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# Allometry of red cell oxygen binding and hematology in larvae of the salamander, *Ambystoma tigrinum*

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**Abstract.** Very few studies have attempted to relate blood characteristics to body mass *within*, rather than between, species. Thus, respiratory and hematological properties of the blood of larval tiger salamanders (*Ambystoma tigrinum*) have been measured in animals ranging in body mass from approximately 2 to 112 g. This amphibian species was chosen because larvae of very different body mass may be of similar developmental stage, minimizing interference from ontogenetic factors. Mean corpuscular volume, mean corpuscular Hb, hematocrit, blood Hb concentration, blood $O_2$ capacity and Hill's n were all positively correlated with body mass ($P < 0.005$). Blood $O_2$ affinity ($P_{50}$) and Bohr shift were not significantly correlated with body mass ($P > 0.20$). The findings are discussed in the context of the general effect of body mass upon metabolic rate in vertebrates, as well as environmental and biological factors specific to larvae of the tiger salamander.

Allometry; Blood; Hemoglobin; $O_2$ capacity; $P_{50}$; Salamander

That resting metabolic rate is related to some power function of the body mass of an animal has been appreciated for nearly 150 years (Sarrus and Rameaux, 1838). Since that time, many so-called 'allometric' studies relating body mass to a variety of morphological structures and physiological processes supporting metabolism have been made (for references see Peters, 1983; Calder, 1984; Schmidt-Nielsen, 1984). Considering the pivotal role of blood in providing for tissue oxygenation, it is hardly surprising to find that many of the hematological and respiratory properties of the blood are also closely related to body mass (for references see Wood *et al.*, 1979; Calder, 1984; Schmidt-Nielsen, 1984). For example, oxygen-hemoglobin ($O_2$-Hb) affinity increases ($P_{50}$ falls) with increasing body mass when comparing different species of mammals, birds, most reptiles and fish. The effect of protons on $O_2$-Hb affinity (Bohr shift) is also

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considerably greater in smaller mammalian species than larger ones, as is the activity of intraerythrocytic carbonic anhydrase. These blood characteristics presumably facilitate the greater tissue $O_2$ delivery and $CO_2$ elimination required to support the higher mass-specific metabolic rate of smaller animals.

Intraspecific correlations between metabolic rate and body mass have been demonstrated repeatedly (see Bartholomew, 1982). Yet, most analyses of the respiratory properties of vertebrate blood made in the context of body mass have been based on *inter*specific rather than *intra*specific comparisons. In theory, blood respiratory properties should be influenced by differences in body mass within a given species. This hypothesis has been only infrequently tested because intraspecific relationships between body mass and blood respiratory properties (as for other physiological and morphological variables) are confounded by large differences resulting from developmental changes that are independent of body mass. For example, the blood of 1-day-old human infant (3 kg) has a $P_{50}$ that is 7–8 mm Hg lower than that of a normal adult (70 kg) (*e.g.* Delivoria-Papadopoulos *et al.*, 1971). Similarly, neonatal hematocrit and blood Hb concentration are respectively about 8% and 4.5 $g \cdot dl^{-1}$ higher than 70 kg adults (Wintrobe *et al.*, 1981). Upon first examination, these data for humans would appear to reflect the negative correlation between body mass and these blood properties that is typical when making interspecific comparisons between adult mammals. However, blood $P_{50}$ rises about 5 mm Hg in the first 6–9 weeks after birth, while body mass shows relatively little increase. Simultaneously, hematocrit and Hb concentration decrease by 12% and 12 $g \cdot dl^{-1}$, respectively. Clearly, any differences in blood respiratory properties that are a function of changing body mass may well be obscured by hematological changes related to development.

To discriminate hematological changes due to body mass from those due strictly to ontogenetic changes, intraspecific allometric analyses of blood respiratory properties should thus be carried out on individuals of comparable developmental stage but different body mass. Unfortunately, the adoption of this criterion will, for most vertebrate species, greatly reduce the mass range over which data may be collected. Whereas scaling relationships can be relatively easily discerned when considering 10–100-fold changes in body mass, it is much more difficult to quantify (or sometimes even to recognize) scaling relationships over narrower mass ranges, simply because of inherent variability in blood respiratory properties among individuals.

In order to test explicitly the hypothesis that blood respiratory properties scale with body mass *within* a single vertebrate species, we have examined the blood $O_2$ binding and hematology of larvae of the tiger salamander, *Ambystoma tigrinum*. Larvae of this salamander occur over a wide body mass range (0.5–150 g), facilitating allometric analysis. In addition, individuals of very different mass can be of similar developmental stage, as evident by their nearly identical external and internal morphology and the ability of larvae of any mass above 10 g to metamorphose spontaneously into healthy, functional adults (unpublished data of the present authors). Some biochemical differences related to development are not evident upon morphological examination in *Ambystoma* (Ducibella, 1974), and consequently the effects of development *per se*

cannot be completely controlled for. Nonetheless, larvae of *A. tigrinum* represent a vertebrate system in which the allometry of blood respiratory properties and hematology can be investigated intraspecifically with minimal interference from developmental factors.

## Materials and methods

*Experimental animals.* Data were obtained from 77 larvae of the tiger salamander, *A. tigrinum* (body mass range 2.6–112.0 g). Specimens were collected in August, 1985, from ponds in Taos County, New Mexico at an altitude of about 2500 m. All animals were transferred to the laboratory, where they were maintained in aerated water at 25 ± 2 °C for 1 to 2 weeks before sampling. Animals were not fed during captivity.

*Experimental procedures.* To sample blood, each animal was sacrificed by a sharp blow to the head, and the heart was carefully exposed via a ventral incision through the chest wall. After blotting the pericardial space, the truncus arteriosus was severed. Blood flowing into the pericardial space immediately was drawn into either a heparinized syringe or heparinized capillary tubes. Total sampling time from first disturbance of the animal was less than 3 min. Preliminary analyses revealed no differences between blood sampled from the pericardial space and blood sampled by direct cardiac puncture with a needle. The former method was adopted for all animals since it facilitated sampling from very small larvae.

The complete series of analyses outlined below required a minimum of 300–400 μl of blood. For larvae in the range of 3–6 g body mass, blood from several individuals was pooled. When blood was pooled, body mass of each contributing individual was within 0.5 g of the mean mass for the pooled individuals. In some instances, blood samples from very small individuals were not pooled, and only a few of the analyses requiring only small amounts of blood (*e.g.* hematocrit, Hb conc., rbc conc.) were performed.

$O_2$ dissociation curves were determined at 25 °C on 2 μl samples of larval blood using a continuous spectrophotometric method (Hem-O-Scan; American Inst. Co.,), as described for the blood of *A. tigrinum* by Burggren and Wood (1981). A curve was determined first at equilibration with 1% $CO_2$ and then at 5% $CO_2$. A separate aliquot (150 μl) from the original blood sample was tonometered with each of the gases used in the Hem-O-Scan, and the pH of this blood subsequently measured with a Radiometer micro pH electrode and PHM72 electrometer. Thus, two curves, each with a known pH within the physiological range for *Ambystoma*, were constructed for each animal. The Bohr shift, Hill's n, and buffer capacity of the blood were then calculated from each curve and/or the associated pH and $P_{CO_2}$ of the blood. To facilitate comparisons of whole blood $O_2$ affinity, blood $P_{50}$ for each animal was then corrected to pH 7.70 using the Bohr factor determined for that animal's blood.

Hemoglobin (Hb) concentration was measured on a 25 μl blood sample, using a

spectrophotometric hemoximeter (Model OSM 2; Radiometer). Although this apparatus uses wavelengths appropriate for human Hb, amphibian Hbs have the same spectra (Wood, 1971). Independent colorimetric determination of amphibian Hb confirmed the accuracy of the hemoximeter. Hematocrit was determined on 10 $\mu$l of blood after centrifugation at 10000 r.p.m. for 3 min. Red blood cell (rbc) counts on 5 $\mu$l samples were determined in the standard fashion using a hemocytometer. From hematocrit, Hb concentration and rbc concentration, then mean corpuscular Hb concentration (MCHC) and mean corpuscular volume (MCV) could be calculated (Wintrobe *et al.*, 1981). Blood $O_2$ capacity of a 20 $\mu$l sample of blood, equilibrated with 30% $O_2$ to ensure full $O_2$ saturation of the Hb, was determined according to the method of Tucker (1967).

*Blood electrophoresis.* Alkaline agarose gel electrophoresis was performed using 1 $\mu$l of a blood hemolysate diluted with distilled water to 1 $g \cdot dl^{-1}$. Samples were electrophoresed at 240 V for 20 min and subsequently stained with Amido Black 10B and cleared with acetic acid. Blood standards consisted of normal and variant human Hbs. The percentage composition of the various Hb fractions that migrated was determined by scanning densitometry.

*Statistical analyses.* Curve fitting analyses of the data revealed that the relationships between body mass and blood properties were equally well described by linear and exponential functions (*i.e.* statistically equivalent regression coefficients from the two analyses). Thus, because there was no compelling reason to transform the raw data, least square error linear regressions of the arithmetic values of all blood variables against body mass were subsequently performed. This analysis determined (1) the regression coefficient, (2) the equation of the line describing the relationship, and (3) the probability that the slope of the line was not significantly different from zero (*i.e.* the probability that a linear relationship does not exist in the data). Where no significant relationship with body mass existed for a particular variable, mean values $\pm$ 1 standard error were calculated from all values of that variable.

## Results

Six of the measured blood variables were positively correlated with body mass. Individual data points and the line of best fit determined by least square error linear regression for these variables are presented in figure 1. The attendant values describing each relationship are indicated in table 1.

Although red blood cell concentration did not significantly correlate with body mass (table 2), MCV increased with increasing body mass, so Hct was significantly higher in larger animals (fig. 1). Because of the larger MCV in larger animals, MCHC also was positively correlated with body mass. Thus, predictably, blood Hb concentration also was positively correlated with body mass. As a consequence of the hematological

TABLE 1

Values describing the equation of the line for each of the six variables in fig. 1, as well as the regression coefficient ($r$) and the significance level ($P$) of the linear regression. See text for further details.

| | n | Line equation | $r$ | $P$ |
|---|---|---|---|---|
| Hematocrit | 74 | Hct = 0.127M + 25.6 | 0.45 | $<0.0005$ |
| Mean corp. vol. | 58 | MCV = 10.031M + 2389 | 0.44 | $<0.001$ |
| Hemoglobin | 69 | Hb = 0.041M + 5.68 | 0.52 | $<0.001$ |
| Mean cell Hb | 37 | MCHb = 2.297M + 564 | 0.56 | $<0.005$ |
| $O_2$ capacity | 57 | $C_{O2}$ = 0.026M + 5.02 | 0.37 | $<0.005$ |
| Hill's n | 32 | n = 0.004M + 2.37 | 0.491 | $<0.005$ |

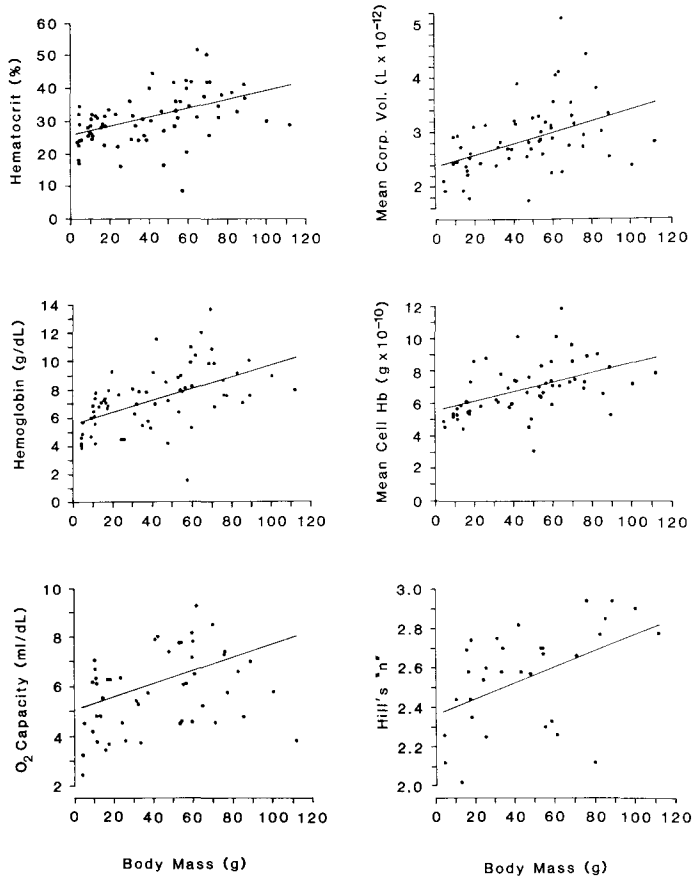


Fig. 1. Relationship between hematological variables and body mass in larvae of the tiger salamander, *A. tigrinum*. Each data point represents a separate animal. The lines through the data points were derived with a least square error linear regression, and are described in table 1. All linear regressions have slopes significantly greater than 0 ($P < 0.005$).

TABLE 2

Mean values ( ± 1 SE) for blood properties not significantly correlated with body mass in larval *A. tigrinum*. The slope of the relationship of the variable regressed against body mass was not significantly different from zero ($P > 0.20$) in every case. See text for further details.

| | n | Mean | SE |
|---|---|---|---|
| Red blood cell concentration (cells · $L^{-1} \cdot 10^{12}$) | 58 | 0.114 | 0.002 |
| $P_{50}$ at pH 7.70 (mm Hg) | 34 | 30.2 | 1.3 |
| Bohr shift | 34 | −0.30 | 0.03 |
| Buffer capacity (mmol · $L^{-1}$ · pH $unit^{-1}$) | 28 | 37.1 | 7.1 |
| $O_2$ capacity/Hb concentration (ml · $dl^{-1}$ · g $Hb^{-1}$) | 58 | 0.80 | 0.02 |

relationships described above, blood $O_2$ capacity was significantly correlated with body mass, rising by over 50% as body mass increased from 3 g (5.0 ml · $dl^{-1}$) to 100 g (7.6 ml · $dl^{-1}$) (fig. 1).

Of the three measured properties of the blood describing $O_2$ binding properties ($P_{50}$, Bohr shift, Hill's n), only Hill's n was significantly correlated with body mass (fig. 1, table 2).

Electrophoretic examination of hemolysates revealed three predominant Hb bands that migrated (fig. 2). Of these, only the percentage of total Hb represented by 'Fraction # 1' was significantly correlated with body mass (fig. 3). No attempt was made to further characterize the Hbs but, relative to human Hb standards that were run simultaneously with the larval blood samples, Fraction # 1 migrated to a position intermediate between that of human fetal Hb and Hb A.

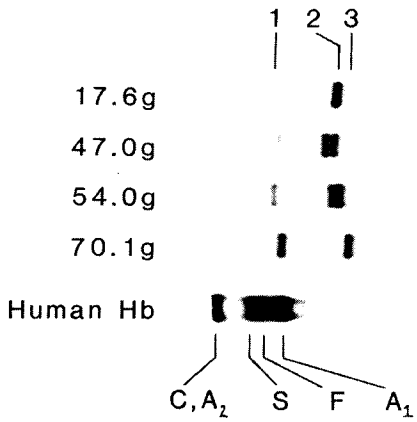


Fig. 2. Representative electrophoretic gels of blood from larvae of *A. tigrinum*. Note the differing proportions of fractions 1, 2 and 3 and their migration with respect to four common human hemoglobin variants (bottom lane). See text for further details.

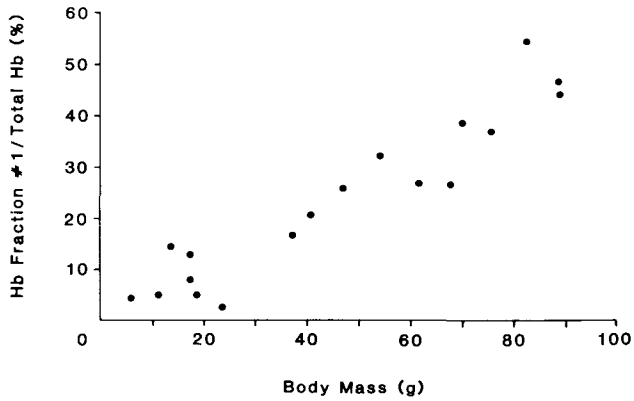


Fig. 3. Changes in hemoglobin fraction '# 1' (expressed as a percentage of total hemoglobin) as a function of body mass in larval *A. tigrinum*.

## Discussion

*Comparisons of blood variables with previous studies.* Several studies of blood variables of *A. tigrinum* or closely related species have been performed (Gahlenbech and Bartels, 1970; MacLean and Jurd, 1971; Ducibella, 1974; Burggren and Wood, 1981; Wood *et al.*, 1982). While metamorphosis from larva to adult has frequently been a theme of these studies, body mass as a variable has not been considered. The present study has demonstrated profound effects of body mass on several important blood variables. Consequently, direct comparisons between studies are somewhat complicated by this finding, particularly when such studies have reported only a single mean value for a given blood variable when animals of widely different body mass were examined.

Comparisons with previous studies of those blood variables that are significantly correlated with body mass can be attempted by calculating the value of the variable from the linear regression equations presented in table 1. For example, we would predict the hematocrit of a 66 g larva of *A. tigrinum* to be 34%. Burggren and Wood (1981) report a mean hematocrit of 32% for a population of larvae with a mean mass of 66 ± 8 g (mean ± 1 SD). Gahlenbech and Bartels (1970) report mean hematocrit and Hb values of 28% and 7.49 g · dl$^{-1}$, respectively, for larvae of *Ambystoma mexicanum* with a mean body mass of 43 ± 3 g. Our predictions from linear regression for ambystomid larvae of 43 g would be Hct = 31% and Hb = 7.44 g · dl$^{-1}$. Thus, even though the values for the regression coefficients given in table 1 indicate that less than 1/3 of the total variance in these hematological parameters is due specifically to body mass, these regression equations have good predictive strength.

Relatively large variation about mean values for blood variables are common in studies of ambystomid salamanders (and other amphibians as well). Our results suggest that some of this variation in previous studies may have resulted from grouping together animals of different body mass. The present study shows, for example, that only a 20 g

difference in body mass can account for nearly a 15% difference in blood Hb concentration (fig. 1). Thus, future investigations of the blood properties of amphibians (and, indeed, probably other lower vertebrates) should either use animals of very similar body mass or perform an allometric analysis over a wide mass range.

Hematological values not significantly correlated with body mass in the present study (table 2) are relatively consistent with previous reports. Noteworthy exceptions are the whole blood $P_{50}$ of 30.2 mm Hg (25 °C, pH = 7.70) and the magnitude of the Bohr shift, − 0.30. Our previous studies on larval *A. tigrinum* have indicated $P_{50}$ values at the same temperature and pH ranging from 25 to 40 mm Hg (Burggren and Wood, 1981; Wood *et al.*, 1982). Previous thermal history as well as intraspecific variation between distinct geographic populations, perhaps relating to environmental oxygen availability, may account for these differences. Similarly, the Bohr shift we measured in this population of *A. tigrinum* is higher than that reported in a previous investigation (Wood *et al.*, 1982). One explanation for the difference between data sets lies in differences in statistical methods by which the Bohr coefficient was calculated. In the earlier study, Wood *et al.* (1982) estimated the Bohr coefficient as the slope of the linear regression of all $P_{50}$ values and the pH at which each $P_{50}$ was measured. In this study, Bohr coefficients were measured for each individual and the mean Bohr coefficient then calculated ( − 0.30, table 2). When these same raw data were analyzed by linear regression analysis according to the techniques of the previous study, a much lower Bohr shift of only − 0.226 was estimated.

While some discrepancies between studies may relate to measurement and/or analytical differences, it must be emphasized that hematological variation between various geographic populations of animals very likely is a real and important phenomenon. Unfortunately, biological factors affecting intraspecific variation in blood properties have not been been systematically determined for *A. tigrinum* or most other vertebrates. There is an important need for future studies to concentrate not on generating a single (artificial) number to describe physiological characteristics, but rather to search for and attempt to understand the wide variations which normally occur (see Feder *et al.* (1988) for further discussion of intraspecific variations).

*Scaling of blood variables with body mass.* A negative correlation between mass-specific $O_2$ consumption and body mass has been demonstrated for a number of amphibians (see Calder, 1984; Schmidt-Nielsen, 1984), including larvae of *A. tigrinum*. In this respect, the metabolism of amphibians is identical to those of virtually every other vertebrate that has been examined in this context. We hypothesized at the outset of this study that (1) since the blood is instrumental in supporting metabolism, and (2) since mass-specific metabolic rate is highest in smaller salamanders, then the properties of the blood of smaller salamanders that alter blood $O_2$ transport (*e.g.* $O_2$ capacity, $O_2$ affinity, *etc.*) should correlate with body mass with a similar power function to that which relates metabolism to body mass.

Whereas a strong linear correlation between body mass and many hematological

variables has been demonstrated for larval *A. tigrinum*, the pattern of change, as well as the variables that were affected, was unexpected. For example, both the Bohr shift and blood $O_2$ affinity were *independent* of body mass over a 40-fold change in body mass in larval tiger salamanders (table 1). Comparisons of whole blood $P_{50}$ both between and within vertebrate species have usually revealed a negative correlation between $P_{50}$ and body mass (see Wood *et al.*, 1979; Calder, 1984; Schmidt-Nielsen, 1984), though occasionally a positive correlation has been reported (Pough, 1977).

A higher $P_{50}$ in smaller animals having higher metabolic rates has generally been viewed as an adaptation favoring $O_2$ unloading at the tissues. In the absence of environmental hypoxia, a right-shifted $O_2$ equilibrium curve can favor $O_2$ unloading without compromising $O_2$ loading at the gas exchange organ(s) (Wood and Lenfant, 1979). However, in considering *A. tigrinum*, it is important to note that larvae are often found in stagnant water at altitudes as high as 3400 m, and are generally abundant to about 3000 m (unpublished observations of the present authors). A low $O_2$ affinity to support greater $O_2$ unloading at the tissues in small larvae, which might be viewed as appropriate for animals at sea level, might in stagnant water at higher altitudes result in sub-optimal $O_2$ loading of Hb in the gas exchange organs. A low $O_2$ affinity in smaller larvae thus might be viewed as potentially maladaptive in larval *A. tigrinum* living at high altitude environments where $O_2$ might become limiting.

The increase in value for Hill's n with increasing body mass in *A. tigrinum*, though of relatively low magnitude, might facilitate $O_2$ delivery in larger animals (see Lutz, 1980). The present study has not attempted to determine the mechanism underlying this change in heme-heme cooperativity exhibited by whole blood. However, it is possible that the various Hb fractions, which show considerable changes in proportion with increasing body mass (fig. 3), have different values for Hill's n.

Obviously, a change in the $O_2$ binding properties is only one of many ways to enhance blood $O_2$ transport. Thus, an increase in Hb concentration, achieved by increases in red cell numbers or mean corpuscular Hb, would increase blood $O_2$ capacity. Either by itself or combined with an increase in cardiac output, a change in blood $O_2$ capacity can be instrumental in supporting an elevated bulk transport of $O_2$ to the tissues.

Indeed, the present study has shown that the hematology of larval *A. tigrinum* is profoundly affected by body mass, but there were a number of unexpected findings. First, arithmetic plots of hematological variables against body mass are as adequately described by linear functions as by exponential power functions (fig. 1). Metabolism and the morphological structures and physiological processes that support metabolism generally scale to body mass with some power function in vertebrates, leading to the common use of log-log plots to describe such relationships (Peters, 1983; Calder, 1984; Schmidt-Nielsen, 1984).

The second, and perhaps most surprising hematological finding, was that all of the characteristics generally regarded as adaptations favoring $O_2$ transport occurred in larger, not smaller, larvae. Thus, mean corpuscular volume, hematocrit, mean corpuscular Hb, blood Hb concentration, Hill's n and blood $O_2$ capacity all were *positively*, not negatively, correlated with body mass (fig. 1). Of greatest importance

physiologically, the present study predicts that blood $O_2$ capacity will increase by about 50% over the approximately 150 g range of body mass normally evident in larval tiger salamanders. This increase in blood $O_2$ capacity is not achieved simply by an increased rate of erythropoiesis, since red blood cell concentration is not related to body mass. Rather there appears to be qualitative changes in red cell production such that cells of both larger volume and higher intraerythrocytic Hb concentration are produced in larvae of larger body mass.

The ratio of blood $O_2$ capacity to Hb concentration was relatively constant at 0.8 in larval *A. tigrinum* (table 2). Typically, this ratio is approximately 1.3 for Hbs in higher vertebrates. It is possible that this low ratio in larval tiger salamanders is due in part to the presence of a significant proportion of non-functional Hb (*i.e.* methemoglobin), a relatively common situation in lower vertebrates (Dessauer, 1970; Wood and Lenfant, 1979). However, we have no data for metHb in the present study. An alternate explanation for low ratios of $O_2$ capacity to Hb concentration in lower vertebrates has been offered by Gruca and Grigg (1980). These authors suggest that the presence in hemolysates of fragments of cellular organelles from nucleated red blood cells could cause optical dispersion and spectral changes not related strictly to Hb concentration when Hb is assayed spectrophotometrically, leading to a consistent underestimate of Hb concentration.

Why should larger larvae of *A. tigrinum* have a higher blood $O_2$ capacity? One answer (admittedly speculative) may lie in the comparatively heavy dependence of amphibians upon cutaneous gas exchange. A larger larva will, relatively to weight, have less surface area for cutaneous respiration than will a smaller larva. Of course, the overall weight-specific respiratory demand of a larger larva also will be proportionally less. Whereas surface area might be expected to scale to body mass with an exponent of 0.65–0.73, metabolism of a variety of salamanders scales to body mass with the exponent of about 0.74–0.89 (for references see Peters, 1983; Calder, 1984; Schmidt-Nielsen, 1984). Thus, mass-specific cutaneous respiratory surface area decreases at a greater rate than mass-specific $O_2$ consumption as an animal's size increases. If an increase in blood $O_2$ capacity is an adaptation favoring blood $O_2$ transport in response to decreased skin surface area, why should larval *A. tigrinum* not simply increase lung ventilation frequency or lung volume and surface area to compensate for this 'loss' of cutaneous respiratory surface area? Air breathing by these aquatic larval salamanders carries with it a significant risk of predation, and has potentially high energetic costs associated with overcoming buoyancy during descent through the water column (see Burggren *et al.*, 1983; Feder, 1984). As an alternative adjustment, marked hematological adjustment (including increase in blood $O_2$ capacity) can certainly occur in response to moderate, acute hypoxia in amphibians (Wood *et al.*, 1982; Pinder and Burggren, 1983). An increase in blood $O_2$ carrying capacity simply might be an energetically effective way of supporting gas exchange in the face of a decrease in weight-specific respiratory surface area.

*In summary*, this study has shown that the blood characteristics of larval tiger salamanders are affected in complex ways by changing body weight. Whereas properties

relating to $O_2$-Hb binding are primarily unaffected by body weight, the $O_2$ capacity of whole blood increases with increasing body weight. These findings are contrary to changes that would be predicted simply on the basis of the relationship between an animal's metabolic rate and weight. Future studies, in which the effects of experimental adjustments of blood characteristics (*e.g.* induced anemia, polycythemia) on metabolic rate, cardiac output, *etc.* are assessed, would be very instructive as to the adaptive significance of increased blood $O_2$ capacity in large larval salamanders.

## References


Bartholomew, G. (1982). Energy metabolism. In: Animal Physiology: Principles and Adaptations, edited by M.S. Gordon, G.A. Bartholomew, A.D. Grinnell, C.B. Jorgensen and F.N. White. New York, MacMillan.

Burggren, W.W. and S.C. Wood (1981). Respiration and acid–base balance in the tiger salamander, *Ambystoma tigrinum*; Influence of temperature acclimation and metamorphosis. *J. Comp. Physiol.* 144: 241–246.

Burggren, W.W., M.E. Feder and A.W. Pinder (1983). Temperature and the balance between aerial and aquatic respiration in larvae of *Rana berlandieri* and *Rana catesbeiana*. *Physiol. Zool.* 56: 263–273.

Calder, W.A., III (1984). Size, Function, and Life History. Cambridge, Harvard University Press.

Delivoria-Papadopoulos, M., N.P. Roncevic and F.A. Oski (1971). Postnatal changes in oxygen transport of term, premature and sick infants. The role of red cell 2,3-diphosphoglycerate and adult hemoglobin. *Pediatr. Res.* 5: 235.

Dessauer, H.C. (1970). Blood chemistry of reptiles: physiological and evolutionary aspects. In: Biology of the Reptiles. Vol. 3, edited by C. Gans and T.S. Parsons. New York, Academic Press.

Ducibella, T. (1974). The occurrence of biochemical metamorphic events without metamorphosis in the axolotyl. *Dev. Biol.* 38: 175–186.

Feder, M.E. (1984). Consequences of aerial respiration for amphibian larvae. In: Respiration and Metabolism in Embryonic Vertebrates, edited by R.S. Seymour. Dordrecht, Junk.

Feder, M.E., A.F. Bennett, W.W. Burggren and R. Huey (1988). New Directions in Physiological Ecology. New York, Cambridge University Press (in press).

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

Gruca, M. and G. Grigg (1980). Methemoglobin reduction in crocodile blood: are high levels of metHb typical of healthy reptiles? *J. Exp. Zool.* 213: 305–308.

Lutz, P.L. (1980). On the oxygen affinity of bird blood. *Am. Zool.* 20: 187–198.

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

Peters, R.H. (1983). The Ecological Implications of Body Size. New York, Cambridge University Press.

Pinder, A.W. and W.W. Burggren (1983). Respiration during chronic hypoxia and hyperoxia in larval and adult bullfrogs (*Rana catesbeiana*). II. Changes in respiratory properties of whole blood. *J. Exp. Biol.* 105: 205–213.

Pough, H.F. (1977). The relationship between body size and blood oxygen affinity in snakes. *Physiol. Zool.* 50: 77–87.

Sarrus and Rameaux (1838). Rapport sur un mémoire adressé à l'Académie royale de médecine. *Bull. Acad. Med. Paris* 3: 1094–1100.

Schmidt-Nielsen, K. (1984). Scaling: Why Animal Body Size is Important. New York, Cambridge University Press.

Tucker, V.A. (1967). Method for oxygen content and dissociation curves on microliter blood samples. *J. Appl. Physiol.* 23: 410–414.

Wintrobe, M.M., G.R. Lee, D.R. Boggs, T.C. Bithell, J. Foerster, J.W. Athens and J.N. Lukens (1981). Clinical Hematology. Philadelphia, Lea and Febiger.

Wood, S.C. (1971). Effects of metamorphosis on blood respiratory properties and erythrocyte adenosine triphosphate level of the salamander *Dicamptodon ensatus* (Eschscholtz). *Respir. Physiol.* 12: 53–65.

Wood, S.C., R. Weber and D. Powers (1979). Respiratory properties of blood and hemoglobin solutions from the piranha. *Comp. Biochem. Physiol.* 62A: 163–167.

Wood, S.C., R.W. Hoyt and W.W. Burggren (1982). Control of hemoglobin function in salamanders (*Ambystoma tigrinum*). *Mol. Physiol.* 2: 263–272.

Wood, S.C. and C. Lenfant (1979). Oxygen transport and oxygen delivery. In: Evolution of Respiratory Processes: A Comparative Approach, edited by S.C. Wood and C. Lenfant. New York, Marcel Dekker, pp. 193–223.