

THE EFFECT OF PROGRESSIVE HYPOXIA ON RESPIRATION IN THE TOAD *BUFO MARINUS*

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SUMMARY

1. As *Bufo marinus* became progressively hypoxic over a period of 90 min, there was a rise in arterial pH, presumably brought about by hyperventilation. The alkalosis gradually disappeared when oxygen levels became very low. It is suggested that this is the result of a respiratory or a metabolic pH adjustment, or both.

2. Hypoxic animals developed a characteristic breathing pattern in which discrete periods of lung ventilations alternated with buccal oscillations or respiratory pauses.

3. A pronounced bradycardia was associated with the concomitant decline of inspired and arterial P_{O_2} .

4. Although respiratory rates were greater than normal resting values in the initial stages of post-hypoxia, the pre-exposure breathing pattern was quickly restored. Following recovery from bradycardia (60 min), the breathing rates, arterial blood gases and pH_a returned to normal within 30 min.

INTRODUCTION

In anuran amphibians, the availability of oxygen decreases during prolonged periods of diving or burrowing. Cardiovascular adjustments include bradycardia, vasodilation of skin capillaries, vasoconstriction of vessels supplying the skeletal musculature, and a decrease in pulmocutaneous blood flow (Poczopko, 1959; Leivestad, 1960; Jones & Shelton, 1964; Jones, 1966; Whitford, 1969; Johansen, Lenfant & Hanson, 1970; Shelton, 1970; Armentrout & Rose, 1971; Emilio & Shelton, 1972). The recovery from diving bradycardia in recently emerged anurans appears to be directly linked to increased respiratory movements by way of lung proprioceptors or presso-receptors in the atria or veins (Jones, 1966). Of further interest is the characteristic breathing pattern (unlike the usual alternation of pulmonary and buccal movements) that develops when frogs (*Rana temporaria*) surface into nitrogen atmospheres (Jones & Shelton, 1964); grouped periods of lung ventilations alternate with buccal ventilations or respiratory pauses. More recent observations, of lung volume in *Rana catesbeiana* (Tenney & Tenney, 1970) and buccal pressure in *Bufo marinus* (Macintyre, 1975), suggest that demands for oxygen during periods of hypoxia may be met in part by high-pressure lung inflations.

The anuran's ability to endure prolonged periods of oxygen deficiency may be interdependent on aerobic and anaerobic processes. Although a reduction in metabolism, as found in submerged *Rana esculenta* (Jones, 1972), may serve to economize on oxygen utilization, there is substantial evidence that anurans utilize anaerobic processes when in an atmosphere of nitrogen (Rose & Drotman, 1967; Armentrout & Rose, 1971). Furthermore, blood gas and pH measurements on *Rana ridibunda* have shown that, upon immersion, the blood oxygen pool is rapidly depleted, P_{a,CO_2} increases and a combined respiratory and metabolic acidosis occurs, the latter of which is thought to be caused by a shift to anaerobic metabolism (Emilio, 1974). It is as yet uncertain whether or not an oxygen debt is repaid upon emergence, as factors other than the need to acquire more oxygen (Jones, 1966; Jones, 1967) are thought to contribute, at least in part, to the increased respiration of anurans at this time.

Although several cardiovascular and respiratory responses have been monitored in diving anurans, comparable data on the more terrestrial non-diving species have been collected while animals were anoxic. In view of this, it was our intent to simultaneously record breathing and heart rates, blood gas tensions and pH during periods of progressive hypoxia in the toad, *Bufo marinus*.

MATERIALS AND METHODS

The experiments were performed on specimens of *Bufo marinus* (350–500 g) collected in Mexico and supplied by a commercial dealer (The Mogul Corp., Oshkosh, Wisc., U.S.A.). Upon arrival, the toads were kept in moistened aquaria at room temperature ($23 \pm 2^\circ\text{C}$). Throughout all experiments, animals were unrestrained and moved freely about the experimental enclosure ($32 \times 32 \times 32$ cm).

Arterial and buccal cannulae were made from Clay-Adams polyethylene tubing (P.E. 100; i.d. 0.086 cm, o.d. 0.152 cm) and were chronically implanted after the animals had been anaesthetized in a 1.5 g/l solution of Sandoz MS-222. A cannula was inserted into the buccal cavity through a small puncture in the cartilaginous portion of the upper jaw and anchored tightly against the roof of the mouth by heat flaring the implanted end. The systemic arch was cannulated in an upstream direction by methods reported elsewhere (Macintyre & Toews, 1976) such that blood flow was not noticeably obstructed. Arterial cannulae were kept filled with heparinized amphibian Ringer (250 i.u./ml) to prevent clotting, 0.1 ml/kg being injected immediately following the operation. Experiments began after a 3 h period of recovery from the anaesthetic.

During each experiment, the animal rested on a moistened platform raised (2–3 cm) above the water level in a large aquarium. A Perspex hood enclosed the platform and provided a gas-tight water seal at the bottom. Three small holes in the hood allowed passages for gases and cannulae. After air was passed through the enclosure for 60–90 min, a controlled flow of pure nitrogen gradually replaced the air such that a repeatable 90 min time course of progressive oxygen depletion took place. This was accurate to ± 5 mmHg/30 min interval ($1 \text{ mmHg} \approx 133.322387 \text{ Pa}$). Following nitrogen exposure, the chamber was flushed with a crisp flow of air and normal pre-exposure oxygen levels were restored in 30 min. All gases were water-saturated and temperature-adjusted (22°C) before being put into the chamber.

At 30 min intervals during each experiment, 200 μ l blood samples were taken from arterial cannulae and analysed for pH_a , P_{a,O_2} , P_{a,CO_2} with a Radiometer BMS-3b blood microsystem and PHM-72 display meter. Chamber gases were similarly analysed for P_{I,O_2} . All electrode calibrations and measurements were made at 22 °C.

Heart and breathing rates were simultaneously recorded on a 2-channel Beckman Type RS Dynograph by connecting the respective cannulae to Statham pressure transducers (Type P23 Db).

RESULTS

Time courses of the mean values of blood parameters, heart rates and breathing rates, recorded from 6 animals, are graphically represented in Fig. 1 and given in Table 1 (which also indicates the time-course of inspired oxygen depletion). Time-courses recorded from individuals, for example that shown in Fig. 2, varied widely from the mean. Breathing and heart rates were recorded from five additional animals. These measurements, and those from animals in Table 1, comprise representative tracings for Fig. 3. Ranges for breathing and heart rates refer to the animals in Table 1.

Respiratory frequency

Normal resting *Bufo marinus* showed three well-defined respiratory movements: oscillations, ventilations and inflations. The mechanics of such movements have been previously considered for *Rana catesbeiana* (DeJongh & Gans, 1969) and *Bufo marinus* (Macintyre & Toews, 1976). The oscillations (oscillatory cycles) are buccal movements which serve to flush air into and out of the oropharyngeal cavity and appear as the small pressure deflexions in Fig. 3. Larger positive pressure changes (Fig. 3) are the ventilations (ventilatory cycles) and correspond to gas exchange in the lungs. Ventilations occur irregularly, alternating with periods of buccal oscillations, although in certain instances they may be grouped into discrete periods which are the inflations (inflation cycles). Inflations recorded from normal resting *Bufo marinus* were identical to those from hypoxic animals (Figs. 3c, d). Each inflation cycle consisted of a series of uninterrupted ventilations, and was recorded in the buccal cavity as five or six positive pressure changes that progressively increased in amplitude. The stepwise increase in buccal pressure is coincident with a progressive increase of intrapulmonic pressure (Macintyre & Toews, 1976). This increased lung pressure is then maintained for a variable length of time (Macintyre & Toews, 1976), during which ventilations are absent and oscillations of decreasing amplitude (Fig. 3c) or respiratory pauses (Fig. 3d) are recorded, until buccal pressure returns to a resting level by means of a single ventilation.

In normal atmosphere, there were 21–100 oscillations/min and 0–56 ventilations/min. Compared to these ranges, inflation cycles were always infrequent. As P_{O_2} levels fell below 60 mmHg, oscillation frequency began to decline (Figs. 1, 2) and continued to do so until (at oxygen tensions of 0–10 mmHg) it was only 5% of the normoxic value (Table 1). Upon return to normal atmosphere, oscillation frequency gradually increased to normal resting levels over a period of 90 min – the delay seemingly caused by a preponderance of ventilations during the first 60 min of recovery.

Table 1. Significance table with mean (\pm S.E.) of parameters recorded from six individuals before, during and after hypoxia, as shown in Fig. 1, and P_{iO_2} ranges/30 min interval

(S indicates whether the difference between adjacent means was significant at the 5% level of Student's t test: + indicates significance, - indicates no significance. Temperature = 22 °C.)

	Pre-exposure	Progressive hypoxia						Post-exposure					
		30 min	S	60 min	S	90 min	S	30 min	S	60 min	S	90 min	
Oscillations/min	68.09 \pm 3.16	-	76.91 \pm 4.57 ^b	+	65.27 \pm 4.65 ^b	+	3.57 \pm 3.10 ^a	-	38.71 \pm 6.47 ^a	-	51.86 \pm 12.22 ^a	-	75.80 \pm 14.55 ^b
Ventilations/min	16.27 \pm 1.38	+	22.34 \pm 2.06 ^a	+	42.12 \pm 3.23 ^a	+	34.94 \pm 2.01 ^a	+	25.00 \pm 4.35 ^a	+	38.91 \pm 5.10 ^a	+	22.29 \pm 5.14 ^b
Heart beats/min	39.91 \pm 0.93	+	44.25 \pm 1.47 ^a	+	36.98 \pm 1.62 ^b	+	22.25 \pm 4.42 ^a	+	31.15 \pm 3.42 ^b	+	39.89 \pm 0.59 ^b	-	38.29 \pm 0.52 ^b
pHa	7.77 \pm 0.02	-	7.84 \pm 0.04 ^b	-	7.89 \pm 0.05 ^a	+	7.76 \pm 0.08 ^b	-	7.66 \pm 0.05 ^b	-	7.72 \pm 0.04 ^b	-	7.75 \pm 0.05 ^b
PaO ₂ (mmHg)	95.84 \pm 6.40	+	59.46 \pm 9.63 ^a	+	22.72 \pm 9.42 ^a	+	6.68 \pm 1.74 ^a	+	59.10 \pm 12.43 ^a	-	82.80 \pm 11.70 ^b	-	102.80 \pm 16.2 ^b
PaCO ₂ (mmHg)	6.07 \pm 0.62	-	4.58 \pm 1.37 ^b	-	4.28 \pm 0.84 ^b	-	4.16 \pm 0.95 ^b	-	4.78 \pm 0.89 ^b	-	3.92 \pm 0.94 ^b	-	4.90 \pm 0.89 ^b
P _i O ₂ (mmHg)	150-155		60-70		20-25		0-10		150-155		150-155		150-155

^a Significantly different from normal means.

^b Not significantly different from normal means.

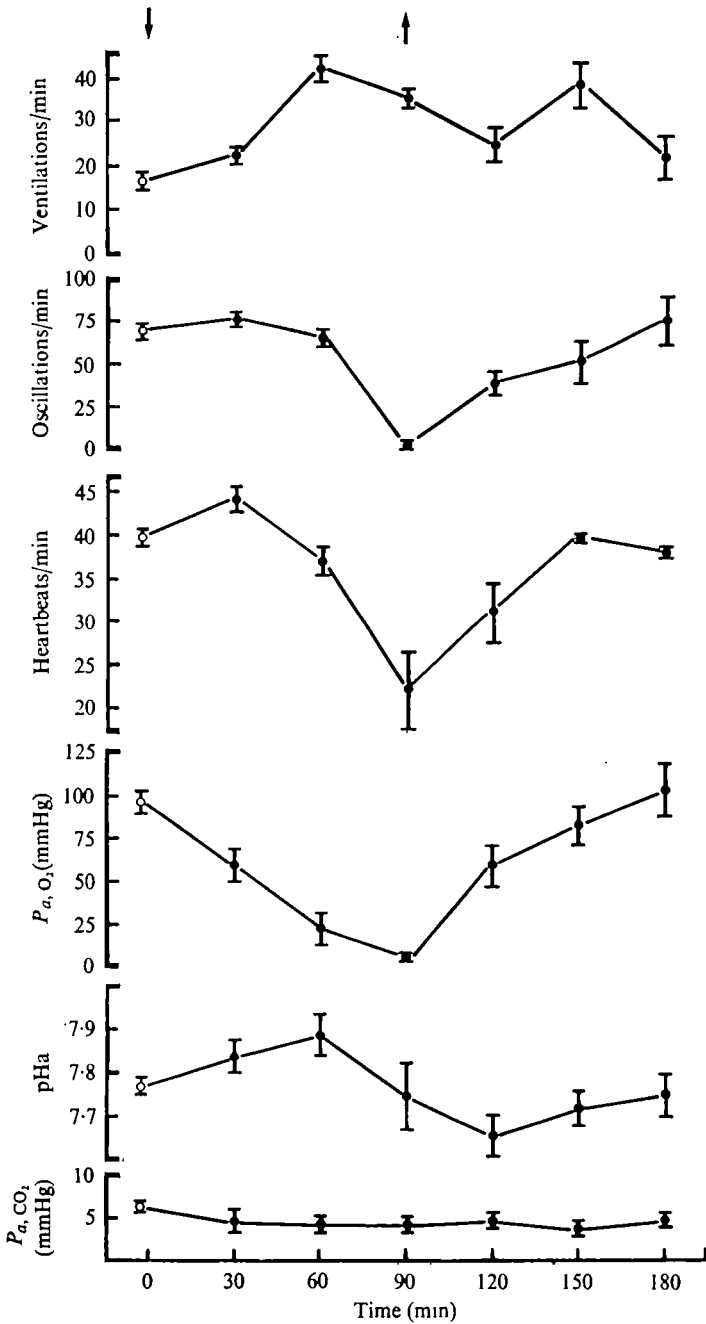


Fig. 1. Simultaneous changes of parameters recorded from 6 *Bufo marinus* during hypoxia and normoxia. Data as in Table 1. Open circles represent mean pre-exposure values. Arrows at top of figure indicate the onset (\downarrow) and termination (\uparrow) of exposure to nitrogen. Zero time indicates the onset of hypoxia. Temperature = 22 °C.

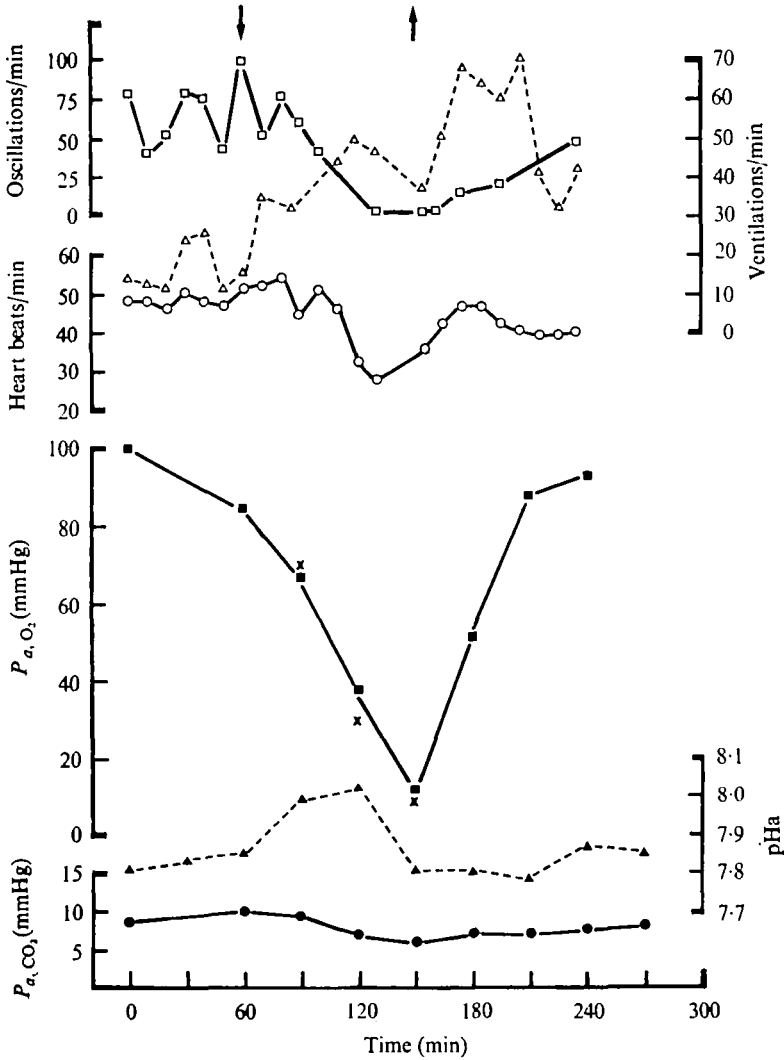


Fig. 2. Simultaneous changes of oscillation frequency (□), ventilation frequency (Δ), heart rate (○), P_{a,O_2} (■), P_{i,O_2} (×), P_{a,CO_2} (●) and pH_a (▲) in a typical experiment on one *Bufo marinus*. Arrows at top of figure indicate the onset (↓) and termination (↑) of exposure to nitrogen. Temperature = 22 °C.

Ventilation frequency progressively increased as P_{O_2} levels fell to 20 mmHg, and then declined as oxygen tensions dropped below this value. Inflation cycles began to occur regularly (2–3 cyc/min) as P_{O_2} tensions fell below 60 mmHg (Fig. 3c). This frequency remained constant until oxygen tensions reached 20–25 mmHg, after which the rate increased to 3–4 cyc/min. At the lower frequency of 2–3 inflations/min, more than half (mean, 24.14; s.e. 2.17) of the total ventilations/min recorded in Table 1 (mean, 42.12; s.e. 3.23) were not directly associated with the inflation cycles. These accounted for those ventilations intermixed with buccal oscillations that preceded each inflation (i.e. Fig. 3c). However, at the higher frequency (3–4 inflations/min) only about a quarter (mean, 8.85; s.e. 3.07) of the total ventilations/min recorded in

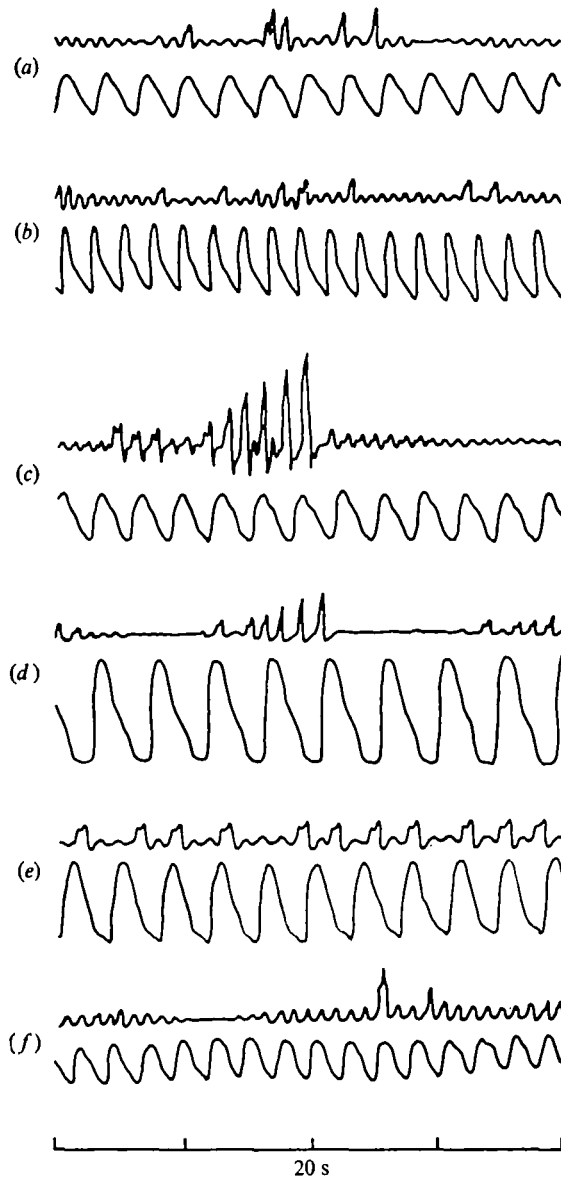


Fig. 3. Typical rate recordings of breathing movements (upper trace) and systemic pressure pulse (lower trace) in free-moving unanaesthetized *Bufo marinus*. (a) Normal pre-exposure rates, (b) P_{I, O_2} of 60-70 mmHg, (c) P_{I, O_2} of 20-25 mmHg, (d) P_{I, O_2} of 0-10 mmHg, (e) 30 min recovery, (f) 90 min recovery. Temperature = 22 °C.

Table 1 (mean, 34.94; S.E. 2.01) were those that preceded each inflation. Therefore, the significant decrease in total ventilations/min between 60 and 90 min exposure (Table 1) was due to a decrease in single ventilations. Accordingly, the number of ventilations forming inflation cycles increased with the increased inflation frequency. Upon return to normal atmosphere, inflation cycles ceased and the breathing pattern became almost that of normal respiration, the difference being an accelerated ventilation

frequency. After 60–90 min recovery (Figs. 1, 2), the high ventilation frequency ceased and normal breathing rates continued thereafter (Fig. 3f).

Heart rate

Although normal pre-exposure heart rates ranged from 26 to 55 beats/min, the low standard error of the mean (mean, 39.91; s.e. 0.93; Table 1) showed that the extreme values were uncommon. In the initial stages of hypoxia, heart rate increased (30 min exposure, Table 1) and then gradually declined as oxygen tensions progressively diminished (Figs. 1, 2 and 3). Bradycardia was quickly relieved following return to normal atmosphere and resting levels were established after 30–60 min recovery.

Arterial pH, P_{O_2} and P_{CO_2}

Normal ranges for arterial pH, P_{O_2} and P_{CO_2} were 7.50–7.89, 49.0–146.3 mmHg and 1.8–11.6 mmHg respectively. All individuals (Table 1) exhibited a significant increase in pH_a over the first 60 min of exposure and a pronounced decrease after 90 min exposure (Figs. 1 and 2). A slight decrease in pH_a occurred upon return to normal atmosphere; resting levels were restored in all animals after 90 min recovery (Figs. 1 and 2). The decline in arterial oxygen tensions paralleled the decrease in P_{I,O_2} and normal pre-exposure levels were re-established after 60 min recovery. P_{a,CO_2} levels were not seen to change significantly during hypoxia (Table 1), but this may have been because measurements were not sensitive enough.

DISCUSSION

Breathing and heart rates recorded from normal resting *Bufo marinus* in this study are similar to those previously reported (Macintyre, 1975; Macintyre & Toews, 1976) and the recorded arterial pH, P_{O_2} and P_{CO_2} values fall within those measurements of Howell *et al* (1970), Rahn & Garey (1973) and Macintyre (1975).

In progressive hypoxia, an increase in ventilation frequency and arterial pH suggested a respiratory induced alkalosis. Measurement difficulties at low partial pressures of CO_2 probably accounted for the apparent lack of a substantial P_{a,CO_2} decrease during hyperventilation (Figs. 1 and 2). In support of this interpretation, Macintyre & Toews (1976) found that *Bufo marinus* relied heavily on pulmonary respiration for the effective elimination of excess CO_2 , and assumed that inflation cycles, similar to those in the present experiments, increased gas transfer per unit time. As oxygen levels became very low (0–10 mmHg), pH_a returned to normal resting levels (Table 1). With inflation cycles being the characteristic breathing pattern at this time, it is questionable whether or not the decline in total ventilations (Table 1) substantially influenced the rate of CO_2 elimination. Consequently, two explanations are advanced. Firstly, the decrease in ventilatory frequency could have been sufficient to cause an increase in P_{a,CO_2} and a respiratory pH adjustment. Alternatively, P_{a,CO_2} could have remained low and a metabolic induced acidosis, as was thought to occur in intensely hypoxic *Rana ridibunda* (Emilio, 1974) could have occurred as a result of anaerobic processes. It is interesting to note that aerial oxygen deficiency in the present work, and diving hypoxia as described by Emilio (1974), had opposite effects (alkalosis and acidosis respectively) on the blood acid-base in anurans.

Our data indicate a connexion between increased heart rate and rapid ventilation in both the early stages of hypoxia and in the recovery from bradycardia. Although breathing rates remained high throughout hypoxia, a slowing of the heart began to occur when P_{O_2} tensions fell below 60 mmHg, implying that the bradycardia was in response to the oxygen lack.

The decline in arterial pH upon return to normoxic conditions (Table 1) could be the result of a re-establishment of skeletal muscle circulation, since vasoconstriction and lactic acid build up in these areas are very common correlates of bradycardia during oxygen stress situations. Breathing and heart rates do not reflect increased oxygen requirements, and normal pre-exposure values were quickly restored following recovery from bradycardia.

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REFERENCES

- ARMENTROUT, D. & ROSE, F. L. (1971). Some physiological responses to anoxia in the great plains toad, *Bufo cognatus*. *Comp. Biochem. Physiol.* **39**, 447-55.
- DEJONGH, H. J. & GANS, C. (1969). On the mechanism of respiration in the bullfrog, *Rana catesbeiana*: a reassessment. *J. Morph.* **127**, 259-90.
- EMILIO, M. G. (1974). Gas exchanges and blood gas concentrations in the frog *Rana ridibunda*. *J. exp. Biol.* **60**, 901-8.
- EMILIO, M. G. & SHELTON, G. (1972). Factors affecting blood flow to the lungs in the amphibian, *Xenopus laevis*. *J. exp. Biol.* **56**, 67-77.
- HOWELL, B. J., BAUMGARDNER, F. W., BONDI, K. & RAHN, H. (1970). Acid-base balance in cold-blooded vertebrates as a function of body temperature. *Am. J. Physiol.* **218**, 600-6.
- JOHANSEN, K., LENFANT, C. & HANSON, D. (1970). Phylogenetic development of pulmonary circulation. *Fedn. Proc.* **29**, 1135.
- JONES, D. R. (1966). Factors affecting the recovery from diving bradycardia in the frog. *J. exp. Biol.* **44**, 397-411.
- JONES, D. R. (1967). Oxygen consumption and heart rate of several species of anuran amphibia during submergence. *Comp. Biochem. Physiol.* **20**, 691-707.
- JONES, D. R. (1972). Anaerobiosis and the oxygen debt in an Anuran Amphibian, *Rana esculenta* (L.). *J. Comp. Physiol.* **77**, 356-81.
- JONES, D. R. & SHELTON, G. (1964). Factors influencing submergence and the heart rate in the frog. *J. exp. Biol.* **41**, 417-31.
- LEIVESTAD, H. (1960). The effect of prolonged submersion on the metabolism and the heart rate in the toad. *Arbok Univ. Bergen (Mat. Nat. Ser.)* **5**, 1-25.
- MACINTYRE, D. H. (1975). Respiratory mechanics and acid-base regulation in *Bufo marinus marinus* (L.) M.Sc. Thesis, Acadia University, Wolfville, Nova Scotia.
- MACINTYRE, D. H. & TOEWS, D. P. (1976). The mechanics of lung ventilation and the effects of hypercapnia on respiration in *Bufo marinus*. *Can. J. Zool.* **54**, 1364-74.
- POCZOPKO, P. (1959). Changes in blood circulation in *Rana esculenta* L. while diving. *Zool. Pol.* **10**, 29-43.
- RAHN, H. & GAREY, W. F. (1973). Arterial CO_2 , O_2 , pH and HCO_3^- values of ectotherms living in the Amazon. *Am. J. Physiol.* **225**, 735-38.
- ROSE, F. L. & DROTMAN, R. B. (1967). Anaerobiosis in a frog, *Rana pipiens*. *J. exp. Zool.* **166**, 427-32.
- SHELTON, G. (1970). The effect of lung ventilation on blood flow to the lungs and body of the amphibian *Xenopus laevis*. *Resp. Physiol.* **9**, 183-196.
- TENNEY, S. M. & TENNEY, J. B. (1970). Quantitative morphology of cold-blooded lungs: Amphibia and Reptilia. *Resp. Physiol.* **9**, 197-215.
- WHITFORD, W. G. (1969). Heart rate and changes in body fluids in aestivating toads from xeric habitats. In *Physiological Systems in Semiarid Environments* (ed. C. C. Hoff and M. C. Riedesal), University of New Mexico Press, Albuquerque, New Mexico.