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Respiratory and Cardiovascular Physiology of the Aquatic Snake, *Acrochordus arafurae*

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Summary. 1. This study examines O_2 and CO_2 exchange and transport, energy production and the biochemical properties of blood and muscle in a sluggish aquatic snake.

2. The hemoglobin (Hb) concentration and hematocrit were low compared to other reptiles (Table 1) but the buffer capacity was higher (Tables 1,2). Hb – O_2 equilibrium curves of whole blood showed high affinity, high Bohr effect, low cooperativity and low temperature sensitivity (Tables 1,3; Figs. 2–4). ATP was the major organic modifier of the Hb; there was no 2,3 DPG (Fig. 1, Table 1).

3. Low myoglobin concentration and relatively high O_2 saturation in the pulmonary artery (Table 5) imply low rates of O_2 uptake by the tissues, even during activity.

4. The high Hb – O_2 affinity favors replenishment of blood O_2 stores but does not limit O_2 delivery to those tissues of low O_2 requirements. Where regional differences in O_2 demands occur, the large Bohr effect compensates for high affinity by releasing more O_2 at higher P_{O_2} in active tissues.

5. Low specific activities of selected glycolytic enzymes from skeletal muscle (Table 4) and the characteristics of lactate dehydrogenase pointed to low capacities for aerobic and anaerobic energy production, confirmed by measurements of O_2 uptake and lactate production during forced activity. Fatigue occurred despite high circulating O_2 reserves (Fig. 9) and blood lactate levels characteristic of other snakes at rest (Table 2).

6. During voluntary apnea, O_2 is recruited at variable rates from the lung and both sides of the circulatory system. CO_2 exchange in the lung quickly ceases and often reverses (Figs. 5,7,8). Much of the CO_2 stored in the blood and tissues during the dive enters the lung during the ventilatory episodes consisting of several breaths. Pulmonary blood flow increases during ventilation (Figs. 5–7) and the duration of the episode ensures that almost the entire blood volume passes through the lung.

Introduction

The Acrochordidae are totally aquatic snakes of the Indo-Pacific region. They are unique in many morphological ways that show them to be highly specialized for aquatic life yet evolutionarily primitive. Their unique, spiked, muscular skin can be flattened to produce a keel for effective swimming but they are practically incapable of locomotion out of water (Dowling 1960; Smith 1914). The snakes are generally sluggish but become active at night (Pough 1973) and rest during the day, anchoring themselves close to the surface. The daytime breathing pattern of *A. javanicus* consists of long (20–30 min) breath-holds broken by a period during which several breaths are taken (Pough 1973; Glass and Johansen 1976). This breathing pattern becomes less distinct when the animals move about.

In an effort to characterize the cardiovascular and respiratory physiology associated with this behavior, several comparative physiologists have used *A. javanicus* or *A. granulatus* to study heart rate and breathing (Pough 1973; Heatwole and Seymour 1976a), cutaneous gas exchange (Standaert and Johansen 1974; Heatwole and Seymour 1975a, 1978), the control of pulmonary gas exchange (Glass and Johansen 1976), muscle enzyme activity (Baldwin and Seymour 1977) and blood O_2 storage (Feder 1980). In a review, Johansen and Lenfant (1972) presented summarized data on blood respiratory properties of *A. javanicus* and related the extremely high Hb – O_2 affinity and Bohr effect to diving behavior.

Useful information on the respiratory physiology of reptiles has been obtained from comparisons of the metabolic machinery used for aerobic and anaero-

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bic energy production (see Bennett and Dawson 1976). Good correlations between patterns of activity and metabolism have been shown in terrestrial and aquatic snakes. Specifically, fast moving snakes that regularly employ sustained activity in the field have high aerobic and anaerobic metabolic scopes whereas snakes characterized by short bursts of intense activity show considerably reduced maximum rates of energy production, both aerobic and anaerobic (Ruben 1976a). Sustained activity has been linked with higher activities of selected skeletal muscle glycolytic enzymes, higher concentration of myoglobin in muscle and more complex morphology of the lung (Ruben 1976b). A study of rate limiting enzymes in skeletal muscle of ten species of terrestrial and aquatic snakes confirmed this correlation by showing a high capability for anaerobic metabolism in highly active snakes and a lower capability in sluggish species, including *A. granulatus* (Baldwin and Seymour 1977).

Against this background, we decided to investigate gas exchange and metabolism in *Acrochordus arafurae,* because this genus was considered to epitomize sluggishness in snakes. Our aim was to reassess the respiratory properties of the blood, examine the rates of O_2 uptake from the lung during rest and activity, and correlate muscle biochemistry with the capacity of this snake for becoming active. By so doing, we were able to obtain information about gas exchange at several levels from the lung to the muscle.

Materials and Methods

Animals. Although the family, Acrochordidae, was originally thought to contain two species, the larger, mainly fresh water *Acrochordus javanicus,* and the small marine or brackish water *A. granulatus* (=*Chersydrus granulatus*), McDowell (1979) recently revised the genus, erecting a new species, *A. arafurae,* from what was called *A. javanicus* in Australia and Papua New Guinea. We initiated the study under the assumption that *A. arafurae* was the same species as *A. javanicus.* The physiological data confirmed the basic similarity of these two species but there are certain differences that distinguish *A. granulatus.*

Seventeen specimens of *A. arafurae* weighing 223–4370 g were collected in the fresh waters of the Alligator River near Darwin, Northern Territory, in August, 1978. They were flown to Adelaide or Melbourne and maintained in aquaria at 25 ± 2 °C. All in vivo experimentation was carried out at this temperature, which was near that of the natural environment at the time of collection (27 °C). Aside from minor skin infections which were controlled by occasional tetracycline injections according to Murphy (1975), they survived captivity well and exhibited large fat reserves, even after four months of voluntary fasting.

Blood Properties. Hemoglobin concentration of whole blood was measured within a week of capture by the spectrophotometric method of Van Kampen and Zijlstra (1961) modified after Drabkin (1932). The millimolar extinction coefficient was assumed to be the same as in man (Antonini and Brunori 1971). Methemoglobin was determined by the method of Evelyn and Malloy (1938).

The hematocrit was determined by centrifuging whole blood in heparinized tubes for 3 min in an International Microcapillary Centrifuge, Model MB. Longer centrifugation did not produce tighter packing. Hematocrits were read to the nearest half per cent on an International Microcapillary Reader.

Organic phosphates (nucleotide triphosphates, 2,3-diphosphoglycerate) in whole blood were measured enzymatically with kits from Boehringer-Mannheim Corporation. ATP and GTP were separated and identified by ion exchange chromatography on Dowex 1×8 (200–400 mesh) formate form, as described by Bartlett (1978). Neutralized trichloroacetic acid extracts of 1.5 ml of washed packed red blood cells were analyzed for total NTP and then chromatographed. Eluted fractions were scanned at 260 nm, and ATP and GTP identified from elution profiles of standards run separately on the same column.

Buffer capacity was measured in vitro by the Astrup method. Whole blood, obtained by heart puncture of resting snakes, was equilibrated for 15 min (Bärtschi et al. 1970) with 3.15 and 6.42% CO_2 in air (Gerin-Portier 1971). These gas mixtures were supplied and laboratory analysed by Commonwealth Industrial Gases, Preston, Victoria. Blood pH was measured with a Radiometer glass electrode, calibrated to 0.01 unit with fresh phosphate buffers. Bicarbonate was calculated with the Henderson-Hasselbalch equation and constants according to Severinghaus et al. (1956) and Severinghaus (1965). Blood buffering was also evaluated by measuring pH, P_{CO_2} and lactate concentration in snakes before and after bouts of forced activity. At least 24 h before an experiment, the aorta was catheterized at the level of the cloaca to facilitate blood collection. A Radiometer digital acid-base analyzer (PHM 72 Mk.2) and blood micro system (BMS 3 Mk.2) provided pH and P_{CO_2} while lactate was measured with Boehringer kits. Buffering capacity was calculated assuming that lactate displaces bicarbonate on an equimolar basis.

$Hb-O_2$ equilibrium curves of whole blood were established by the mixing technique of Edwards and Martin (1966) after Haab et al. (1960). Briefly, known volumes of completely oxygenated and deoxygenated blood of identical P_{CO_2} were mixed in a 500 µl Hamilton microsyringe and the resulting P_{O_2} measured with a Radiometer O_2 electrode in a thermostatted cell. The electrode was calibrated with pure N_2 and snake blood equilibrated with air. Tonometers, consisting of 25 ml round flasks, rotated in a water bath maintained with ± 0.2 °C precision. Deoxygenation occurred when humidified N_2-CO_2 mixtures were passed through the flask; equilibration was found to be complete within 15 min. Oxygenation occurred faster and employed mixtures of air and N_2 at constant P_{CO_2}. The curves were based on six points and 100% saturation was assumed in blood equilibrated with air. Cooperativity (Hill's n) was determined from Hill plots of 25, 50 and 75% saturation. The Bohr effect was calculated from whole blood curves at the P_{50}, and from washed red blood cell suspensions in 0.05 M bis-tris or tris HCl buffer made with 0.01 M NaCl, pH ranging from 6.9 to 8.0.

The presence of multiple hemoglobin types was shown by isoelectric focussing of washed red blood cell hemolysates on LKB Ampholine PAG plates, pH 3.5–9.5.

Skeletal Muscle Properties. Myoglobin concentration in the body wall musculature was determined according to Reynafarje (1963).

Maximum activities of lactate dehydrogenase, hexokinase, phosphorylase and phosphofructokinase in skeletal muscle were determined at 25 °C as described by Baldwin and Seymour (1977). The electrophoretic isoenzymes of LDH and the relative proportions of M and H subunits in skeletal muscle were determined as described by Muller and Baldwin (1978).

Gas Tensions and Blood Flow in vivo. Three snakes were successfully catheterized non-occlusively with soft P.V.C. tubing so that blood

could be sampled from the pulmonary artery and pulmonary vein at the confluence of the anterior and posterior branches near the heart. Three other animals were catheterized in the caudal portion of the aorta only. Details of surgical technique and analysis of blood gases are presented elsewhere (Seymour 1978). The catheters were tolerated well and, when occasionally flushed with heparinized saline, remained patent for up to 3 weeks.

Two of the snakes with pulmonary catheters and two others without catheters were fitted with chronic Doppler blood flow cuffs on the common pulmonary artery. This vessel was carefully dissected away from its attachments to the pericardium and placed within a sterilized, custom made polystyrene cuff similar to those supplied by Parks Instrument Co. (White et al. 1974). The wires from the transmitting and receiving crystals were exteriorized at different points on the body to reduce cross-talk. These short wires led immediately to a miniature four-pin connector which could be attached to long coaxial cables leading to Parks Doppler Flowmeters (Models 803 and 806). The output of each flowmeter was recorded on a Grass Model 7D polygraph. Once the controls on both instruments had been set, they were not changed until the flow cuffs were calibrated at the end of experimentation. It required at least 3 days for the cuffs to become stabilized by granulation tissue to the vessels whereupon a clear, constant signal was produced despite movement of the snake. The best signals appeared after a week, so most experiments were run during the second and third weeks of implantation. Absolute calibration of the cuffs was performed by anaesthetizing a snake with Ketalar and collecting blood from beyond the cuff in a heparinized graduated cylinder. The appropriate blood pressure was maintained at the site of the cuff by establishing a down stream pressure head. Autopsy showed the cuffs firmly encased in clean tissue, the pulmonary vessels unrestricted, and the sites of catheterization clean and healthy.

Many measurements of blood flow rate, pH, gas content and lactate concentration were made at random while the animals rested under water during the day. To stimulate them into intense activity, they were severely pinched on the neck and tail causing, at first, rapid squirming and swimming movements. The stimulation was usually continued until the snakes did not right themselves when turned on their backs. During and after this period, which lasted 2–10 min, additional measurements of the blood parameters were taken. At least 48 h was allowed for the snake to recover before another bout of activity was enforced.

Results

Blood Properties

Some properties of *A. arafurae* blood are summarized in Table 1. Our values for hemoglobin, hematocrit and mean cellular hemoglobin concentration are virtually identical to those presented for *A. javanicus* by Johansen and Lenfant (1972). By comparison to the bulk of reptiles studied, including *A. granulatus* (Pough 1979; Feder 1980), the O_2 carrying capacity of the blood of *A. arafurae* is low. This results from a lower hematocrit, mean cellular hemoglobin concentration being similar to that in other reptiles (Wood and Johansen 1974). Methemoglobin is low in *A. arafurae,* despite a technique possibly prone to produce erroneously high values in reptiles (Gruca and Grigg 1980).

Total nucleotide triphosphate (NTP) is 7.9 μ mole per ml of red blood cells, or 1.7 mole NTP per mole of hemoglobin. ATP represents 92% of the NTP, the remainder being GTP (Table 1, Fig. 1). A high proportion of ATP is consistent with data from the yellow rat snake (*Elaphe obsoleta*) and boa constrictor (*Boa constrictor*) which showed 7.7 and 8.2 μ mole ATP but only 0.8 and 1.7 μ mole GTP per ml of red cells, respectively (Bartlett 1978). *A. javanicus* is reported by Johansen and Lenfant (1972) to have 7.5 μ mole ATP and also considerable 2,3-DPG. Whereas their ATP concentration is similar to our value, we found no DPG whatsoever. Indeed, DPG is almost always absent in the red blood cells of adult squamates (Bartlett 1978, 1980; Rapoport and Guest 1941).

Buffer capacity was similar in blood titrated with CO_2 (Table 1) and lactic acid (Table 2). Unfortunately, in vivo blood buffering based on P_{CO_2} and pH was not conclusive because of variability between animals and relatively small ranges of P_{CO_2} in resting snakes. Buffering tended to be high compared to many diving reptiles (Wood and Johansen 1974) but the individual values overlap the published data considerably. It appears that, in addition to hemoglobin,

Table 1. Respiratory properties of the blood of *A. arafurae*

Parameter	Mean	S.D.	*n* animals
Hemoglobin (g/100 ml)	6.18	1.45	8
Hematocrit (%)	21.6	6.5	8
Cellular hemoglobin (g/100 ml)	29.2	2.8	8
Methemoglobin (% Hb)	3.3	1.2	4
Nucleotide triphosphates (μmole/ml RBC[a])	7.9	1.7	5
ATP (% NTP)	92	–	1
GTP (% NTP)	8	–	1
Molar ratio (NTP/Hb_4)	1.7	0.3	5
2,3-DPG	0	0	3
Buffer capacity Slykes (m mole HCO_3^-/l·pH)	28.4	18.2	3
Buff. Cap. (Slykes/g Hb)	0.47	0.31	3
ΔH (kJ/mole)	− 8.8 (pH = 7.37) −12.8 (pH = 7.59)	– –	6 6

[a] RBC, red blood cells

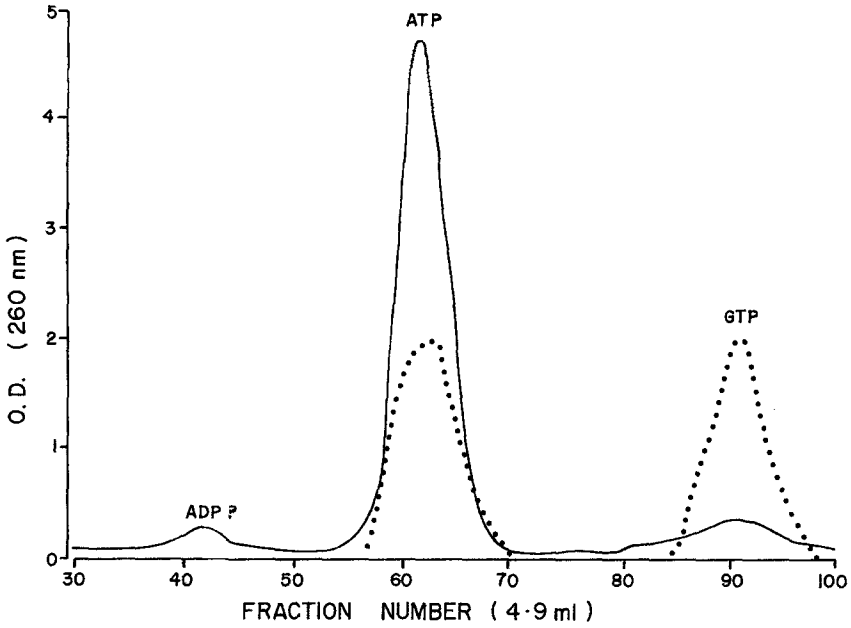

Fig. 1. Absorbance at 260 nm of elution fractions of washed, packed red blood cells (solid line) compared to ATP and GTP standards (dotted line). The small peak is probably ADP

Table 2. Effect of forced activity on blood pH and lactate in *A. arafurae* at 25 °C

Animal	pH (aorta)		ΔpH	Lactate mmole/l		Δ lactate mmole/l	Buffer cap. Slykes
	Rest	Recovery (low)		Rest	Recovery (max)		
1	7.453	7.343	0.110	1.19	3.67	2.48	22.5
1	7.410	7.351	0.059	0.49	2.26	1.77	29.8
2	7.417	7.275	0.142	0.81	4.59	3.78	26.5
2	7.476	7.342	0.134	0.50	3.60	3.10	23.1
3	7.400	7.265	0.135	0.78	4.71	3.93	29.1
Mean	7.431	7.315	0.116	0.75	3.77	3.01	26.2
S.D.	0.032	0.042	0.034	0.29	0.99	0.90	3.3

Table 3. Data from Hb–O_2 equilibrium curves of *A. arafurae*

Temperature	P_{CO_2} (Torr)	pH ($\bar{x} \pm$ S.D.)	P_{50} (Torr) ($\bar{x} \pm$ S.D.)	Bohr effect $\frac{\Delta \log P_{50}}{\Delta pH}$	Cooperativity at P_{50} (n_H)
10	23.6	7.61 ± 0.05	11.4 ± 0.7		
	48.3	7.39 ± 0.07	19.3 ± 0.3	−1.04	–
20	12.4	–	9.5 ± 0.5		
	23.4	7.59 ± 0.03	13.2 ± 0.9[a]		
	47.7	7.35 ± 0.01	22.2 ± 1.0	−0.94	2.10
25	12.4	7.80	9.5[b]		
	23.2	7.58 ± 0.05	15.0 ± 0.5		
	47.3	7.37 ± 0.02	23.3 ± 0.6	−0.91	1.83

[a] Three animals except ($n = 5$)
[b] Three animals except ($n = 1$)

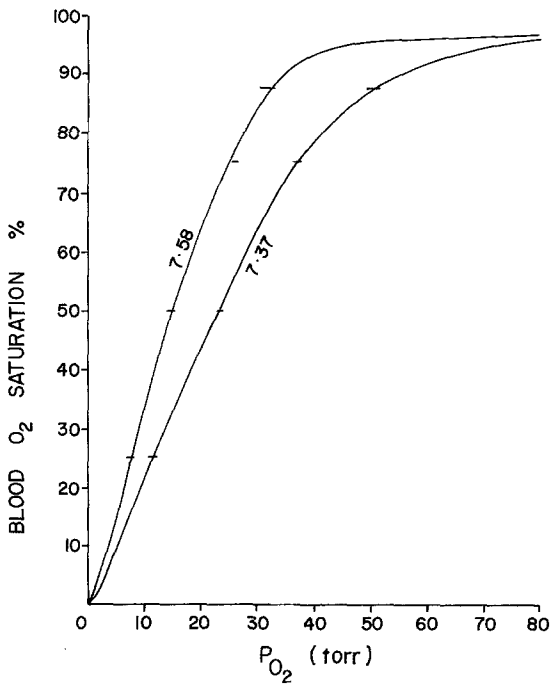

Fig. 2. Hb–O_2 equilibrium curves for whole blood of *Acrochordus arafurae* at 25 °C and pH 7.37 and 7.58. The horizontal lines indicate the range of the data

there are other buffers because the specific buffer capacity of 0.47 Slykes/g Hb is much higher than the range (0.1–0.3) seen in other reptiles (Wood and Johansen 1974; Seymour and Webster 1975; Seymour 1976).

The Hb–O_2 equilibrium curves for whole blood are characterized by high affinity, high Bohr effect, and low cooperativity (Table 3, Fig. 2). The data

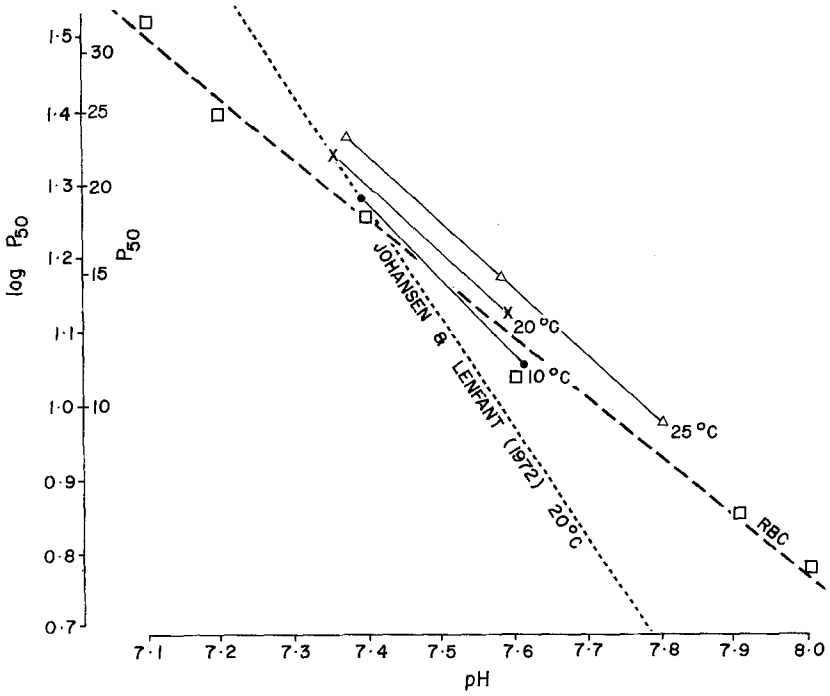

Fig. 3. Bohr effect in *Acrochordus arafurae* whole blood at three temperatures (solid lines) compared to washed red blood cell suspensions in buffer solutions and to data for *A. javanicus* whole blood at 20 °C (Johansen and Lenfant 1972)

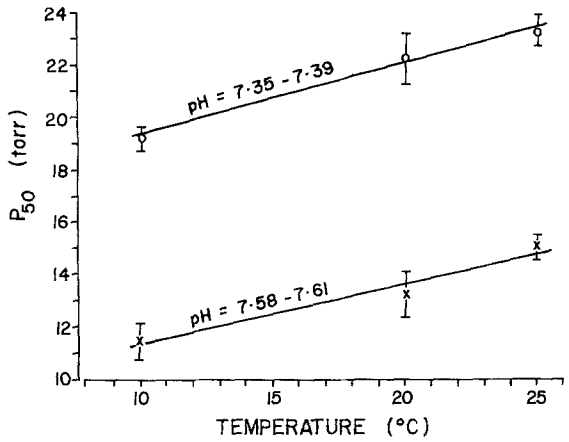

Fig. 4. Temperature sensitivity at Hb−O_2 affinity of whole blood at two levels of pH

agree essentially with those presented for *A. javanicus* by Johansen and Lenfant (1972) except that the Bohr effect in our animals (−0.94) is not as high as in theirs (−1.47 to −1.64). Pough (1977) lists the P_{50} of *A. javanicus* blood as 40 Torr and we cannot explain why the affinity was so low in his snakes. With Pough's spectrophotometric technique, Seymour and Webster (1975) found the P_{50} in *A. granulatus* to be 14 Torr, a value similar to the present data.

The Bohr effect in whole blood equilibrated with different CO_2 was similar to the effect in washed red blood cell suspensions in the absence of CO_2 (Fig. 3). Higher temperature reduces the affinity (Fig. 4). The heats of oxygenation ('enthalpy'), calculated according to Roughton (1936), varied with pH such that ΔH changed from −8.8 kJ/mole at pH = 7.35–7.39 to −12.8 kJ/mole at pH = 7.58–7.61. Although ΔH increased in value at higher pH, temperature sensitivity, expressed as $\Delta P_{50}/\Delta T$, decreased from 0.27 to 0.24 (Fig. 4).

Isoelectric focussing of washed red cell hemolysates revealed four hemoglobin bands. The isoelectric points of the three major bands were at pH 7.7, 7.5 and 7.3. A faint band at pH 7.1 was also detected.

Skeletal Muscle Properties

Myoglobin concentration in skeletal muscle of three animals ranged from 0.19 to 0.28 ($\bar{x}$=0.22) mg/g wet weight. Myoglobin is important, not only as a potential store of O_2 but also as an agent that facilitates O_2 transport from the blood to muscle (Hemmingsen 1963, 1965). It is significant that, in *A. arafurae*, skeletal muscle myoglobin concentration is lower than in the three terrestrial snakes (0.38–1.85 mg/g) studied by Ruben (1976b).

The specific activities of hexokinase, phosphorylase, phosphofructokinase and lactate dehydrogenase in skeletal muscle of *A. arafurae* are shown in Table 4, together with data from 10 other species of snake including *A. granulatus*. The activities of these four enzymes are low in *A. arafurae*, with values similar to those reported for *A. granulatus*, *Laticaude laticaudata* and *L. colubrina*, which also are sluggish, docile snakes. Electrophoretic analysis of skeletal muscle

Table 4. Specific activities[a] of selected glycolytic enzymes from skeletal muscle of *Acrochordus arafurae*, *A. granulatus* and nine other snakes

Species	Hexokinase	Phosphorylase	Phosphofructokinase	Lactate dehydrogenase	DH ratio[e]
A. arafurae[b]	0.12 (0.10–0.15)	11.6 (9.3–12.9)	13.5 (6.8–16.9)	104 (74–145)	1.1
A. granulatus[c]	0.20	11	12	280	0.9
Other snakes[c,d]	0.34 (0.09–0.85)	68 (10–290)	112 (8–566)	657 (272–1,040)	1.0 (0.8–1.3)

[a] μ moles substrate $min^{-1}g^{-1}$ wet muscle, 25 °C
[b] Values presented as the mean with the range in parentheses for 3 individuals
[c] Data from Baldwin and Seymour (1977)
[d] Mean and range from nine species of terrestrial and aquatic snakes
[e] Ratio of lactate dehydrogenase activity; 0.33 mM pyruvate: 10 mM pyruvate

lactate dehydrogenase showed that M subunits predominated (73 percent), with the M_4 isoenzyme accounting for 60 percent of the total lactate dehydrogenase activity. The low pyruvate inhibition ratio obtained for total lactate dehydrogenase in the skeletal muscle homogenate (Table 4) indicates that the reaction functions preferentially in the direction of lactate formation (Wilson et al. 1963; Dawson et al. 1964).

Blood Gases and Pulmonary Blood Flow

After several days of recovery from catheterization, blood samples from the pulmonary artery and vein of resting snakes revealed high saturation of the hemoglobin in both sides of the circulation (Table 5). In fact the lowest P_{O_2} observed in the pulmonary artery during undisturbed ventilation was 20.8 Torr which corresponds to a hemoglobin saturation of about 54%. Once, after a bout of forced activity, P_{O_2} in the pulmonary artery eventually dropped to 14.0 Torr (Fig. 5), the lowest value ever observed. Blood pH was 7.50 on this occasion and the hemoglobin saturation was about 33% just prior to breathing.

Table 5. Blood gases, pH and Hb–O_2 saturation (S_{o_2}) in four *A. arafurae* resting underwater at 25 °C. The values were taken without reference to duration of apnea

	Mean	Range	*n*
Pulmonary artery			
P_{O_2}(Torr)	46.6	14.0–92.4	41
P_{CO_2} (Torr)	37.0	21.6–46.2	39
pH	7.517	7.467–7.579	14
S_{O_2} (%)	79.7	33–97	41
Pulmonary vein			
P_{O_2} (Torr)	76.3	26.2–129.3	55
P_{CO_2} (Torr)	36.6	25.3–48.4	49
pH	7.499	7.439–7.574	17
S_{O_2} (%)	94.3	72–99	55

Table 6 shows the mean pulmonary blood flow during long periods of voluntary ventilation in three snakes equipped with Doppler flow cuffs. It is noteworthy that pulmonary blood flow in *A. arafurae* is quite low compared to other reptiles (Crawford et al. 1976).

Only one snake in Table 6 (2.7 kg) provided simultaneous sampling of arterial and venous blood, so in the other two, an estimate of the a-v difference (0.0146 ml O_2/ml blood) is based on the data from the other snakes with catheters. Thus the prevailing rate of O_2 uptake from the lung is about 0.19 ml O_2/kg·min. At a slightly lower temperature (20–22 °C), Standaert and Johansen (1974) measured pulmonary O_2 uptake to be 0.144 ml O_2/kg·min in *A. javanicus*. With an assumed Q_{10} of 1.75, the data from these two studies agree well. However, with the same Q_{10}, Glass and Johansen's (1976) data for *A. javanicus* indicate a total O_2 consumption of about 0.37 ml O_2/kg·min at 25 °C.

Table 6. Average pulmonary blood flow measured during periods of at least two hours in three undisturbed *A. arafurae* in shallow aquaria. The rate of oxygen uptake ($\dot{V}_{O_2}$) is calculated from blood flow ($\dot{Q}$), a−v saturation difference $(a-v)S_{O_2}$ and mean oxygen capacity[a] according to the Fick principle

Snake weight kg	$\dot{Q}$ ml/min	$\dot{Q}$ ml/kg·min	$(a-v)S_{O_2}$ %	$(a-v)C_{O_2}$ ml O_2/ml	$\dot{V}_{O_2}$ ml O_2/kg·min
2.70	23.76	8.80	31.34	0.02634	0.232
2.88	31.33	10.88	16.56[b]	0.0146[b]	0.159
4.37	54.80	12.54	16.56[b]	0.0146[b]	0.183
Mean		10.74			0.191
S.D.		1.87			0.037

[a] Oxygen capacity includes bound and dissolved gas in blood equilibrated with air: (0.0618 g Hb/ml) (1.36 ml O_2/g Hb) + 0.004 = 0.088 ml O_2/ml
[b] Values include data from Table 5 and from pure oxygen breathing experiments

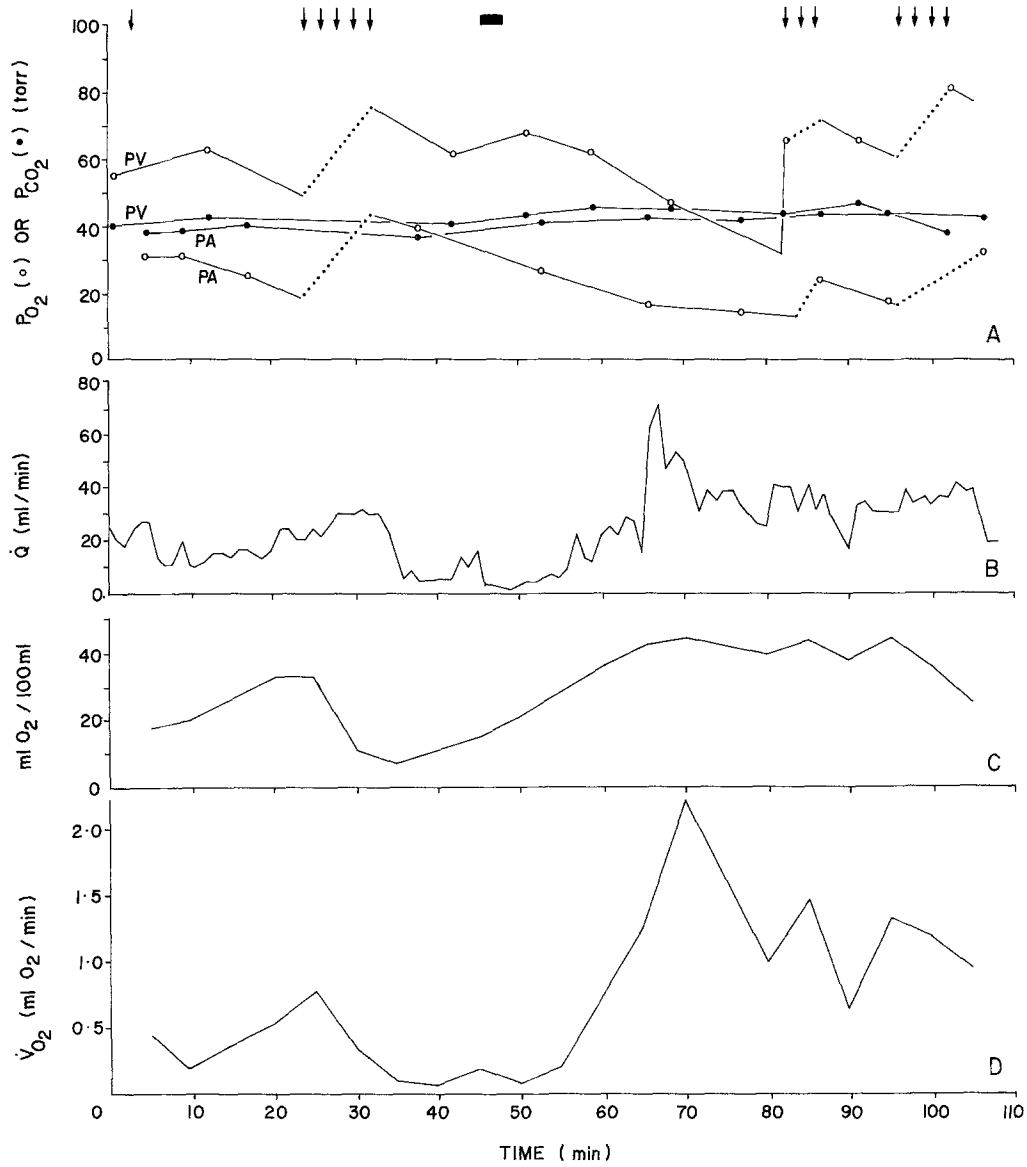

Fig. 5A–D. Gas tensions and pulmonary blood flow in *Acrochordus arafurae* during voluntary breath-holding and forced activity at 25 °C. **A** P_{O_2} and P_{CO_2} in the common pulmonary artery and vein. Notice that the P_{CO_2} is consistently higher in the vein except during the final breathing episode. Individual breaths are indicated by arrows; the bar indicates a 2.5 min period of forced activity. **B** Pulmonary blood flow is high during breathing episodes and tends to fall during apnea. Notice the anticipatory increase prior to breathing. **C** a-v difference calculated from gas tensions, pH and Hb–O_2 equilibrium curves. **D** Rate of O_2 uptake from the lung, calculated from a-v difference and blood flow

Notice that the O_2 uptake from the lung is not constant, even in resting snakes. This occurs primarily because of changes in pulmonary blood flow that are usually coincident with breathing episodes (Figs. 6 and 7). During normal ventilatory periods, blood flow remained high for an average of 7.7 (±3.6 SD) min in 110 observations. The changes in O_2 uptake from the lung can be calculated from blood flow and a-v difference (Fig. 5). When O_2 uptake from the lung is low during apnea, more of the O_2 used by the snake appears to come from the blood reserve and the difference in a-v O_2 content increases. This is particularly apparent in the illustrated case where pulmonary blood flow remained low despite a 2.5 min episode of forced activity during a dive (Fig. 5). Following the activity, blood flow gradually increased as the a-v difference stabilized.

There was never a great difference in P_{CO_2} between the pulmonary artery and vein. The similarity shown in Table 5 is not a result of pooling the data because analysis of 33 paired measurements (taken within 5 min of each other and in random order) showed

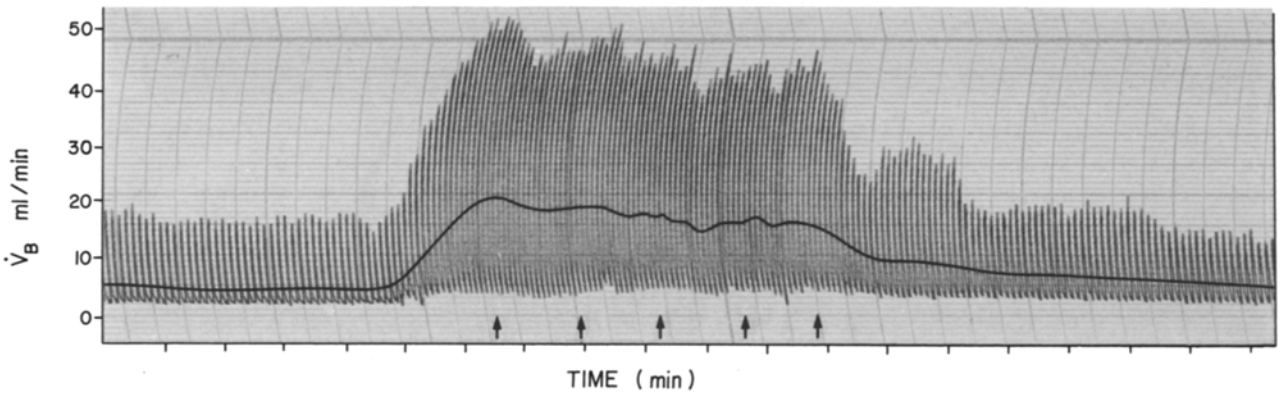

Fig. 6. Example of the change in pulmonary blood flow occurring with breaths (arrows). The mean flow is superimposed on instantaneous flow

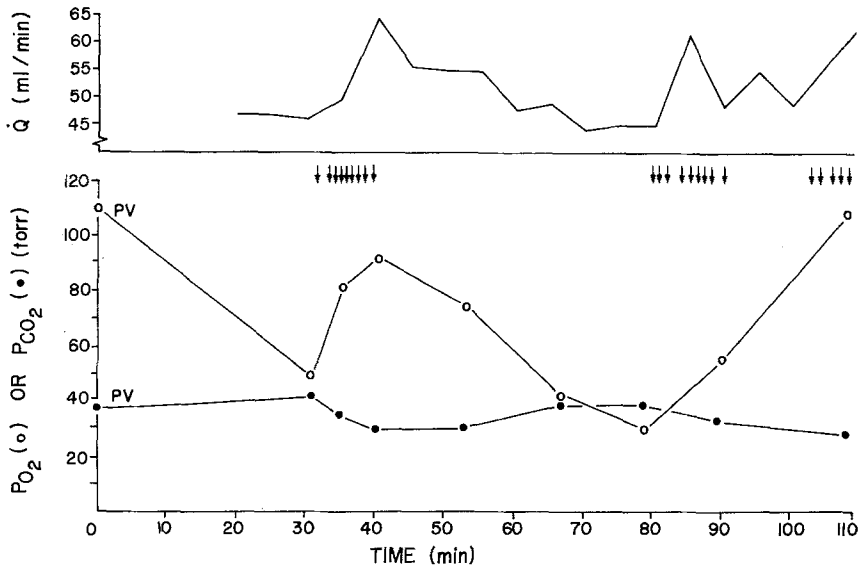

Fig. 7. P_{O_2} and P_{CO_2} in the pulmonary vein and pulmonary blood flow during voluntary breath-holding at 25 °C. Individual breaths are indicated by arrows

that the P_{CO_2} difference was not significantly different from zero ($\bar{x}=0.76\pm2.45$ Torr). However, it is noteworthy that in many cases, the P_{CO_2} in the pulmonary vein was consistently a few Torr higher than that in the pulmonary artery (Fig. 5). This was correlated with a significantly lower pH in the vein. Thirteen pairs of pH values showed the difference to be 0.030 (±0.017 SD) units. It should be recognised that most of these data were taken during breath-holding because the snakes spend about 90% of their time underwater during the day (Glass and Johansen 1976). Therefore our data characterize the apneic condition in which little or no CO_2 exchange occurs across the lung. P_{CO_2} gradients favoring CO_2 exchange can develop, however, during and immediately after a bout of ventilation (Fig. 5).

The prevailing levels of P_{CO_2} in *A. arafurae* (Table 5) appear high compared to other snakes. In four species of sea snake, for example, aortic P_{CO_2} is maintained between 18 and 32 Torr (Seymour and Webster 1975; Seymour 1978) and in the terrestrial snakes, *Pseudechis porphyriacus* and *Boiga dendrophila* it is about 19 ± 4 and 13 ± 3 Torr, respectively (Seymour, unpublished). A high circulating P_{CO_2} might seem incongruous in a snake with a significant cutaneous gas exchange capability like *Acrochordus* (Standaert and Johansen 1974). Although aquatic CO_2 exchange tends to reduce blood P_{CO_2}, the long breath-holds in this snake simply override this tendency.

Not only was there little a-v P_{CO_2} difference, P_{CO_2} did not increase much in the blood during breath-holding (Figs. 5 and 7). Usually it went up a few Torr and the maximum deviation observed in 17 long breath-holds was 10 Torr. This corresponds to a pH decrease of 0.095 unit. These small changes represent the combined effects of cutaneous CO_2 loss and CO_2 buffering by the blood and body. Fig. 8 shows how small the rise in P_{CO_2} is by relating P_{CO_2} with P_{O_2}

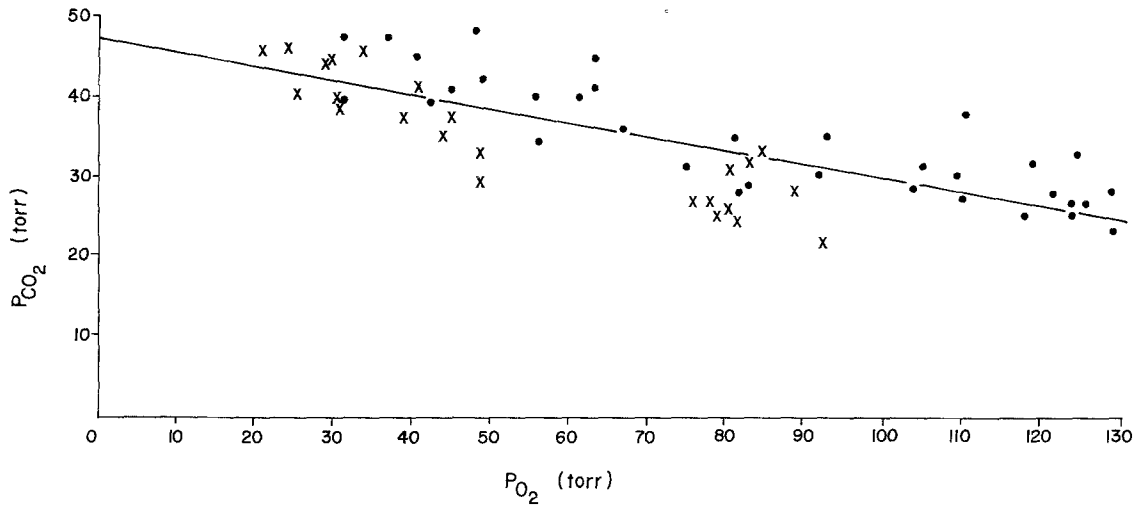

Fig. 8. The relationship between P_{CO_2} and P_{O_2} in the common pulmonary vein (•) and pulmonary artery (x) during normal voluntary breathing. Notice that P_{CO_2} tends to be higher in the pulmonary vein and that P_{CO_2} is only weakly dependent on P_{O_2}

in the pulmonary vessels during normal breathing. There is a high degree of independence of P_{CO_2} and P_{O_2}, and a similar situation occurs in other diving reptiles (Ackerman and White 1979; Burggren and Shelton 1979).

Forced Activity

When pinched on the neck skin and tail, the snakes reacted vigorously at first, thrashing about quickly and attempting to swim away. Strong struggling lasted about 2 min and thereafter the movements gradually weakened. The quick onset of reflexogenic death feigning that occurs in some reptiles under threat did not occur. There was no bradycardia (Table 7). After about 5–10 min, we could turn a flaccid animal on its back without an immediate righting response, and we assumed that it was fatigued. The snakes eventually righted themselves, albeit slowly, but would not display further activity. We do not know if this evoked activity is similar to any voluntary behavior in nature. Even when captured in the field, the snakes reacted less violently than when first pinched.

Table 7. Heart rate and pulmonary blood flow during rest, activity and recovery in three *A. arafurae* at 25 °C. Means ± S.D.

Condition	Heart rate (beats/min)	Blood flow (ml/kg·min)	Stroke flow (ml)
Rest, eupnea	14.1 ± 2.2	13.61 ± 4.21	0.96
Rest, apnea	12.6 ± 2.1	6.79 ± 2.57	0.54
Forced activity (2 min)	13.1 ± 5.6	2.89 ± 1.05	0.22
Rest, post activity (2 min)	14.7 ± 5.8	5.08 ± 3.12	0.34

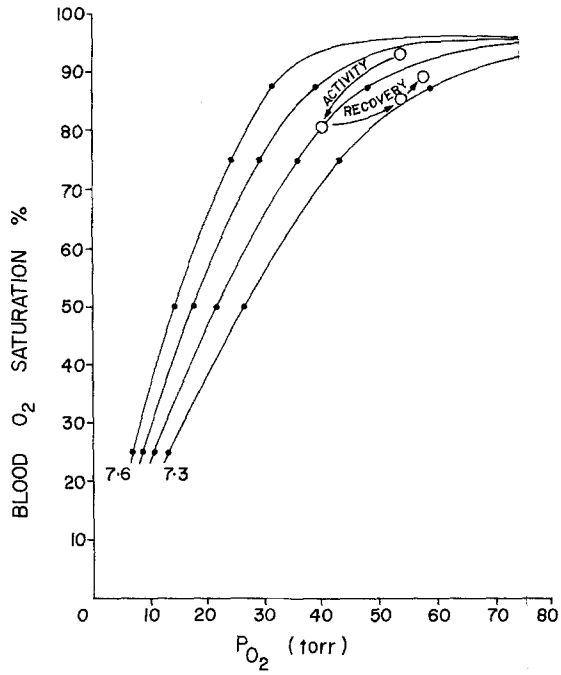

Fig. 9. Changes in saturation of blood from the aorta associated with 10 min of forced activity and recovery. The first point is immediately before exercise and represents a resting condition, the second is just after exercise but before a breath, and the third and fourth are 20 and 40 min into the recovery period during which breaths were taken

The effect of 2 min of activity on blood gases was negligible (Fig. 5). Despite activity during apnea, the blood remained almost completely saturated in the pulmonary vein ($P_{O_2} > 60$ Torr) and it was always over 50% saturated in the pulmonary artery ($P_{O_2} > 30$ Torr). Forced activity to apparent fatigue, furthermore, was accomplished without the exhaustion of systemic arterial O_2 reserves (Fig. 9). Metabolic aci-

dosis shifted the $Hb-O_2$ equilibrium to the right and tended to favor O_2 unloading at a higher P_{O_2} in the tissues during recovery. Fatigue in the face of ample pulmonary O_2 supplies was impressively demonstrated by one snake that tired after 5 min of activity despite the fact that it was breathing pure O_2 immediately prior to the exercise.

Systemic arterial blood samples, taken periodically during recovery, showed blood lactate concentrations rising to an average of 3.7 mmole/l at 20 min after the activity and then stabilizing or dropping (Table 2). This was accompanied by a decline in pH averaging 0.116 unit.

Most bouts of activity resulted in a pronounced decrease in pulmonary flow that occurred despite a fairly constant heart rate (Table 7). This implies a marked increase in resistance in the pulmonary circuit. Blood flow gradually increased as these snakes rested after the exercise.

Discussion

This study on *A. arafurae* and others on *A. javanicus* converge to characterise the respiratory physiology of a group of extremely sluggish aquatic snakes with low rates of overall energy utilization.

Biochemical Properties of the Blood and Muscle

Our work substantiates the first report that *Acrochordus* blood is exceptional in its high affinity for O_2 and high Bohr effect (Johansen and Lenfant 1972). $Hb-O_2$ affinity seems better correlated to patterns of activity than to diving behavior in snakes, and the Bohr effect does not appear to be related to diving, per se, because several sea snakes show low Bohr shifts while all three *Acrochordus* species show high shifts (Heatwole and Seymour 1975b; Seymour and Webster 1975; Seymour 1976).

We view the properties of *Acrochordus* hemoglobin as an adaptation to long quiescent dives in a sluggish snake. A high affinity would maintain a high P_{O_2} difference between the gas and blood in the lung during the ventilatory periods when pulmonary blood flow is high and the blood entering the lung is only slightly less than saturated. *Acrochordus* normally takes several breaths during one breathing episode, presumably to maintain lung P_{O_2} high, further favoring a large P_{O_2} gradient from lung gas to blood. However, a high affinity must also reduce the P_{O_2} gradient into the tissues and unload O_2 slowly. The relatively high saturation of systemic venous blood indicates that the major resistance to O_2 delivery lies not at the pulmonary or cardiovascular level but at the tissue level in *A. arafurae*. Low concentrations of myoglobin in skeletal muscle are also consistent with a slow diffusion of O_2 into the tissues.

The huge Bohr effect (Table 3) partly compensates for the disadvantage of the inherently high $Hb-O_2$ affinity. Although pH was usually lower in the pulmonary vein than in the pulmonary artery, the effect may be of use in the conventional sense of facilitating O_2 transport at relatively high P_{O_2} as the blood circulates from lung to active tissues. Regional metabolic rates and blood flows may vary, to produce local P_{CO_2} and pH changes that are large enough to make the Bohr effect significant. Because the mixed venous blood in the pulmonary artery contains fractions from active tissues as well as from the skin where CO_2 loss occurs, however, the effect of the Bohr shift in the active muscles is obscured.

The large Bohr shift also can be moderately effective in releasing O_2 at higher P_{O_2} as CO_2 and other acid metabolites build up in the body during a strenuous dive when the muscles need more O_2. Blood pH decreased about 0.116 units during forced exercise (Table 2). This pH change increased the P_{O_2} about 10 Torr in blood 90% saturated and 5 Torr in blood 50% saturated and thus it caused a 20–25% increase in the driving force for O_2 diffusion into the tissues. During single voluntary dives without strenuous activity, however, pH never changed more than 0.095 units and the advantage of the Bohr effect appeared small indeed.

Acrochordus is known to exchange a small but significant amount of O_2 through the skin (Standaert and Johansen 1974; Heatwole and Seymour 1975a). A high $Hb-O_2$ affinity may be related to this ability because it keeps systemic arterial P_{O_2} relatively low and favors cutaneous O_2 uptake in well oxygenated water and reduces O_2 loss to anoxic water. Some sea snakes also exchange gas cutaneously and keep arterial P_{O_2} low but they do it by possessing a large right to left shunt rather than a high $Hb-O_2$ affinity (Seymour 1978).

The apparent heat of oxygenation, ΔH, is a measure of the sensitivity of $Hb-O_2$ to temperature. *A. arafurae* hemoglobin is notably insensitive (Fig. 4, Table 1). In fact, none of the 18 snake species considered by Pough (1980) has a lower value. Pough (1980) and Wood (1980) recently considered the possible adaptive significance of differences in ΔH among reptiles. Pough showed a tendency for thermal specialists (reptiles with a narrow range of preferred body temperatures when active) to have a lower ΔH than thermal generalists. *A. arafurae* in the Alligator River system of Australia is considered to be a thermal specialist because the water temperatures usually remain between about 25 and 32 °C diurnally and annually (T.D. Walker and P.A. Tyler, unpublished). This

snake's low ΔH, therefore, is consonant with Pough's observations.

A low ΔH in *Acrochordus* may be functionally linked with the high Bohr effect because the ΔH value is affected by not only the thermodynamics of Hb–O_2 binding but also the heat of solution of O_2 and the binding of various ligands (e.g. CO_2, H^+, organic phosphates) to Hb. As Powers (1980) also demonstrates in fish, a lower pH reduces the ΔH value in *Acrochordus* blood (Table 1). Consistent with this idea is the reduction in Bohr effect at higher temperature (Table 3), which indicates a lower affinity of Hb for protons.

The low concentrations of myoglobin, low activities of hexokinase, and predominance of M subunit lactate dehydrogenase isoenzymes displaying little substrate inhibition at high pyruvate concentrations, are indicative of a white skeletal muscle with low O_2 demands and a limited capacity for aerobic energy production. In addition, the relatively low activities of the rate limiting glycolytic enzymes, phosphorylase and phosphofructokinase, and low activity of lactate dehydrogenase, suggests a low maximum glycolytic flux during bursts of anaerobic muscle work (Wilson et al. 1963; Burleigh and Schimke 1968, 1969; Crabtree and Newsholme 1972; Baldwin 1975).

O_2 Supply and Metabolic Scope

A. arafurae does not appear capable of a high rate of energy utilization during activity. In the lab, all voluntary movements were slow and deliberate and in the field the snakes could be easily collected by hand because they made little effort to escape. The experiments involving forced activity confirmed this picture. After a period of violent movement, the snakes gradually fatigued. The buildup of lactate in the blood was only 3.0 mmole/l in 5–10 min (Table 2). In the squamates examined so far, lactate represents more than 95% of the anaerobic metabolite production (Bennett 1978). If the ratio of blood lactate to whole body lactate is about 0.72 as in other snakes (Ruben 1976a), and each mmole of lactate is associated with 1.5 mmole of ATP produced (Bennett and Licht 1972), a 1 kg *A. arafurae* would produce 3.2 mmole ATP before exhausting. By comparison, exercise for 5 min resulted in an anaerobic ATP production of 16.2 mmole ATP/kg in *Crotalus viridis*, 29.1 mmole/kg in *Coluber constrictor* and *Masticophis flagellum* and 5 mmole/kg in the sluggish *Lichanura roseofusca* (Ruben 1976a). Forced activity for 10 min yielded between 17.7 and 30.2 mmole/kg in the aquatic snake, *Nerodia rhombifera* at 25 °C (Gratz and Hutchison 1977). It is interesting that the described pattern of activity during stimulation is similar in *N. rhombifera* and *A. arafurae* yet the former produced much more ATP anaerobically.

Because our snakes held their breath, it was impossible to measure total O_2 uptake during forced activity in the usual way. However rough estimates of O_2 uptake, based on the small changes in blood and lung O_2 content during activity, show that the aerobic metabolic scope is also very small in *A. arafurae*.

Gas Storage and Exchange During Voluntary Breath-Holds

One of the many peculiarities of *Acrochordus* species is the obvious enlargement of veins in the body cavity that probably represent the origin of a high blood volume. We did not measure this in *A. arafurae* but Feder (1980) found that *A. granulatus* had a blood volume nearly twice as great as that in other aquatic snakes. It is noteworthy that the pulmonary arterial blood, derived from the systemic vein, was typically more than 80% saturated (Table 5). Because left to right intraventricular shunting is absent or small in squamates during apnea (White 1976; Seymour 1978), this suggests that the venous side of the systemic circulation is a capacious store of O_2 in resting snakes breathing voluntarily. On one occasion, when a snake was disturbed and probably frightened, the P_{O_2} fell to 14 Torr yet the saturation was 33% when the apnea was broken (Fig. 5). Thus the O_2 reserves seem to be always substantial, even when conditions might favor continued submergence and depletion of reserves. We therefore cannot support the proposition that apnea is broken when the O_2 reserves are practically exhausted (Glass and Johansen 1976).

Another approach to this question is to compare the apneic duration with rates of O_2 consumption and lung volume in *A. javanicus*. Let us assume that the snake has a lung volume of 66 ml/kg (Standaert and Johansen 1974) and begins a long dive with 15% O_2 in its lung, a conservative value based on P_{O_2} in the pulmonary vein measured in the present study. A 1 kg snake would therefore begin a dive with 9.9 ml O_2 in the lung. If the blood volume in this species is similar to the value of 133 ml/kg for *A. granulatus* (Feder 1980), and all the hemoglobin (6.18 g/100 ml, Table 3) is saturated, there is $6.18 \cdot 1.33 \cdot 1.36 = 11.2$ ml O_2 in the blood. The total reserve is 21.2 ml. At 25 °C the rate of O_2 consumption of *A. javanicus* is about 0.22 ml/kg·min (Standaert and Johansen 1974) or 0.37 ml/kg·min (Glass and Johansen 1976). The former study involved more animals and is closer to the rate measured in *A. granulatus* (Heatwole and Seymour 1975a). The total O_2 reserve would last $21.2/0.22 = 96$ min or $21.2/0.37 = 57$ min, depending on

which study is accepted. Because normal dives at 25 °C last an average of 29 min (Glass and Johansen 1976), it is clear that at least half of the O_2 reserves remain when the apnea is broken. This pattern of maintaining a substantial O_2 reserve is similar to the situation in sea snakes and turtles breathing voluntarily in shallow aquaria (Seymour and Webster 1975; Burggren and Shelton 1979). It also leads to the conclusion that routine dives in *Acrochordus* are essentially aerobic.

We believe that the pattern of ventilation in resting *A. arafurae*, characterized by long apnea broken by a ventilation period when several breaths are taken, is suited to replenish the substantial O_2 reservoir in the venous blood. In a snake having a tidal volume greater than 50% of lung volume (Glass and Johansen 1976; Standaert and Johansen 1974) there would seem little reason to take an average of 5.5 breaths during the ventilation period if renewal of the lung gas were the main objective. Three breaths would replace over 90% of the lung gas. Furthermore, there are always pauses of 0.5–2 min between the breaths during the ventilatory period. Our data suggest that the ventilatory period is lengthened to accommodate a large fraction of the snake's blood passing through the lung. Periodic breaths maintain a high availability of O_2 during this time and the rate of pulmonary blood flow remains high for an average of 7.7 min (Fig. 6). With an eupneic blood flow of 13.61 ml/kg·min (Table 7) and an assumed blood volume of 133 ml/kg (Feder 1980), it would require 9.8 min to pass the entire blood volume through the lung. Therefore the data indicate that most of the blood is exposed to lung gas before the snake begins its long apneic period. The value of this pattern of ventilation is that the snake can fully saturate its blood reserves and still begin a long dive with a lung high in O_2. Apparently much of the O_2 exchange in the lung occurs during the relatively brief ventilatory period. Intermittent ventilation also occurs in aquatic chelonians, some of which show progressive changes in end tidal P_{O_2} and P_{CO_2} indicating that the blood continues to change its gas content throughout each breathing episode (Glass et al. 1978).

Although O_2 is metered from the lung during a long breath-hold, there is little CO_2 exchange in the lung during the nonventilatory period. This is verified by the small changes in P_{CO_2} in the pulmonary vein. Assuming this represents the P_{CO_2} in the lung, the observed maximum increase in P_{CO_2} of 10 Torr in a 39 min dive (Fig. 5) indicates a maximum exchange of 0.023 ml CO_2/kg·min or about 14% of the rate of CO_2 production (Standaert and Johansen 1974). Further, if we accept that about 33% of the CO_2 exchange occurs cutaneously (Standaert and Johansen 1974), it follows that more than 50% of the total CO_2 production occurs across the lung during a ventilatory period lasting only 10% of the total time. This pattern of cyclic CO_2 exchange probably occurs in all diving reptiles and has been analyzed in detail in the turtle, *Pseudemys scripta* (Ackerman and White 1979; Burggren and Shelton 1979).

The shift of the lung from a sink to a source of CO_2 during long breath-holding is rarely observed in voluntary breathing vertebrates. In *Acrochordus*, however, the pulmonary vein usually had higher P_{CO_2} and lower pH than the pulmonary artery. A similar observation was made by Lenfant et al. (1970) on the aquatic turtle, *Chelys fimbriatus*. This may result from a combination of the CO_2 concentrating effect of O_2 absorption from the pulmonary gas, CO_2 production by pulmonary tissue, and acidification of the blood by oxygenation of hemoglobin.

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