with the same environmental history.

The present investigation was designed to elucidate the respiratory functions of *A. trigrinum* in the face of natural variations in temperature, oxygen availability and developmental state. Data are presented on O_2 uptake, O_2 transport, and acid-base balance as a function of thermal acclimation and metamorphosis from gilled neotenes to gill-less adults.

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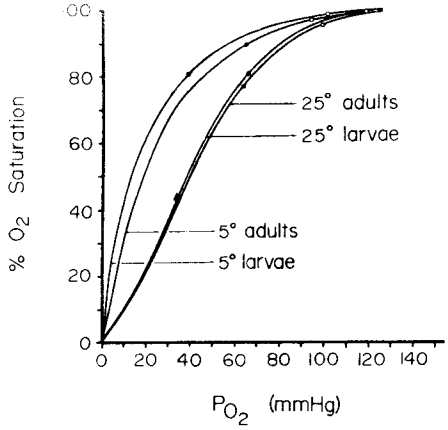

Fig. 1. Representative oxygen dissociation curves of blood from larval and fully transformed adult *Ambystoma tigrinum* acclimated at 5 and 25 °C. All dissociation curves were measured at in vivo temperature on blood at pHa (see Table 1). Mean values of lung P_{O_2} for each group are indicated on each curve with an open circle, while corresponding values for arterial blood are represented by solid circles. A possible value for systemic venous blood P_{O_2} is indicated for the 25 °C larvae by a triangle (see Discussion for further details)

Table 1. In vivo values ($\bar{x} \pm 1$ s.d.) of arterial blood pH (pHa), $[HCO_3^-]$, and blood P_{50} in larval and adult *Ambystoma tigrinum* acclimated to 5 or 25 °C. Values in parenthesis refer to numbers of animals when different from those indicated in the first row

Acclimation and measurement temperature (°C)	Larvae		Adult	
	5 °C	25 °C	5 °C	25 °C
Number of animals	8	10	5	9
Body mass (g)	65 ±7	66 ±8	23 ±11	21 ±9
In vivo pHa at acclimation temperature	7.97 ±0.05 (6)	7.70 ±0.09	7.78 ±0.06 (4)	7.51 ±0.05 (5)
In vivo $[HCO_3^-]$ (mM/l)	16.6 ±1.18 (6)	11.2 ±2.6	14.3 ±1.9 (4)	20.9 ±2.0 (5)
P_{50} at pHa (mmHg)	13 ±4	39 ±4	19 ±4	41 ±2

Materials and Methods

Animals. Experiments were performed on 45 *Ambystoma tigrinum*. The larval neotenes and adults had weight ranges of 51–79 g and 12–34 g, respectively. They were collected during the spring from natural ponds and cattle 'dug-outs' in New Mexico and Arizona, transferred to aquaria in the lab, and divided into two populations containing a mixture of neotenes and adults. One population was then kept at 5 ± 1/2 °C, the other at 25 ± 1 °C. Animals in both populations were acclimated for at least 2 weeks (often greater than 4 weeks) before any experimentation. Some neotenes in the warm population transformed to adults. Animals in the process of metamorphosis (beginning of regression of external gills or development of characteristic skin mottling of the adult) were not used and all adults used were at least 3 weeks post-metamorphosis. Adults were kept in open aquaria with shallow water and access to a platform so they could leave the water.

Blood Sampling and Analysis. Blood was sampled in one of two ways. In one group, undisturbed larvae or adults were instantly killed by a sharp blow to the head. The chest was quickly opened, and blood withdrawn from the truncus arteriosus into a heparinized syringe. From first disturbance of the animal to termination of blood sampling usually required 30–45 s. In the second group, animals were anaesthetized with MS 222 and the femoral artery or vein was then occlusively cannulated in an upstream direction with a PE 10 or PE 20 tubing. Collateral circulation must exist for the hind limbs, since femoral venous or arterial cannulation did not impair hind limb function. The animals were then allowed to recover for at least 18–24 h before blood sampling was started. In several animals the left lung was also cannulated through the chest wall with a PE 60 tubing for the purpose of drawing lung gas samples. All catheter positions were confirmed post-mortem.

Oxygen dissociation curves for larval neotene and adult blood were determined at 5 or 25 °C on 2 µl whole blood samples with an Aminco Hem-O-Scan apparatus. The pH of blood samples in this apparatus was determined by tonometry (BMS-2; Radiometer) with the same P_{CO_2} used in the determination of oxygen dissociation curves. Duplicate runs were performed on each blood sample, with the mean P_{50} of each animal determined by averaging the two values. The discrepancy was rarely more than 1 mmHg.

In vivo pH was measured anaerobically in blood obtained from rapid arterial puncture or from cannulae. Arterial P_{CO_2} was calculated using the Astrup technique (Siggard-Anderson et al. 1960). In vivo HCO_3^- concentrations were calculated from in vivo P_{CO_2} and pH with the Henderson-Hasselbalch equation using values of pK′ (5 °C: 6.32; 25 °C: 6.15) and CO_2 solubility coefficients (5 °C: 0.076; 25 °C: 0.040) from Reeves (1976).

In vivo blood and lung P_{O_2} was measured on 100 µl samples drawn anaerobically into a glass syringe, and injected into the blood gas analyzer. Lung ventilatory movements of the larvae and adults were monitored visually.

Oxygen Uptake. Animals were placed individually in 500 ml Erlenmeyer flasks containing 300 ml of water, through which air was bubbled. The exhaust line from the flask was the input for an O_2 analyzer (Applied Electrochemistry 53A) whose output was recorded on a chart recorder. The agitation provided by the airstone kept water and gas P_{O_2} in equilibrium. Any reduction of the oxygen concentration in the exhaust gas was therefore the additive effect of aquatic and aerial respiration. A precision reduction valve and 'soap bubble' flowmeter were used to control and measure air flow. Flow was regulated to provide a reduction in O_2 concentration of the exhaust gas of 0.4–0.6%. The entire flask was placed in a thermostatted water bath at either 6 or 25 °C. Following a 12 h acclimation period, oxygen uptake was measured during an ensuing 24 h. Since the animals were fasting, an assumed respiratory exchange ratio (R) of 0.7 was used to correct calculated values of O_2 uptake for $R < 1$ (Hill 1972).

Results

O_2 Affinity of Blood

The oxygen dissociation curve for each experimental group is shown in Fig. 1 for in vivo conditions of temperature and pH (Table 1). The in vivo pH of adult blood was 0.2 units less than that of neotene blood at both 5 and 25 °C. When the O_2 affinities (P_{O_2} at 50% saturation) are corrected for the pH

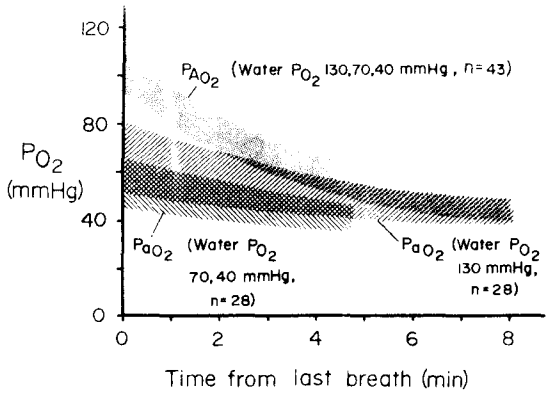

Fig. 2. Lung P_{O_2} ($P_{A_{O_2}}$) and arterial P_{O_2} (Pa_{O_2}) during intermittent lung ventilation in undisturbed, unrestrained larval *A. tigrinum* acclimated to, and measured at, 25 °C. Lung and blood P_{O_2}'s were measured at a water P_{O_2} of 130, 70 and 40 mmHg. The different hatched areas include all data points for the particular water condition indicated. *n* number of individual samples made from an overall total of 9 larvae

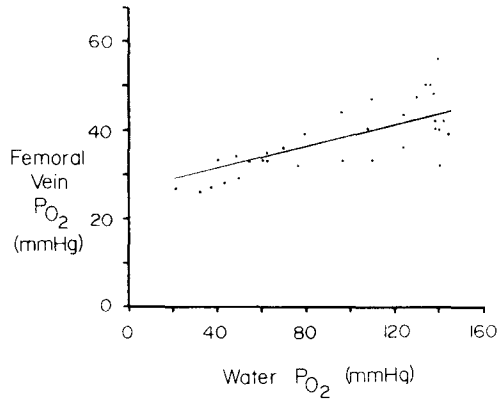

Fig. 3. The oxygen partial pressure of blood in the femoral vein as a function of water P_{O_2} in 4 undisturbed, unrestrained larval *A. tigrinum* acclimated to 25 °C. The linear regression line was calculated by the method of least squares

differences using a Bohr factor of -0.08 $d\log P_{50}/d\text{pH}$ (Wood et al., to be published) there is no significant difference between the blood of larvae and adults at either 5 or 25 °C. The influence of temperature acclimation on O_2 affinity was assessed by comparing P_{50} values at constant pH (7.7) and temperature (25 °C). All P_{50} values convert to about 40 mmHg except the cold-acclimated larvae ($P_{50}=25$ mmHg). The cellular mechanisms controlling this change in O_2 affinity are described elsewhere (Wood et al., to be published).

O_2 Transfer in the Lungs

Figure 2 shows the results of lung and arterial P_{O_2} measurements in 17 larvae. The animals were unrestrained in shallow water of varying P_{O_2}. After gulping air at the surface they submerged for apneic periods of 3 to 8 min. Lung P_{O_2} ($P_{L_{O_2}}$) was 40–50 mmHg above arterial P_{O_2} (Pa_{O_2}) immediately after a breath and decreased to values approaching Pa_{O_2} during the apneic period. When water P_{O_2} was reduced to 70 or 40 mmHg, Pa_{O_2} but not $P_{L_{O_2}}$ decreased. When the same experiments were repeated at 5 °C the apneic periods were 30 min or longer. The results at 5 °C were (mean ± SD):

P_{O_2} air ($P_B \simeq 630$ mmHg)	P_{O_2} H_2O	$P_{L_{O_2}}$	Pa_{O_2}
122 ± 5	120	102 ± 20	41 ± 4
122 ± 5	90	102 ± 20	20 ± 5
122 ± 5	45	102 ± 20	23 ± 8

Lung and arterial P_{O_2}'s were measured in adults under normal, terrestrial, conditions. The values immediately after a breath were:

	$P_{L_{O_2}}$ (mmHg)	Pa_{O_2} (mmHg)
5 °C	95 ± 11	65 ± 12
25 °C	120 ± 17	63 ± 2

Because of the small size of these animals we were unable to obtain mixed venous blood to assess overall tissue O_2 delivery. However, measurements of femoral venous P_{O_2} provided some data relevant to cutaneous O_2 uptake. As shown in Fig. 3, femoral venous P_{O_2} (Pv_{O_2}) was, like Pa_{O_2}, dependent on water P_{O_2} ($P<0.05$). In contrast to Pa_{O_2}, there was no effect of apnea on Pv_{O_2}.

Acid-Base Balance

The effects of temperature on acid-base balance were determined both in vitro and in vivo. The temperature coefficient of blood pH ($d\text{pH}/dT$) was identical, at 0.0135, for both conditions in both larvae and adults (Table 1). However, the absolute values differed significantly with the adults being 0.2 units lower at both temperatures. Buffer capacities of whole blood ($d\,HCO_3^-/d\text{pH}$, mmol/l·pH) were:

	Acclimation and measurement temperature	
	5 °C	25 °C
Larvae	20 ± 4	21 ± 6
Adults	30 ± 5	44 ± 4

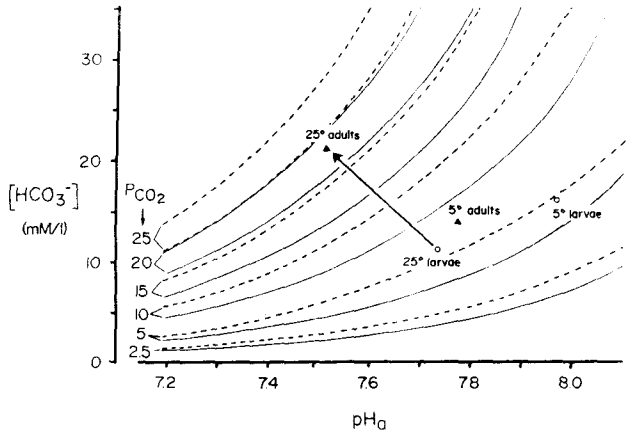

Fig. 4. A Davenport diagram revealing changes in acid-base balance occurring both during changes in acclimation temperature and during metamorphosis in *A. tigrinum*. The curved lines indicate P_{CO_2} isopleths (mmHg) with the solid lines calculated for 25 °C and the dotted lines for 5 °C. The large arrow indicates the changes with metamorphosis in acid-base status

The significantly higher values of adult blood are correlated with higher hematocrits. Thus, hematocrits at 5 and 25 °C were 35±3% and 40±3%, respectively for adults, and 30±4% and 32±4%, respectively, for larvae.

The patterns of change in acid-base balance which follow transformation from neotenic to adult form are shown in Fig. 4. The points indicate the in vivo pH, P_{CO_2}, and $[HCO_3^-]$ for larvae and adults at 5 and 25 °C (measurement and acclimation temperature). Since metamorphosis did not occur in any larvae kept at 5 °C, an arrow indicating the effect of transformation is applied only to the 25 °C larvae.

O_2 Uptake

The measurements of O_2 uptake (Table 2) in all experimental groups reveal four relationships, e.g. the effects of temperature, temperature acclimation, metamorphosis, and activity. 'Maximum' values are the highest O_2 uptakes measured during any 30 min of the total 24 h measurement period. The same applies to 'minimum' values which dominated the 24 h record. Reading the table vertically, the effects of temperature and activity are significant ($P<0.05$) for all 4 experimental groups. Reading the table horizontally, the effect of metamorphosis on minimum $\dot{V}_{O_2}$ is significant only for the 25 °C acclimated animals measured at 25 °C. The effect of metamorphosis on maximum $\dot{V}_{O_2}$ is significant for the 5 °C acclimated animals measured at both temperatures and for the 25 °C acclimated animals measured at 25 °C. Also reading horizontally there was no significant effect of temperature acclimation on minimum $\dot{V}_{O_2}$ of larvae or adults at either measurement temperature. The same was true for maximum $\dot{V}_{O_2}$ with exception of the 25 °C acclimated adults.

Table 2. 'Minimum' and 'Maximum' Oxygen uptake and Q_{10} in larval and adult *Ambystoma tigrinum*, under varying acclimation and measurement temperatures. Mean values ± 1 s.e.m. are given, units for $\dot{V}_{O_2}$ in µl O_2 STPD/g·h. The notation within the parenthesis following $\dot{V}_{O_2}$ for adults indicates whether the $\dot{V}_{O_2}$ determined under the same acclimation and measurement temperature is significantly higher than for larvae at the 0.05 level (*sig.*), or not significantly different (*n.s.*)

	Measurement temperature (°C)	Acclimation temperature (°C)			
		Larvae		Adults	
		5 °C ($n=8$)	25 °C ($n=11$)	5 °C ($n=5$)	25 °C ($n=7$)
Minimum	6 °C	6±2	14±5	12± 5 (n.s.)	17±2 (n.s.)
	25 °C	44±5	52±6	63±10 (n.s.)	86±7 (sig.)
Q_{10}		2.85	2.00	2.39	2.35
Maximum	6 °C	14± 2	24± 4	27±5 (sig.)	25± 3 (n.s.)
	25 °C	107±10	137±16	172±6 (sig.)	236±20 (sig.)
Q_{10}		2.50	2.50	2.65	3.26

Note: Within either the larval or adult group, values of $\dot{V}_{O_2}$ for the cold acclimated populations are not significantly lower ($P>0.10$) than those for the warm acclimated populations at the same measurement temperature. The single exception was in the case of the maximum $\dot{V}_{O_2}$ at 25 °C in adults, in which case the $\dot{V}_{O_2}$ of the warm acclimated adults was significantly higher ($P<0.05$) than that in the cold acclimated adults

Discussion

O_2 Affinity of Blood

The present data, showing no difference in standard O_2 affinity between neotenic larvae and adults, contrast with all previous studies of ontogenetic changes in P_{50} of salamander blood. The axolotl, *Ambystoma mexicanum*, when induced to transform from a neotene to a gill-less adult, shows a significant decrease in O_2 affinity (Gahlenbeck and Bartels 1970) with no change in hemoglobin type (Maclean and Jurd 1971). Another ambystomid salamander, *Dicamptodon ensatus*, also has a decrease in blood O_2 affinity following spontaneous transition from neotene to adult (P_{50} from 46 to 62 mmHg at pH 7.8, 25 °C) with, but not due to, a change in hemoglobin type (Wood 1971). However, in considering the present results, it is important to note that *A. trigrinum*

larvae undergo a 'biochemical' metamorphosis at age 4–5 months. At this time the hemoglobin type does change (Ducibella 1974). It is also important to note that the neotenic forms, in spite of extensive gill structure, are obligatory air-breathers (at 25 °C) and have the same Hb type and red cell organic phosphates as the transformed adults (Wood et al., to be published).

The differences between larval and adult blood O_2 affinity under in vivo conditions is due entirely to temperature effects and, for the 5 °C acclimated neotenes, a decrease in organic phosphates. Because of a small Bohr effect in *A. trigrinum* blood (Wood et al., to be published) the pH difference plays a negligible role in the in vivo P_{50} differences.

O_2 Transport

Regardless of metamorphic state or acclimation temperature, systemic arterial blood was 80–90% saturated (Fig. 1). Without data on O_2 saturation of central venous blood it isn't possible to estimate intracardiac shunting. Such calculations are complicated by the fact that breathing is tri-modal in neotenes and bi-modal in adults. In both forms, however, the skin is thin and highly vascularized, and apparently contributes to an elevation of venous blood P_{O_2} when water P_{O_2} is high (Fig. 3). Using these data for O_2 saturation of mixed hind limb blood derived from both the skin and hind limbs, a *maximum* venous O_2 saturation of about 40–45% is indicated (triangle on oxygen dissociation curve in Fig. 1). This suggests a substantial O_2 extraction by the resting tissues.

Rates of oxygen depletion from the lungs and blood of larval *A. tigrinum* during apnoea (Fig. 2) were similar to those in other intermittently breathing amphibians (Emilio 1974; Emilio and Shelton 1974). The decreasing difference between $P_{A_{O_2}}$ and $P_{a_{O_2}}$ during breath-holding occurs because cutaneous O_2 uptake in oxygenated water sustains a minimum $P_{a_{O_2}}$ while lung O_2 declines. In severely hypoxic water the $P_{a_{O_2}}$ of 25 °C neotenes exceeded water P_{O_2} by 10–20 mmHg, raising the possibility of cutaneous O_2 loss. There is evidence in anuran amphibians that skin perfusion, and therefore cutaneous gas exchange, may be regulated (see Smith 1976; Moalli et al. 1980; Burggren, unpublished observations), but no such ability has yet been demonstrated for the Urodela. Clearly, however, any mechanism to maintain at least partial blood O_2 saturation when in hypoxic water would be highly selected for in *Ambystoma tigrinum*, which inhabits both eutrophic sloughs and high altitude ponds up to 3500 m above sea level (Delson and Whitford 1973; Heath 1976).

Acid-Base Balance

The temperature coefficients of in vitro and arterial pH were not affected by metamorphosis and are consistent with the 'relative alkalinity' model of ectothermic acid-base regulation (Rahn 1966). Although confirmation requires data at more than 2 temperatures, the present study indicates that this model applies to urodele as well as the anuran amphibians studied by Baumgardner and Rahn (1967), Howell et al. (1970), and Reeves (1972).

The CO_2 content (mostly as HCO_3^-) of the blood of the neotenic larvae remains largely unchanged in the face of a temperature rise, as in the bullfrog *Rana* (Reeves 1972) and the turtle *Chelydra* (Howell and Rahn 1976). In *Pseudemys*, in vitro HCO_3^- levels increase at the rate of 8%/10 °C between 10 and 30 °C (Robin 1962), a rate slightly exceeded in vivo by the blood of adult *A. trigrinum*. Unfortunately, comparison of CO_2 content during temperature change in *A. tigrinum* with that in other vertebrate ectotherms is complicated by the pronounced shift from aquatic gas exchange at low temperatures to predominantly aerial gas exchange at high temperatures (Whitford and Sherman 1968). A shift towards aerial respiration results in increasing difficulty in excreting CO_2 (see Randall et al. 1980). In a sense then, a temperature rise results in adjustments in acid-base status relating not only to temperature alone, but also to what must be considered a 'functional metamorphosis' to a more terrestrial animal. Examination of the somewhat less complex situation of metamorphosis at 25 °C reveals very similar changes in acid-base status (i.e. increased P_{CO_2}, increased HCO_3^-, decreased pH) to those in the anuran amphibian *Rana catesbeiana* during metamorphosis into a lunged adult (Erasmus et al. 1970/71; Just et al. 1973) and in amphibious fish when air exposed (cf. Wood and Lenfant 1976).

O_2 Uptake

Resting and maximum values of oxygen uptake for neotenic and adult *A. tigrinum* at 5 and 25 °C (Table 2) agree closely with those in the literature for *A. tigrinum* and other species of *Ambystoma* (Whitford and Sherman 1968; Feder 1976, 1977). Q_{10} values in poikilotherms are usually larger in the heavier individuals of a species (Bullock 1955; Rao and Bullock 1954), so the lower Q_{10} value of the larvae, which weigh nearly 3 times as much as the adults is noteworthy. The adjustments in metabolic rate which occur during the process of acclimation at 5 or 25 °C in *A. tigrinum* are unusual among vertebrate poikilotherms, where acclimation generally results in a significant reduction of the Q_{10} calculated for unac-

climated populations (Prosser 1973). No significant change in Q_{10}, or even a rise in Q_{10} during temperature acclimation, as suggested by the data for *A. tigrinum*, often indicates that oxygen availability or other environmental factors may be influencing metabolic acclimation (Prosser 1973). Certainly, this pattern of temperature acclimation is common to both neotenic and adult *A. tigrinum*, so a factor other than metamorphic state is indicated.

This research was supported in part by NSF Grants PCM-03752 (W.W.B.) and PCM-24246 (S.C.W.).

References

Baumgardner FW, Rahn H (1967) Normal blood pH of the toad at different temperatures as a function of the ionization constant of water. Physiologist 10:121

Bullock TH (1955) Temperature adaptation in poikilothermic animals. Biol Rev 30:311–342

Delson J, Whitford WG (1973) Critical thermal maxima life history stages in desert and montane populations of *Ambystoma tigrinum*. Herpetologica 29:352–355

Ducibella T (1974) The occurrence of biochemical metamorphic events without anatomical metamorphosis in the axolotl. Develop Biol 38:175–186

Emilio MG (1974) Gas exchange and blood gas concentrations in the frog *Rana ridibunda*. J Exp Biol 60:901–908

Emilio MG, Shelton H (1974) Gas exchange and its effect on blood gas concentrations in the amphibian, *Xenopus laevis*. J Exp Biol 60:567–579

Erasmus BDW, Howell BJ, Rahn H (1970/71) Ontogeny of acid-base balance in the bullfrog and chicken. Respir Physiol 11:46–53

Feder ME (1976) Lunglessness, body size and metabolic rate in salamanders. Physiol Zoöl 49:398–406

Feder ME (1977) Oxygen consumption and activity in salamanders: Effect of body size and lunglessness. J Exp Zool 202:403–414

Gahlenbeck H, Bartels H (1970) Blood gas transport properties in gill and lung forms of the axolotl (*Ambystoma mexicanum*). Respir Physiol 9:175–182

Heath AG (1976) Respiratory response to hypoxia by *Ambystoma tigrinum* larvae, paedomorphs, and metamorphosed adults. Comp Biochem Physiol 55A: 45–49

Hill RW (1972) Determination of oxygen consumption by use of the paramagnetic oxygen analyzer. J Appl Physiol 32:261–263

Howell BJ, Rahn H (1976) Regulation of acid-base balance in reptiles. In: Gans C, Dawson WR (eds) Biology of the Reptilia. vol 5A, Physiology. Academic Press, New York

Howell BJ, Baumgardner FW, Bandi K, Rahn H (1970) Acid-base balance in cold blooded vertebrates as a function of body temperature. Am J Physiol 218:600–606

Just JJ, Gatz RN, Crawford EC (1973) Changes in respiratory functions during metamorphosis of the bullfrog, *Rana catesbeiana*. Respir Physiol 17:276–282

Maclean N, Jurd RD (1971) Electrophoretic analysis of the hemoglobins of *Ambystoma mexicanum*. Comp Biochem Physiol 40B: 751–755

Moalli R, Meyer RS, Jackson DC, Millard RW (1980) Skin circulation of the frog, *Rana catesbeiana*: Distribution and dynamics. Respir Physiol 40:137–148

Prosser CL (1973) Comparative animal physiology. Saunders, Philadelphia, pp 1–966

Rahn H (1966) Acid-base regulation and temperature in the evolution of vertebrates. Proc Int Union Physiol Sci 8:91–92

Randall D, Burggren W, Farrel A, Haswell M (1980) The evolution of air breathing in vertebrates. Cambridge University Press, Cambridge

Rao KP, Bullock TH (1954) Q_{10} as a function of body size and habitat temperature. Am Nat 88:33–44

Reeves RB (1972) An imidazole alphastat hypothesis for vertebrate acid-base regulation: tissue carbon dioxide content and body temperature in bullfrogs. Respir Physiol 14:219–236

Reeves RB (1976) Temperature-induced changes in blood acid-base status: pH and P_{CO_2} in a binary buffer. J Appl Physiol 40:752–761

Robin EE (1962) Relationship between temperature and plasma pH and carbon dioxide tension in the turtle. Nature 195:249–251

Siggard-Anderson, O, Engel K, Jorgensen K, Astrup P (1960) A micromethod for determination of pH, carbon dioxide tension, base excess, and standard bicarbonate in capillary blood. Scand J Clin Lab Invest 12:172–176

Smith DG (1976) The innervation of the cutaneous artery in the toad *Bufo marinus*. Gen Pharmacol 7:405–409

Whitford WC, Sherman RE (1968) Aerial and aquatic respiration in axolotl and transformed *Ambystoma tigrinum*. Herpetologica 24:233–237

Wood SC (1971) Effects of metamorphosis on blood respiratory properties and red cell ATP in the salamander, *Dicamptodon ensatus*. Respir Physiol 12:53–65

Wood SC (1980) Adaptation of red blood cell function to hypoxia and temperature in ectothermic vertebrates. Am Zool 20:163–172

Wood SC, Lenfant CJM (1976) Physiology of fish lungs. In: Hughes GM (ed) Respiration of amphibious vertebrates. Academic Press, London, pp 257–270