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BLOOD PHYSIOLOGY AND OXYGEN TRANSPORT DURING ACTIVITY IN TWO LIZARDS, *VARANUS GOULDII* AND *SAUROMALUS HISPIDUS*

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Abstract—1. Aspects of blood physiology—hematocrit, oxygen capacity and affinity, lactate content, pH and composition of blood buffers—were investigated in the lizards *Varanus gouldii* and *Sauromalus hispidus* during activity at different temperatures.

2. Although oxygen capacity and affinity, resting pH and lactate levels, bicarbonate and phosphate concentrations are nearly identical in both species, only *Sauromalus* sustains a decrease in blood pH during activity, accompanied by a decrease in oxygen capacity and affinity, high levels of lactate production and exhaustion.

3. Non-carbonic blood buffers prevent a change in blood pH in *Varanus*. Lungs of great surface area facilitate exchange of oxygen and carbon dioxide, and *Varanus* undergoes only moderate lactate generation and remains aerobic during activity.

INTRODUCTION

MOST species of reptiles rely principally on anaerobic metabolism for short bursts of rapid activity (Moberly, 1968a, b; Bennett, 1971, 1972b; Wilson, 1971; Bennett & Licht, 1972; Bennett & Dawson, 1972, 1973). This anaerobiosis entails high levels of lactate generation and rapid exhaustion; oxygen debt is high and recovery is slow. Reliance on anaerobiosis is necessary since the aerobic capacities of these animals are low: levels of oxygen consumption during rest and activity are much less than those of comparably sized homeotherms (Bartholomew & Tucker, 1963; Bennett & Dawson, 1973). The oxygen capacity (Dawson & Poulson, 1962) and oxygen affinity of the blood (Pough, 1969) are low in comparison to mammalian levels, as are the activities of the aerobic enzymes (Bennett, 1972a). Reptilian activity, therefore, appears to be limited to either slow movements sustainable by limited aerobic capacities or rapid bursts supported anaerobically.

Notable exceptions to the former generalizations are the monitor lizards (genus *Varanus*). These animals are carnivorous and are highly active predators. They are capable of high-speed pursuit and sustained activity. Aerobic scope is exceptionally high (Bartholomew & Tucker, 1964; Bennett, 1972b). Resting

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levels of oxygen consumption are, however, identical to those of other reptiles of equal size (Bennett, 1972b). Since maintenance costs are equal, it appears that *Varanus* possesses specific capabilities for sustained oxygen utilization during activity which other lizards lack. An extensive study (Bennett, 1971) was undertaken to determine how varanid lizards are able to function aerobically in contrast to other anaerobic saurians. Previously published portions of that study (Bennett, 1972a, b, 1973) have indicated a lack of specialization in the ventilatory, circulatory or enzymatic systems, suggesting that the blood physiology of varanids may be responsible for their greater oxygen transporting capacity. For instance, a greater blood oxygen capacity or affinity might account for the difference. This study reports measurements of hematocrit and oxygen capacity of the sand goanna, *Varanus gouldii*, and an iguanid lizard, the spiny chuckwalla, *Sauromalus hispidus*, animals of equal size (approximately 0·5 kg) and thermal preferendum (37–38°C). The effects of activity on blood pH, lactate content and oxygen affinity are also examined, as well as measurements of the blood buffering capacity and composition of both animals.

MATERIALS AND METHODS

Experimental animals

Fifteen adult *S. hispidus* (mean weight, 574 g) and ten adult *V. gouldii* (mean weight, 674 g) were used in these experiments. Conditions of animal maintenance have been described elsewhere (Bennett, 1972b). Experiments were run on *Varanus* in February–March and on *Sauromalus* in June–July, the summer active period for both species. All animals were fasted at least 2 days before experimentation.

Hematocrit and oxygen capacity

Blood samples of approximately 0·3 ml were obtained by ventricular heart puncture from twelve *Sauromalus* and nine *Varanus*. Coagulation was prevented by the addition of crystaline sodium heparin, and the samples were thoroughly mixed before analysis. Hematocrit was obtained by centrifuging approximately 50 μl of blood for 10 min at 3000 rev/min in flame-sealed heparinized capillary tubes. The percentage of red blood cell volume in the total volume of the sample was recorded as the hematocrit.

The oxygen capacity of the blood was measured by fully oxygenating the sample with air and measuring the total amount of oxygen bound at 25°C. A 0·25-ml sample of whole blood was injected into a small glass tonometer (25 cm^3 capacity). This was attached to the shaft of a small motor and rotated at 20 rev/min, so that the blood sample formed a thin layer around the inside of the chamber and achieved maximum equilibration with the gas sample. A gas mixture of 95% air and 5% carbon dioxide saturated with water vapor (P_{O_2} = 145 torr, P_{CO_2} = 36 torr, P_{H_2O} = 24 torr) was metered into the tonometer at 250 cm^3/min for 7 min. The tonometer was then sealed, and the sample equilibrated for another 8 min. The entire apparatus was enclosed in a constant-temperature cabinet at 25°C. At the end of the 15-min equilibration period, the sample was removed and analyzed for oxygen content according to the method of Roughton and Scholander (1943). The oxygen content was expressed as vol % (cm^3 of dry O_2 carried in 100 ml of whole blood), corrected to STPD conditions.

Blood lactate content and pH

Blood lactate and pH were measured immediately before and after a period of enforced activity. Five or more animals of each species were placed in a constant-temperature

cabinet overnight and equilibrated to 25, 30, 35 or 40°C. They were then removed individually and 0·35 ml of blood was rapidly obtained by heart puncture. Elapsed time between first handling and sample procurement was less than 30 sec; struggling by the animal during this time was minimal.

The pH of a 0·25-ml sample was measured immediately with either a Beckman blood pH microelectrode apparatus and physiological gas analyzer or a Metrohm pH meter equipped with a Leeds and Northrup miniature pH electrode assembly. The temperature of the electrodes was regulated at the body temperature of the animal by a recirculating water bath. The pH was recorded to ±0·01 pH unit. This measurement was judged to be the pH of the blood of the resting animal.

A 0·1-ml sample of blood was precipitated in an equal volume of 3·5% perchloric acid, mixed and analyzed according to the procedure of Bennett & Licht (1972). Resulting concentrations of lactate were expressed as mg % (mg lactate in 100 ml of whole blood).

The lizard was then stimulated for 7 min by general handling of the legs and tail and by infrequent electrical shocks to the hind limbs. The latter were delivered by a Grass stimulator through safety-pin electrodes. The stimulation was identical to that administered during measurements of aerobic scope, heart rate increment and ventilation volume in previous experiments (see Bennett, 1972b, 1973). Struggling by all animals was intense; *Sauromalus*, unlike *Varanus*, often became refractory to further stimulation. Blood samples were collected by heart puncture immediately after activity and 5 and 10 min post activity. Samples were analyzed for pH and lactate as described previously. Maximum lactate concentrations and minimum pH values were always observed immediately after activity or 5 min post activity.

Blood buffers

Measurements were made to determine the composition and efficacy of the blood buffering systems of both species of lizards. The bicarbonate–carbonic acid buffer system was assayed by the determination of bicarbonate concentration under standard conditions. This was done manometrically, according to the method outlined by Umbreit *et al.* (1964). Bicarbonate concentrations were assayed in whole blood samples from 6 *Sauromalus* and 5 *Varanus* at 38°C in an atmosphere of 5% carbon dioxide at 95% air saturated with water vapor (P_{O_2} = 145 torr, P_{CO_2} = 35 torr, P_{H_2O} = 50 torr).

A titration curve for all non-carbonic buffers in whole blood was constructed by varying P_{CO_2} of the atmosphere to which the sample was exposed and measuring the resulting pH. A 3·0 ml sample of heparinized blood was obtained for each species by pooling samples from resting individuals. This sample was mixed thoroughly and stored on ice between determinations. The equilibration system and pH apparatus described previously were utilized. The temperature of the system was regulated at 35°C. Gas mixtures of varying proportions of CO_2, O_2, and N_2, saturated with water vapor, were prepared in a 2-liter capacity brass spirometer (P_{CO_2} = 8–175 torr, P_{O_2} = 148 torr, P_{N_2} = 385–552 torr, P_{H_2O} = 42 torr). A 0·3 ml sample of whole blood was placed in the tonometer and equilibrated with the gas mixture as described previously for oxygen capacity measurements. The pH was measured immediately at the end of the equilibration period.

The phosphate buffers were assayed by determination of the total amount of inorganic phosphate by the spectrophotometric method outlined by Hawk *et al.* (1947). The increment in the optical density of samples and standard solutions (2–4 mM/l.) was read on a Beckman DB spectrophotometer at 660 nm.

Oxygen affinity

The information obtained in the previous experiments was used to determine oxygen affinity of whole blood under conditions of rest and activity at a series of temperatures. In these experiments, a sample of whole blood was exposed to gas mixtures of varying oxygen and carbon dioxide content; the latter was used to regulate the pH of the blood sample.

The oxygen content of the sample after equilibration at any given temperature was determined by the Roughton–Scholander method and compared to that of a fully oxygenated sample at 25°C. The percentage of potential saturation was expressed as a function of P_{O_2}.

Blood was withdrawn in 2·0-ml samples by heart puncture from two lizards of the same species and pooled to provide sufficient blood for analysis. The pooled sample was heparinized, stored in ice water and mixed thoroughly before analysis. The oxygen capacity of this pooled sample was measured at 25°C as described previously.

The pH of the blood sample was regulated at the levels measured in resting and active animals at each temperature. The correct P_{CO_2} to establish the desired pH had to be determined separately for each sample of blood because of varying levels of alkaline reserve between samples. This was facilitated by reference to the titration curve for non-carbonic buffers. The slopes of these curves were nearly identical between samples within a species, although their position on the P_{CO_2} axis varied considerably between blood samples. To determine the desired P_{CO_2}, the sample was exposed to a known P_{CO_2} and the resulting pH measured; the desired P_{CO_2} could then be estimated by reference to the slope of the titration curve. The sample was then exposed to the predicted P_{CO_2} to check the accuracy of the prediction; pH was regulated at the desired level $\pm 0{\cdot}02$ pH units. A given P_{CO_2} maintained a constant pH throughout the entire experiment on a sample. Values of P_{CO_2} required to regulate the desired pH levels were 25–40 torr and 86–150 torr to simulate rest and activity, respectively, in *Sauromalus* and 23–32 torr for both conditions in *Varanus*.

To determine the oxygen saturation of the sample at a given P_{O_2} and P_{CO_2}, a gas mixture was made in the spirometer, 0·20 ml of the pooled sample of whole blood was placed in the tonometer, and both were permitted to equilibrate to chamber temperature for 5 min. Equilibration with the gas mixture proceeded as previously described, for a total exposure of 15 min. At the end of this period, the temperature of the sample was measured with a thermocouple connected to a Honeywell recorder. The sample was rapidly withdrawn and analyzed for oxygen content according to the Roughton–Scholander method. The resulting volume was corrected to STPD, and the percentage saturation was determined by dividing the latter volume by the oxygen capacity of the sample. Saturation of each blood sample was measured at six or more different partial pressures of oxygen for each pH desired; two separate curves were constructed for each temperature at which the pH of the blood of the active animal was different from that of the resting animal. At least two equilibration curves were made for each condition and the data were pooled to form composite curves. The variability between curves constructed for different samples under identical conditions was very small; substantially identical curves were obtained from different pooled samples.

Statistics

All linear regressions reported are the best computed least-squares fit to the data. Mean values are reported with standard errors; 95 per cent confidence limits are used to estimate significance. Difference between mean values was tested by Mann–Whitney U-tests. Mean values for pH determinations were made by converting data to hydrogen ion concentrations, performing statistical manipulations and reconverting the data to pH notation.

RESULTS

Hematocrit and oxygen capacity

The average hematocrit of *Sauromalus*, 33% ($\pm 1{\cdot}3$), is not significantly greater ($P > 0{\cdot}1$) than that of *Varanus*, 29% ($\pm 2{\cdot}4$). Oxygen capacity of the blood, however, is significantly greater ($P < 0{\cdot}02$): *Sauromalus*, 9·7 vol % ($\pm 0{\cdot}6$); *Varanus*, 8·0 vol % ($\pm 0{\cdot}5$).

Lactate content of the blood

The difference in lactate concentration of the blood of resting *Sauromalus*, 6·9 mg % (±1·0), and in resting *Varanus*, 8·1 mg %(±1·9), is not significant ($P > 0{\cdot}2$). The resting level of blood lactate is temperature independent.

In active *Sauromalus*, the maximum lactate concentration in the blood is a direct function of body temperature (Fig. 1) over the range of 25–40°C (correlation

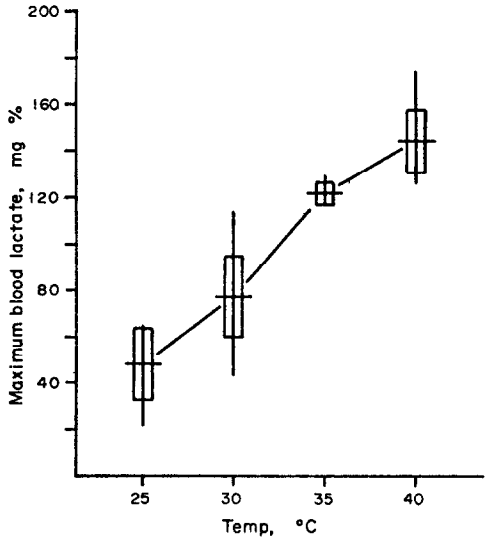

FIG. 1. Maximum lactate concentration in the blood of active *S. hispidus* as a function of body temperature. Horizontal bar indicates mean; vertical rectangle, twice the standard error of the mean; vertical bar, range. The linear regression describing these data is mg % lactate = $-117{\cdot}7 + 6{\cdot}60T_b$.

coefficient = 0·90). The mean maximum level at 40°C is 144 mg %. In contrast, maximum blood lactate in *Varanus* is temperature independent and considerably lower (Fig. 2): the mean value of all observations is 58·8 mg % (±6·6). Although the utilization of anaerobic metabolism is not significantly different at 25 and 30°C, *Sauromalus* relies upon anaerobiosis to a much greater extent than does *Varanus* at normal activity temperatures (35–40°C).

Blood pH

The blood pH of *Saurolamus* decreases only slightly with increasing body temperature in resting animals, but decreases greatly in active ones (Fig. 3). The pH differences in resting and active animals at any given body temperature is temperature dependent. The blood pH at 25°C remains unchanged during activity, but a significant difference appears at 30°C and increases at 35 and 40°C.

The pH notation obscures the magnitude of the observed change: at 40°C, the hydrogen ion concentration of the blood doubles during activity.

The relationship between blood pH and body temperature in resting *Varanus* is complex (Fig. 4), suggestive of two temperature plateaus of 7·22 at 25–30°C and 7·33 at 35–40°C. Increasing temperature generally increases resting blood pH. The pH of the blood does not change during activity in *Varanus*.

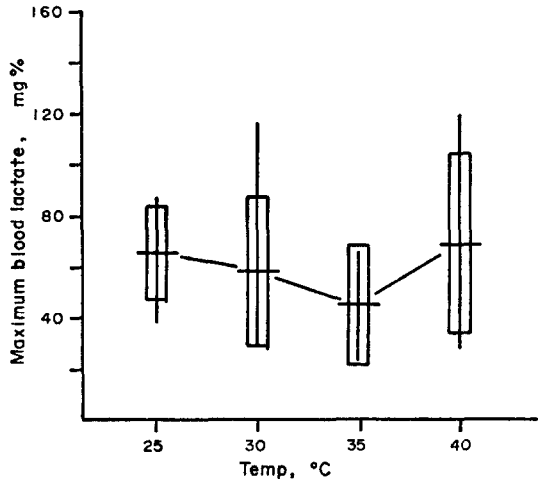

FIG. 2. Maximum lactate concentration in the blood of active *V. gouldii* as a function of body temperature. Symbols as in Fig. 1.

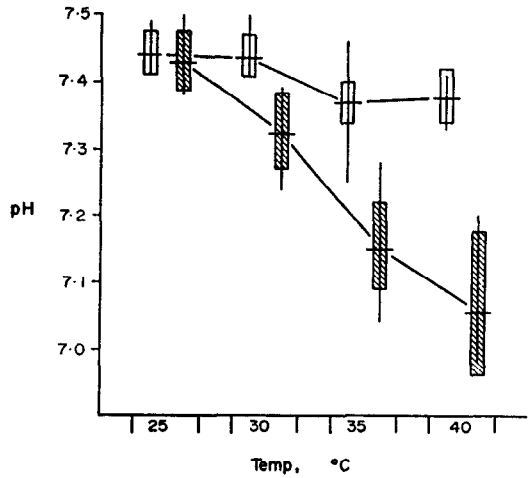

FIG. 3. Blood pH in resting and active *Sauromalus* (open and hatched rectangles, respectively). Symbols as in Fig. 1. The linear regressions describing these data are $pH = 7{\cdot}59–0{\cdot}0059T_b$ and $pH = 8{\cdot}09–0{\cdot}0263T_b$ for resting and active animals, respectively.

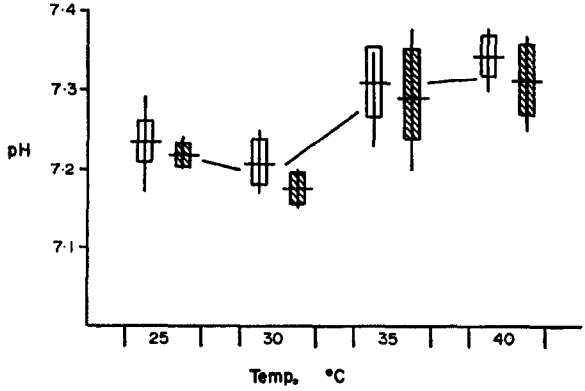

FIG. 4. Blood pH in resting and active *Varanus* (open and hatched rectangles, respectively). Symbols as in Fig. 1. The linear regression describing resting pH is $pH = 7{\cdot}02 + 0{\cdot}0079T_b$.

Over the normal operating thermal range, 35–40°C, the blood pH of resting *Sauromalus* and *Varanus* is quite similar (approximately 7·35). Varanid blood is considerably more acidic at 25–30°C than that of *Sauromalus*. During activity at high body temperatures, the blood of *Sauromalus* reaches a much lower pH than that of *Varanus*: 7·06 and 7·33, respectively, at 40°C.

Blood buffers

The concentration of bicarbonate in *Sauromalus* blood, 13·7 mM/l. (±0·8), is not significantly different ($P = 0{\cdot}2$) from that of *Varanus*, 16·4 mM/l. (±1·0).

The titration curves of non-carbonic blood buffers for both lizards are given in Fig. 5. Siggaard-Andersen (1964) has found that linear approximation represents a satisfactory description of this relationship of pH as a function of log P_{CO_2}. The non-carbonic buffering strength of *Sauromalus* blood is $-2{\cdot}26$ log P_{CO_2} per unit pH change. For *Varanus*, the non-carbonic buffering strength is $-8{\cdot}55$ log P_{CO_2}/pH unit. Large alterations of P_{CO_2} are ineffective in changing the pH of varanid blood. The non-carbonic buffering systems of *Varanus* are, therefore, considerably more effective than those of *Sauromalus*.

Concentrations of inorganic phosphate in the blood of *Sauromalus*, 2·05 mM/l. (±0·17), and *Varanus*, 2·22 mM/l. (±0·16) are not significantly different ($P > 0{\cdot}2$). The low levels of inorganic phospate compared to those of bicarbonate and protein support the general conclusion that phosphates contribute little to the overall buffering ability of the blood.

Oxygen affinity

The relationship between oxygen affinity of whole blood, pH and temperature is given for *Sauromalus* and *Varanus* in Figs. 6 and 7, respectively. Values for the oxygen tension at which the pigment is half-saturated with oxygen (P_{50}), the maximum saturation achieved and the heme–heme interaction (n) were calculated

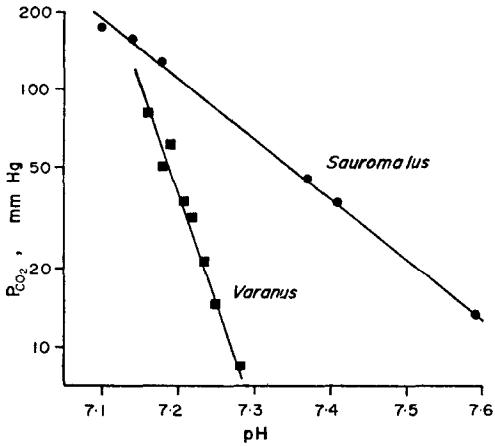

FIG. 5. The pH of whole blood of *Sauromalus* and *Varanus* (circles and squares, respectively) at 35°C as a function of P_{CO_2}. Linear descriptions of these data are pH = 7·78–0·442 log P_{CO_2} for *Sauromalus* and pH = 7·39–0·117 log P_{CO_2} for *Varanus*.

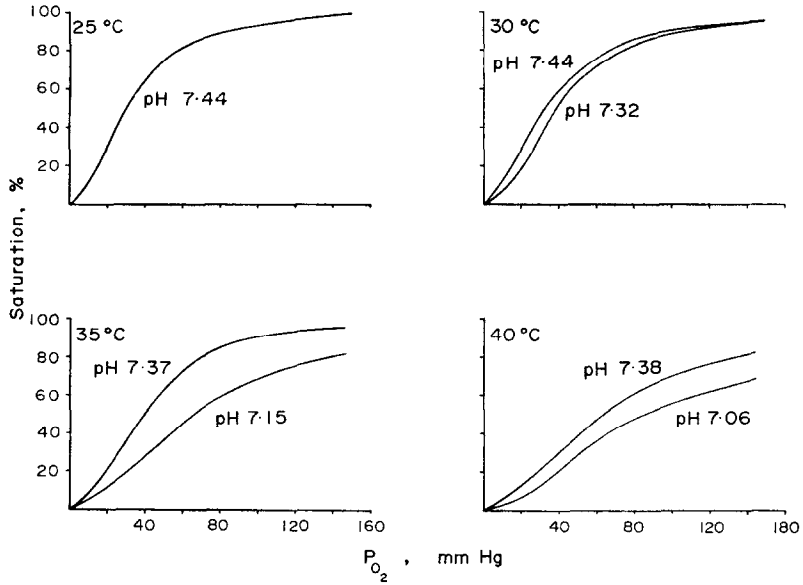

FIG. 6. Oxygen affinity of whole blood as a function of temperature and pH in *S. hispidus*.

from these data according to Hill's equation and are reported in Tables 1 and 2.

For *Sauromalus*, increasing temperature and decreasing pH during activity drastically decrease oxygen affinity, maximum saturation and heme unit interaction. The Bohr shift as a result of pH change during activity is quite pronounced, with a mean value of $-0{\cdot}65$ log P_{50}/unit pH.

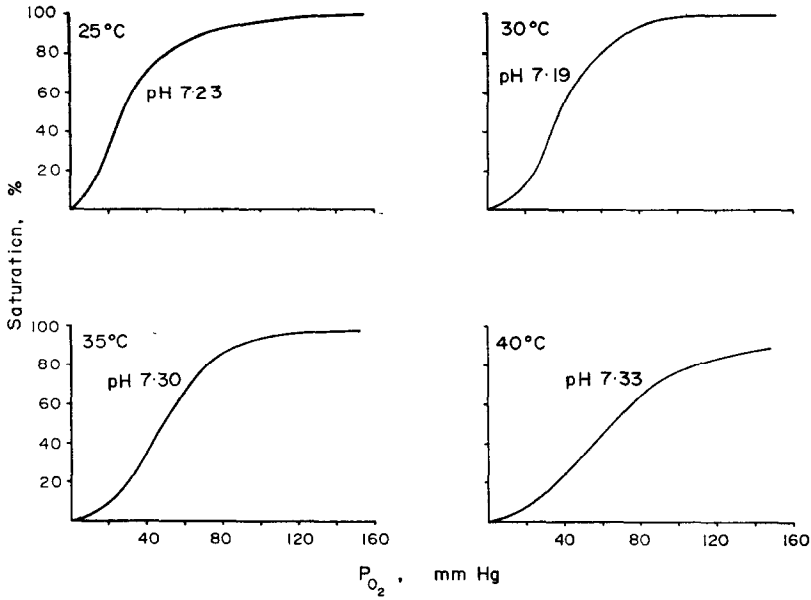

FIG. 7. Oxygen affinity of whole blood as a function of temperature and pH in *V. gouldii*.

TABLE 1—VARIABLES RELATING TO THE OXYGEN AFFINITY OF WHOLE BLOOD OF *Sauromalus hispidus*

	Rest				Activity			
T_b (°C)	pH	Max. sat. (%)	P_{50} (torr)	N	pH	Max. sat. (%)	P_{50} (torr)	N
25	7·44	100	29	1·93	—	—	—	—
30	7·44	96	32	2·15	7·32	96	38	2·41
35	7·37	96	39	2·37	7·15	82	64	1·92
40	7·38	83	62	1·91	7·06	69	87	1·63

TABLE 2—VARIABLES RELATING TO THE OXYGEN AFFINITY OF WHOLE BLOOD OF *Varanus gouldii*

T_b (°C)	pH	Max. sat. (%)	P_{50} (torr)	N
25	7·23	100	27	2·34
30	7·19	99	37	3·22
35	7·30	97	48	2·87
40	7·33	89	65	2·58

Only one oxygen equilibrium curve was constructed at each temperature for *Varanus*, since pH does not change during activity. The oxygen affinity of the blood is very similar to that of resting *Sauromalus*, in spite of the considerable pH differential at 25 and 30°C. Increased temperature also decreases affinity in this species and depresses maximum saturation. The facilitation in oxygen loading or unloading due to heme unit interaction is considerably greater in varanid blood. The maximum saturation observed at ambient P_{O_2} is quite similar in both species. The principal differences between the oxygen affinities of the two bloods appear to result from the decrease in blood pH during activity in *Sauromalus*.

DISCUSSION

Hematocrit and oxygen capacity

The hematocrits and oxygen capacities measured are very similar to those reported for congeneric species (*Varanus griseus*, 35%, Nair, 1955; 32%, Khalil & Abdel-Messeih, 1961; *Sauromalus obesus*, 30% and 11·8 vol%, Dill *et al.*, 1935) and are close to the mean values reported for lizards as a group: 9·8 vol % (Dawson & Poulson, 1962). It is clear from these experimental results and those of other workers that the greater aerobic capacities of varanids cannot be explained by a greater blood hemoglobin content or oxygen-carrying capacity than other lizards. *Sauromalus* has a greater oxygen capacity and a lower aerobic scope. It is possible that such factors as blood viscosity set an upper limit on the hematocrit values and higher oxygen demands must be compensated by other factors.

Blood lactate

The concentration of blood lactate has been shown to be a good indicator of total anaerobic energy generation during activity (Bennett & Licht, 1972). The levels of blood lactate indicate that *Sauromalus* relies on anaerobic metabolism to a far greater extent than *Varanus* does under identical conditions of activity. The former species often became refractory to stimulation at high body temperatures; *Varanus* never did and could respond with intense struggling to continuous stimulation for over an hour.

Anaerobic dependence under these conditions of stimulation is strongly temperature dependent in *Sauromalus*. This condition contrasts to the thermal independence of maximum blood lactate in *Iguana iguana* (Moberly, 1968a) and total anaerobic metabolism in several species of small lizards (Bennett & Licht, 1972). *Sauromalus* forms excessive (>100 mg %) amounts of lactate at 35 and 40°C, its normal range of activity temperatures, and *Varanus* experiences only moderate and sustainable anaerobiosis (see Bennett & Dawson, 1973, for a review of activity level and blood lactate concentrations). Most reptiles have a great reliance on and tolerance of anaerobic metabolism during rapid or strenuous activity. Varanid lizards appear to constitute a significant exception to this generalization.

Blood pH

The effect of controlled activity on blood pH has been poorly investigated in non-mammalian vertebrates. The failure to control or minimize activity is probably responsible for the great variety and range of pH values reported for reptiles in the literature. Wilson (1971) found a significant decrease in blood pH during strenuous activity in five species of lizards (agamids and skinks); these decrements are quite similar to those found for *Sauromalus* in this study. Fish also decrease blood pH during activity (Auvergnat & Secondat, 1942; Black *et al.*, 1959; Garey, 1972). *Varanus* appears exceptional in its ability to prevent acidosis during physical exertion. This regulation has important consequences for the maintenance of aerobiosis during activity.

The response of resting pH to temperature change in *Varanus* and *Sauromalus* in this study does not correspond to that established for other poikilothermous vertebrates by Reeves (1969) and Howell (1970). They have found that blood pH decreases with increasing body temperature in such a manner that the ratio of hydroxyl to hydrogen ions remains constant. This study indicates that *Varanus* and *Sauromalus* maintain a considerable degree of independence of resting pH and body temperature. The question of pH or pOH/pH regulation in lizards is not completely settled. Further work on this problem should, as Garey (1972) points out, carefully control the effects of activity because of its consequent acidosis in most of these animals.

Blood buffering

The bicarbonate-carbonic acid system constitutes the principal blood buffer preventing pH alteration during activity and its consequent generation of lactic acid, according to the following relation:

$H^+lactate^- + Na^+HCO_3^- \rightleftharpoons Na^+lactate^- + CO_2 + H_2O$. This system, as assayed by the bicarbonate content of the blood, appears no better developed in varanids than in other lizards. The values obtained here are quite similar to those reported for *Sauromalus obesus* (19·0 mM/l., Dill *et al.*, 1935) and for other lizards (13–21 mM/l., Edwards & Dill, 1935; Hernandez & Coulson, 1951; Dessauer, 1952).

The greater buffering ability of varanid blood is manifested in the titration curve for non-carbonic buffers. The buffering value for *Varanus*, −8·55 log P_{CO_2}/pH, is far greater than that reported for other lizards (−1·25 to −5·00, Dill *et al.*, 1935; Edwards & Dill, 1935; Verjbenskaya, 1944; Wood & Moberly, 1970; Wilson, 1971; Bennett & Wilson, unpublished data), crocodilians (−1·33 to −2·08, Dill & Edwards, 1931, 1935) or turtles (−1·03 to −1·27, Southworth & Redfield, 1926; Wilson, 1939; Verjbenskaya, 1944; Gaumer & Goodnight, 1957). These buffering values are relatively temperature independent. The intercept of the titration curve is influenced by temperature, recent feeding (alkaline tide) or the addition of non-volatile acids (e.g. lactic acid) (Dill & Edwards, 1935; Siggaard-Andersen, 1964), but the slope of the curve is thought to reflect the

concentration and composition of the non-carbonic blood buffers: hemoglobin, plasma proteins, phosphates and non-protein thiol groups.

Since inorganic phosphate levels are low in varanid blood, it might be concluded that the protein buffers are responsible for its greater pH stability. Hemoglobin levels have been shown to be no higher than in other lizards, but the amino acid composition of varanid hemoglobin and plasma proteins has not been investigated. Another possibility is the presence of high concentrations of non-protein thiol compounds. Fantl (1972) has recently reported extremely high levels of these compounds (over ten times mammalian concentrations) in the plasma of a skink, *Tiliqua scincoides*, and a turtle, *Chelodina longicollis*. An investigation into their presence and function in the blood of other reptiles, particularly varanids, is clearly indicated.

Oxygen affinity of the blood

The oxygen affinity of the blood of *Sauromalus* and *Varanus* is nearly identical under similar conditions of pH and temperature. These relationships suggest that the greater oxygen transporting ability of varanid blood is not the result of increased oxygen affinity of the hemoglobin molecule. It is instead the avoidance of metabolic acidosis during activity, with its consequent depression of carrying capacity, oxygen affinity and heme-heme interaction through the Bohr effect.

It has long been suggested that the low oxygen capacities and affinities and high thermal preferenda of desert lizards might create difficulties in the oxygen transporting function of the blood (Dill, 1938; Prosser & Brown, 1961; Dawson, 1967). Dill (1938) estimated that arterial blood of *S. obesus* and *Heloderma suspectum* could reach only 50 and 40 per cent maximum saturation, respectively, at a hypothetical body temperature of 50°C. The measurements in this study constitute the first demonstration in reptiles of a depression of oxygen capacity at normal activity temperatures and an additional depression due to acidosis during activity. These conditions are analogous to the Root effect in the blood of some fishes, in which the potential capacity of the blood is never realized at low pH values. Most previous studies on the oxygen affinity of whole reptilian blood (Dill & Edwards, 1931, 1935; Edwards & Dill, 1935; Dill *et al.*, 1935; Verjbenskaya, 1944; Pough, 1969; Wood and Moberly, 1970) have assumed that blood exposed to ambient oxygen pressures (140–150 torr) is completely saturated at all body temperatures. This assertion must be accompanied by measurement of the capacity at lower temperatures. If a thermal depression of oxygen capacity does exist, the assumption of complete saturation and the establishment of an oxygen affinity curve on that basis will cause an overestimation of the heme-heme interaction (n), an overestimation of the oxygen affinity at any given P_{O_2}, and an underestimation of the Bohr shift and thermal dependence. The only other study which has examined the effect of temperature on oxygen affinity in reptiles is that of Greenwald (1971) on the gopher snake, *Pituophis melanoleucus affinis*. No depression of capacity was found at temperatures up to 35°C at pH 7·4. The resting blood pH and the effect of activity on blood pH in *Pituophis* are unknown.

The oxygen affinities of *Sauromalus* and *Varanus* blood are similar to those of other lizards of comparable size. Sufficient data now exist to permit examination of the dependence of oxygen affinity on body size in this group. All reported measurements of saurian oxygen affinity (P_{50}) of whole blood at pH 7·4 or P_{CO_2} = 40 torr at 37–38°C (or interpolated to this temperature) are given in Table 3 and Fig. 8. When mean weight values are not reported, body weight is estimated by

TABLE 3—VALUES OF P_{50} AT 37–38°C FOR WHOLE BLOOD OF LIZARDS AT P_{CO_2} = 40 torr OR pH = 7·4

Species	Weight (g)	P_{50} (torr)	Reference
Amphibolurus barbatus	375	49*	Bennett & Wilson (unpublished data)
Dipsosaurus dorsalis	45	69	Pough, 1969
Gerrhonotus multicarinatus	25	72	Pough, 1969
Heloderma suspectum	1250†	50	Edwards & Dill, 1935
Iguana iguana	1300†	49	Wood & Moberly, 1970
Sauromalus hispidus	574	48	Present study
Sauromalus obesus	210‡	58	Dill *et al.*, 1935
Sceloporus occidentalis	11	72	Pough, 1969
Tiliqua rugosa	500	57*	Bennett & Wilson (unpublished data)
Uma notata	30	68	Pough, 1969
Varanus gouldii	674	50*	Present study

* Estimated assuming Bohr shift of $-0{\cdot}50$ log P_{50}/pH unit.
† Estimated from median of weight range.
‡ Estimated from standard adult body weight (Pough, 1973).

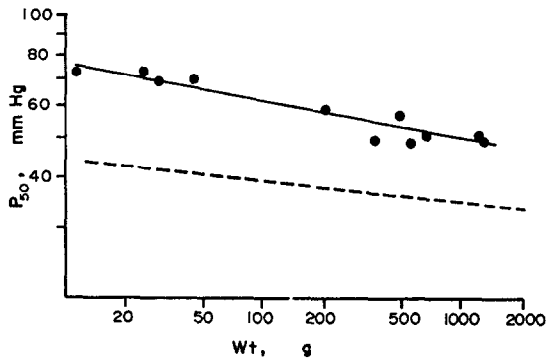

FIG. 8. Relation of P_{50} for whole blood of lizards at 37–38°C to body weight. Data are taken from Table 3. Solid regression line is based on Equation (1); dashed line is the regression of Schmidt-Nielsen & Larimer (1958) for oxygen affinity of mammalian blood [Equation (2)].

the median of the weight range or by reference to standard adult body weight (Pough, 1973). The least-squares regression of this relationship is

$$\log P_{50} = 1{\cdot}973 - 0{\cdot}0936 \log W \quad (1)$$

($N = 11$, $r = -0{\cdot}95$, 95% (confidence limits of slope = $\pm 0{\cdot}024$),

where P_{50} is measured in mm Hg P_{O_2} and W is body weight in g. It can be seen that the affinity of neither *Sauromalus* nor *Varanus* blood is exceptionally high or low in comparison to other saurians. Species of smaller body size have a lower blood oxygen affinity and may possess the lowest affinity of any group of vertebrates. Schmidt-Nielsen & Larimer (1958) have calculated a similar regression for mammalian blood at 37°C and $P_{CO_2} = 40$ torr:

$$\log P_{50} = 1{\cdot}702 - 0{\cdot}054 \log W. \quad (2)$$

The size dependence of oxygen affinity in lizards is much greater than in mammals, and the affinity itself is much lower: the P_{50} of a 10-g lizard is 70 per cent greater than that of a 10-g mammal; the differential is 41 per cent for 1-kg animals. It is apparent that oxygen affinity of the blood increased greatly during the evolution of the mammals, in support of increased metabolic demands. Varanid lizards have not adopted this solution to their demands for increased oxygen consumption.

Pough (1969) reported a narrow range of P_{O_2} values (68–72 torr) for five lizard species at their normal activity temperatures. The biological significance of this value is unclear: it may reflect no more than the small size range of the animals investigated. Exclusive of *Dipsosaurus* in the spring, his data show a similarly narrow range of values of P_{50} at 37·5°C, irrespective of preferred body temperature. *S. hispidus* and *V. gouldii* have P_{50} values of 48 and 50 torr, respectively, at their normal activity temperatures.

Effect of activity on oxygen transport

It is now possible to attempt a composite picture of the function of all these interrelated factors in both lizards during activity. *Varanus* and *Sauromalus* are almost identical in most factors affecting oxygen transport and utilization: resting metabolic rate, resting and active ventilation rate, active heart rate, hematocrit, blood oxygen capacity and affinity, resting blood pH and lactate content, blood bicarbonate and phosphate concentrations and aerobic enzymatic activities in the liver and skeletal muscle. The physiological superiorities of *Varanus* are few but critical: a complex lung structure with a much greater surface area, excellent non-carbonic blood buffers and high levels of myoglobin in the skeletal muscles (Bennett, 1971, 1972a, b, 1973).

A reasonable hypothesis of the physiological events accompanying activity in *Sauromalus* is as follows. The energy required in the initial burst of activity is generated by lactate production, as it is in all vertebrates investigated, because of the inherent lag times associated with increasing oxygen transport. Low myoglobin levels delay the transport of oxygen from the blood to the muscle cells.

The skeletal muscles of *Sauromalus* have a high activity of phosphofructokinase, the rate-limiting enzyme of glycolysis, doubtless facilitating energy liberation through lactate formation. The lactic acid thus formed and the carbon dioxide formed aerobically diffuse into the blood, where the former is buffered by bicarbonate ions. The bicarbonate–carbonic acid system is a highly efficient buffer, as long as P_{CO_2} remains constant or decreases. It is probable, however, that P_{CO_2} increases because of aerobically and anaerobically generated CO_2. Release of this gas would be hampered by the simple, lightly vascularized lung structure of *Sauromalus*, as is an increment in oxygen uptake. An increment in P_{CO_2} would increase the concentration of hydrogen ions in the blood (decrease the pH), as is observed during activity. The latter effect greatly decreases the oxygen affinity, oxygen-carrying capacity and heme unit interaction of the blood, which already has a low affinity because of *Sauromalus*' high thermal preferendum. All these factors considerably hamper oxygen uptake in the lungs and its discharge in the tissues, the latter being further reduced by the low myoglobin content in the muscles. Deprived of an adequate supply of oxygen, *Sauromalus* must rely on continued anaerobiosis and lactate formation. A cycle is thus established, in which increased reliance on anaerobiosis further decreases the ability to transport oxygen for aerobic metabolism and creates further utilization of anaerobic metabolism. This situation results in an explosive rise in lactate and hydrogen ion concentrations and consequent exhaustion. These factors also are responsible for the high oxygen debt of *Sauromalus*, since they delay lactate elimination and the re-establishment of muscle phosphagens and oxygen stores.

Varanus escape this anaerobic condition by the efficacy of its non-carbonic blood buffers in preventing a decrease in blood pH and of its complex lung structure, with its greater ability to release carbon dioxide and to maintain the functioning of its bicarbonate buffers. The lung is also more efficient in oxygen uptake, and high levels of myoglobin, equivalent to those of most mammals, facilitate the rapid transfer of oxygen from the blood into the muscle fibres. Aerobiosis is thus sustained and lactate production is maintained at tolerable levels. These factors likewise minimize the extent and increase the rate of repayment of oxygen debt. The principal features of these models which require further substantiation are the behavior of the P_{CO_2} of the blood of both species, the rate of CO_2 release, and the arterial-venous differences in oxygen concentration before, during and after activity.

The maintenance cost of the lung structure, blood buffers and myoglobin by varanids cannot be great, since standard metabolism of these animals is no greater than that of other saurians (Bennett, 1972b; Bennett & Dawson, 1973). The demands for high levels of continuous activity in this group have created adjustments in the basically anaerobic reptilian metabolic pattern to maintain high levels of oxygen acquisition. The oxygen consumption capacities of varanids are, of course, limited: they can reach but only barely exceed those of resting mammals and birds. The latter homeothermic groups are able to utilize far greater amounts of oxygen for a greater level of sustained work. Such a selective

benefit entails the increased cost of the maintenance of more energetically expensive and complex physiological systems, even under resting conditions.

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Key Word Index—Activity; aerobic scope; anaerobiosis; bicarbonate; blood; blood buffering; hematocrit; hemoglobin; lactate; lizard; oxygen affinity; oxygen capacity; pH; phosphate; reptile; *Sauromalus*; temperature; *Varanus*.