

RESPIRATION IN THE AFRICAN LUNGFISH *PROTOPTERUS AETHIOPICUS*

II. CONTROL OF BREATHING*

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INTRODUCTION

The process of respiratory gas exchange and gas transport in vertebrates shows adaptive adjustments correlated with aquatic and aerial modes of breathing. Such adjustments are explicit structurally in the design of the gas-exchanging organs and functionally in a number of features such as respiratory properties of blood and ventilation characteristics (Lenfant & Johansen, 1966; Lenfant, Johansen & Grigg, 1966; Steen & Kruyssen, 1964; Rahn, 1966; Hughes, 1966; Robin & Murdaugh, 1966).

Relatively little work has appeared on the phylogenetic development of respiratory control mechanisms (Hughes & Shelton, 1962). The lungfishes hold key positions in this development in as much as they utilize both aquatic and aerial gas exchange with different emphasis on these among species from the three genera available. Respiratory control has earlier been evaluated for the Australian lungfish, *Neoceratodus forsteri*, a predominantly aquatic breather (Johansen, Lenfant & Grigg, 1967). This present paper aims at a similar evaluation for *Protopterus aethiopicus* which depends primarily on aerial breathing for gas exchange. In a previous paper we have described the respiratory properties of blood and normal pattern of gas exchange in *P. aethiopicus* (Lenfant & Johansen, 1968).

MATERIAL, METHODS AND EXPERIMENTAL PROCEDURES

Eighteen specimens of *P. aethiopicus* were used in the present investigation. The fish ranged in weight from 500 g. to 6 kg. Part of the material was transported by air from Lake Victoria, Uganda, to Seattle, Washington, U.S.A., while some parts of the investigation were made on freshly caught material from Lake Victoria, at the Makerere University College in the Department of Physiology in Kampala, Uganda.‡ All fishes were fasting during the periods of observation and experimentation.

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Blood gases and pH were measured by means of a Beckman 160 gas analyser using an oxygen macro-electrode and a Severinghaus-type CO₂ electrode mounted in special micro-cuvettes. Blood pH was measured with a Beckman micro-assembly. Heart rate and blood pressures were measured using Statham pressure transducers connected directly to chronically indwelling catheters. Blood velocity was measured using an ultrasonic Doppler-shift blood-flow meter after Franklin, Watson, Pierson & van Citters (1966).

Branchial breathing rate was measured by recording of pressure changes behind an operculum via a chronically implanted catheter. The same catheter allowed sampling of exhaled water and injection of drugs to the immediate vicinity of the gills. The frequency of air breathing was apparent as distortions from the normal pattern in the blood-pressure record, but could also be counted by visual observation. All recordings were made on an Offner Dynograph recorder.

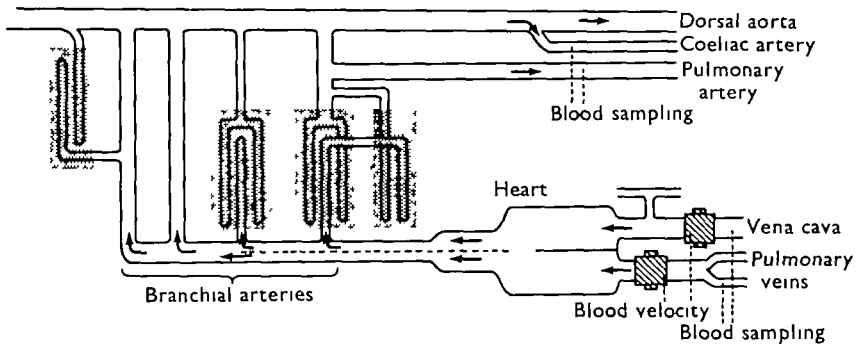


Fig. 1. Schematic drawing of the central circulation in *Protopterus*. Sites of catheter cannulation and placement of blood-velocity transducers are marked in.

Blood samples were obtained from a pulmonary artery, a pulmonary vein, a systemic artery and the vena cava. In some experiments additional blood samples from an afferent branchial artery and pulmonary air samples were acquired. All samples were obtained via chronic cannulations which proved patent for periods extending up to 12 days. Details of anaesthesia, surgical techniques and methods of cannulation and placement of blood-velocity transducers are found in earlier papers (Lenfant & Johanson, 1968; Johansen, Lenfant & Hanson, 1968). Figure 1 shows a schematical arrangement of the central circulation in *Protopterus* with sites of cannulation and blood-velocity transducer placements. Following recovery from anaesthesia several hours were allowed to pass before any sampling or experimental procedures were carried out.

Measurements related to breathing and circulation were recorded and correlated with sampling of blood and lung gases. Studies were made on resting fish in aerated water and during air exposure, as well as during hypoxic, hyperoxic and hypercarbic conditions in the ambient water or gas phase. Additional experiments included pharmacological stimulation of breathing.

RESULTS

Undisturbed breathing in aerated water

It was demonstrated in an earlier paper (Lenfant & Johansen, 1968) that *P. aethiopicus* has a bimodal gas exchange when living in water. Oxygen absorption is mainly carried out by the lungs, while CO₂ is chiefly eliminated through gills and skin.

A marked irregularity was apparent in the rhythms of both branchial and aerial breathing when the fish rested in aerated water. Most commonly the intervals between air breaths ranged from 2 to 6 min., but intervals as short as 30 sec. and longer than

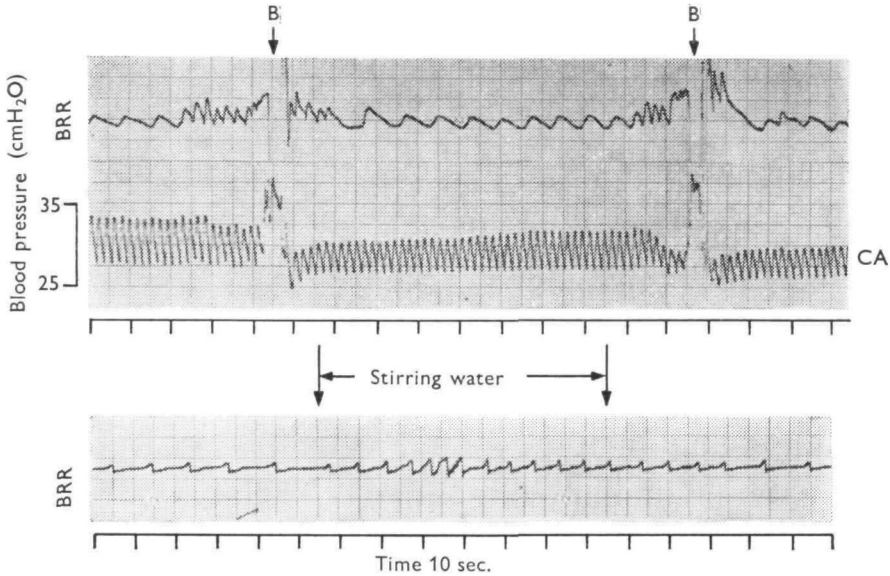


Fig. 2. Top tracing: coupling of branchial (BRR) and pulmonary (B) breathing in undisturbed *Protopterus*. Lower tracing: increase in rate of branchial breathing depending on agitation of the water.

1 hr. also occurred. Branchial breathing was equally labile both in frequency and vigour. In general the ventilatory movements were so shallow that water ventilation was too small to be measured with accuracy. The frequency of branchial pumping was normally higher than the frequency of air breathing. At times long periods prevailed when branchial pumping ceased altogether.

It became apparent that the two modes of breathing were normally interrelated in their pattern. It was particularly noteworthy that an increased rate and depth of branchial water pumping commonly preceded an air breath. The intensified branchial breathing started 20–60 sec. prior to the air breath (Fig. 2).

It was known from previous work on the South American lungfish, *Lepidosiren paradoxa* (Johansen & Lenfant, 1967), that an extremely marked lability in the branchial breathing rate could depend on mechanical agitation of the water. Figure 2 (lower tracing) demonstrates a similar dependence of branchial breathing rate on stirring of the water for *P. aethiopicus*.

Breathing behaviour during air exposure

The African lungfish is known to aestivate out of water for several months during periods of drought. The ability of the fish to withstand air exposure as measured by the pattern of gas exchange has also been studied experimentally (Lenfant & Johansen, 1968).

Experimental air exposure by draining the tank water elicited a conspicuous increase in the frequency of air breathing (Fig. 3).

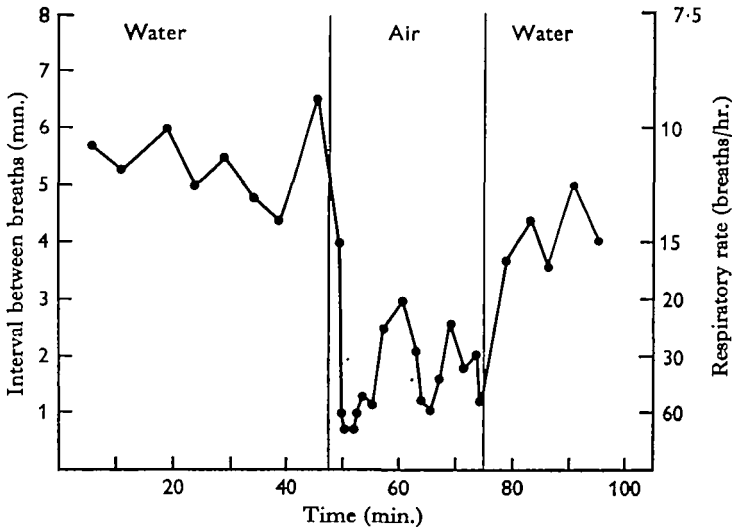


Fig. 3. Change in frequency of airbreathing as a result of air exposure in *Protopterus*.

The dramatic increase in the rate of air breathing appeared to be elicited by the physical act of the exposure to air. The onset of the response was too prompt to allow time for internal changes to occur. The powerful stimulus of air exposure was also revealed in fish recovering from anaesthesia in water. If the head and mouth of such fish were air-exposed by gentle lifting, an air-breath was taken long before voluntary motor movements and before any spontaneous attempt to surface for an air breath. Air breathing could also be evoked by air exposure during recovery from anaesthesia before a withdrawal response to painful stimuli, like pricking with a needle, was apparent.

Blood gas tensions during undisturbed breathing and during air exposure

It was considered essential to correlate the undisturbed breathing pattern in resting non-anaesthetized fish in water and also the breathing pattern in air, with gas tensions in the blood and in the pulmonary air.

Figure 4 A and B show two plots relating pulmonary gas and blood gas tensions to time during undisturbed breathing in aerated water. The sampling was started right after an air breath and was repeated at close intervals till the next breath. The vertical arrows indicate the time of other air breaths.

It is apparent that the P_{O_2} drops at about the same rate in all cycles. Note, however,

the great variability in the duration of the breathing cycles and the consequent variability in the arterial P_{O_2} at which another breath is taken. These data are suggestive that the absolute level of arterial O_2 tension does not represent a strong triggering stimulus to aerial breathing.

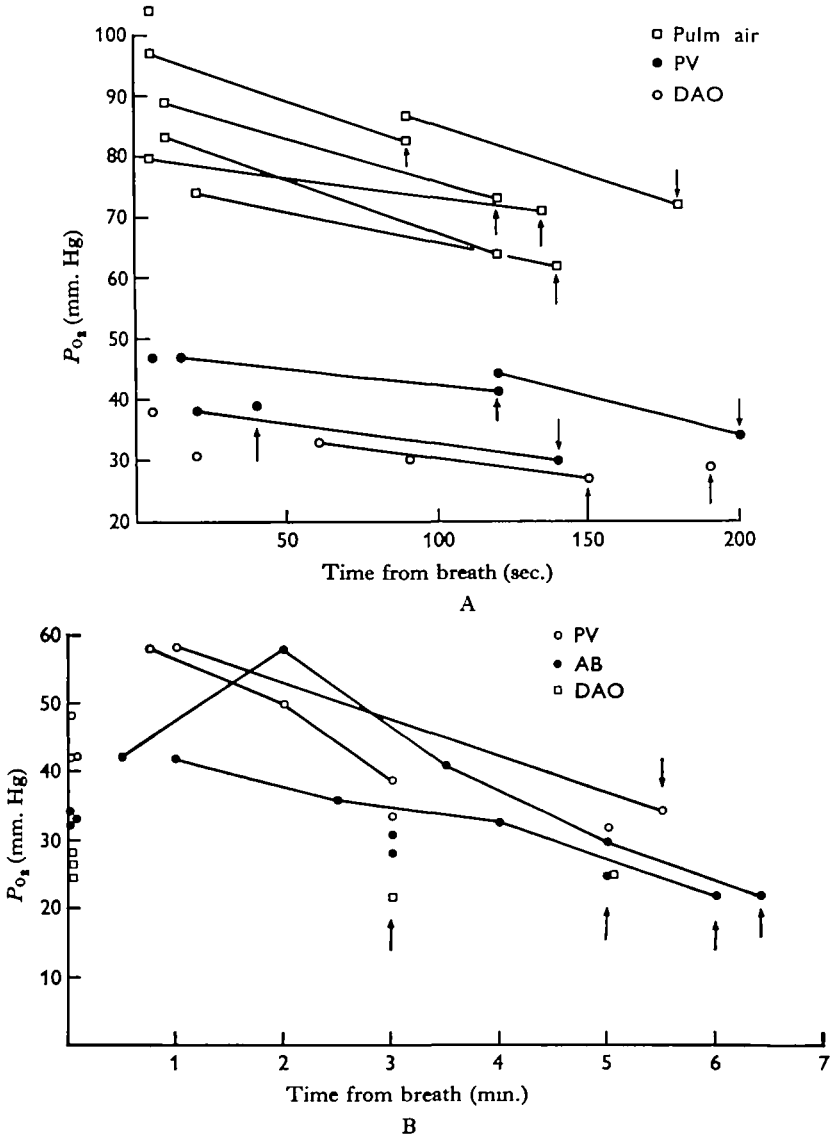


Fig. 4. Relationship of O_2 tension in pulmonary gas or blood during the interval between consecutive air breaths in *Protopterus*. The vertical arrows indicate time of another breath. A and B represent analyses from two different individuals. AB, afferent bronchial; DAO, dorsal aorta; PV, pulmonary vein.

Figure 5 A and B correlate pH in arterial blood with arterial oxygen tension for a series of breath cycles of variable duration.

The time course of changes in the blood pH between breaths presents a variable

picture but suggests no tendency for the occurrence of a new breath to be correlated with a reduction in arterial pH.

These results indicate that air exposure was associated with a general increase in the level of O_2 tension and CO_2 tension in all vessels sampled (Fig. 6).

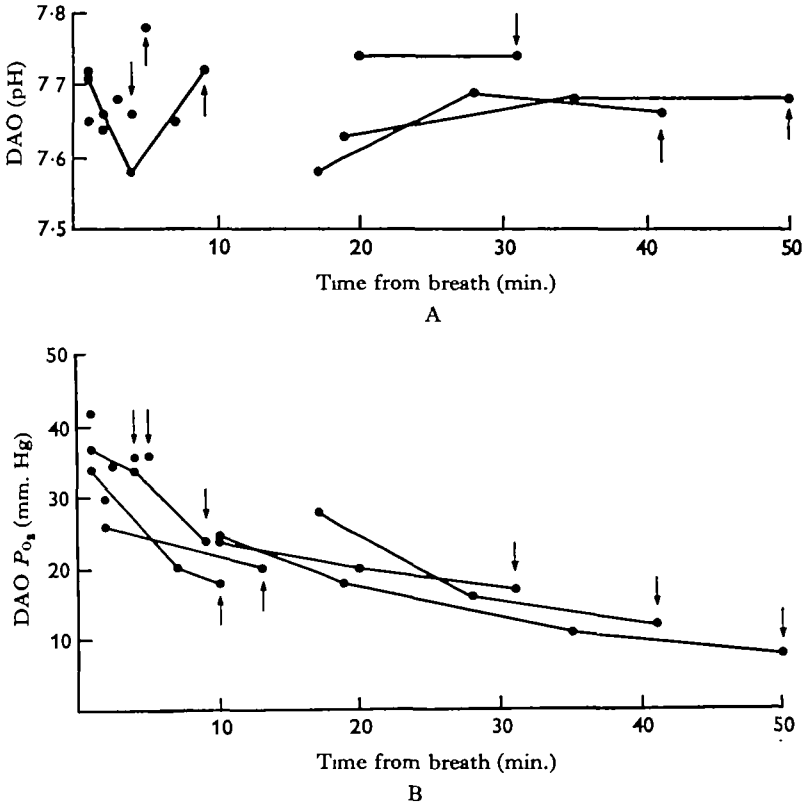


Fig. 5. Time course of arterial pH (A) and systemic arterial O_2 tension (B) during consecutive intervals between air breaths in *Protopterus*. The vertical arrows mark the time of another breath. DAO, dorsal aorta.

The time required for blood sampling limits the possibility of correlating air breathing closely with changes in blood gas composition. A fish may typically take an air breath within a few seconds after air exposure, with successive breaths following at close intervals commonly up to 20 times more frequently than prior to air exposure. Return to water after prolonged air exposure was associated with an increased vigour and frequently of branchial breathing while the rate of air breathing rapidly returned to its value prior to air exposure.

Breathing response to hypoxic water

Lungfishes were exposed to hypoxic water by bubbling nitrogen in the aquaria while monitoring the decline in water P_{O_2} , or by transferring fish to tanks with O_2 deficient water of known concentration. During these procedures the branchial respiratory rate and the frequency of air breathing were recorded and correlated with

changes in blood gas tensions and pH. Exposure to hypoxic water never evoked any compensatory breathing response, suggestive that the P_{O_2} of ambient water plays a normal role in the control of breathing.

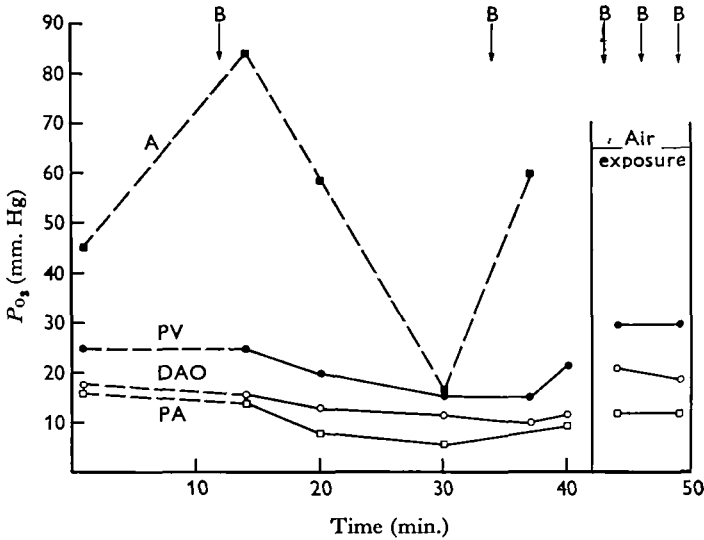


Fig. 6. Time course of pulmonary O_2 tension and blood O_2 tension before and during a period of air exposure in *Protopterus*. The arrows indicate air breaths. A, air in lung; DAO, dorsal aorta; PA, pulmonary artery; PV, pulmonary vein.

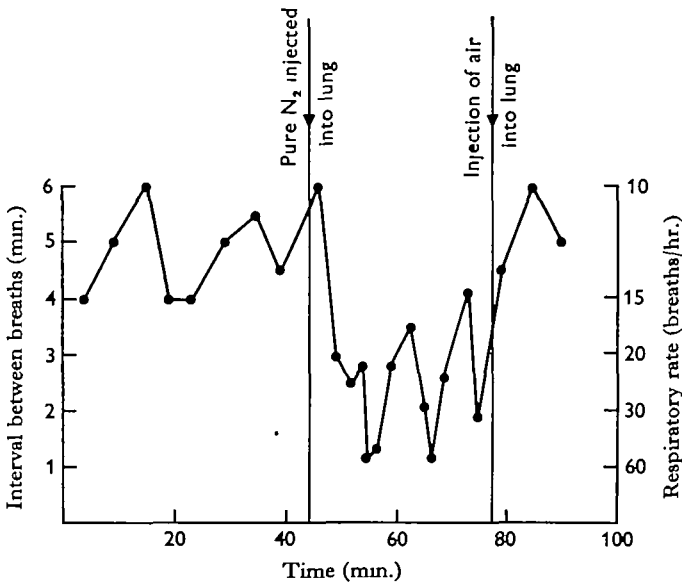


Fig. 7. Change in frequency of air breathing when nitrogen was injected into the lung of *Protopterus*.

Breathing response to hypoxic gas in the lung

Fishes were made to surface into an hypoxic atmosphere, or hypoxic gas mixtures were carefully injected directly into the lungs via chronically indwelling catheters.

Both procedures elicited an increased frequency of air breathing. Figure 7 illustrates a typical response. It is noteworthy that the accelerated breathing following injection of hypoxic gas into the lung was elicited at arterial P_{O_2} levels higher than most of those occurring in the late phase of normal spontaneous breathing cycles.

Breathing response to increased ambient CO_2

When 5% CO_2 in air was bubbled carefully through the aquarium a consistent reduction in the rate of branchial water pumping occurred. Sometimes the response was evoked promptly, at other times more slowly. Correlated with the reduced

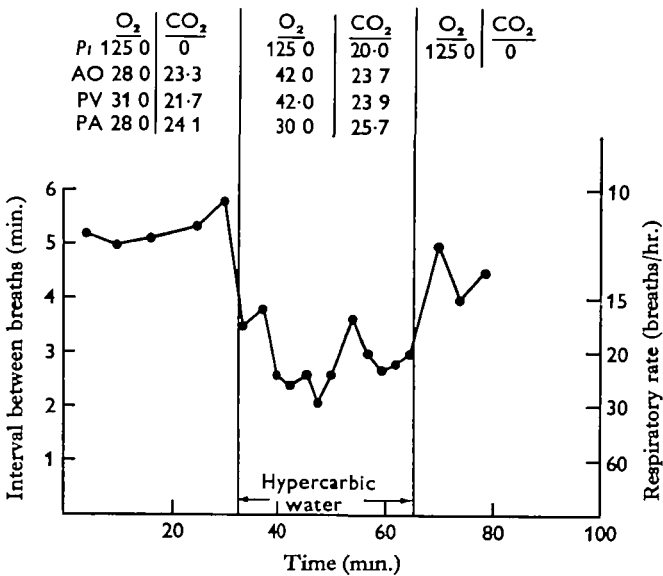


Fig. 8. Change in frequency of air breathing in *Protopterus* during bubbling of 5% CO_2 in air into the aquarium. At the top of the figure is listed gas tensions in ambient water (P_1), dorsal aortic blood (AO), pulmonary venous blood (PV) and pulmonary arterial blood (PA).

branchial breathing was an increase in the frequency of air breathing. The data disclosed that no significant changes occurred in the CO_2 tension of arterial blood, while the O_2 tension commonly showed an increase in all vessels sampled (Fig. 8).

Figure 9 provides additional information on blood pH changes and branchial breathing rate while CO_2 was bubbled through the water. Again a stimulation of air breathing is apparent while branchial breathing is depressed. The accelerated air breathing does not correlate with a reduced pH, but such a reduction occurs in the recovery period.

Figure 10 correlates branchial breathing rate and frequency of air breathing with blood velocity in the pulmonary artery. The top tracing was recorded when the fish rested in aerated water with slow bubbling of CO_2 through the water. The water pH had been reduced by one unit during the CO_2 bubbling. The branchial breathing rate was two per minute before bubbling, but ceased altogether in response to the increase of CO_2 in the water. The rate of air breathing increased concurrently, reducing the interval between breaths from more than 4 min. to about 1 min. The pulmonary blood

flow was reduced, but it is not known whether the reduced blood flow resulted from a general reduction in cardiac output or from a redistribution of the regional blood flow.

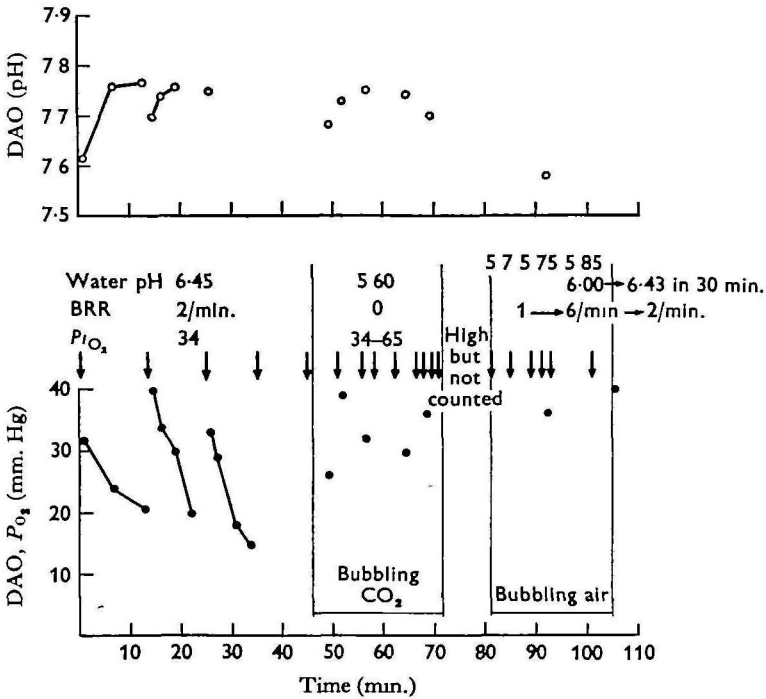


Fig. 9. Time course of arterial pH and P_{O_2} in *Protopterus* before and after bubbling of 5% CO_2 in air into the water. Branchial breathing rate (BRR) and rate of air breathing (arrows) are marked in. DAO, dorsal aorta.

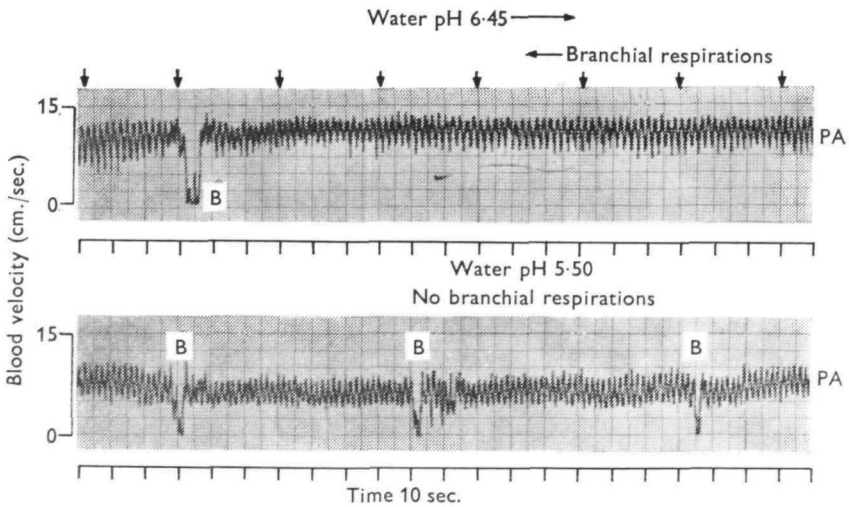


Fig. 10. Correlation of branchial breathing rate (small arrows), air breathing rate (B) and pulmonary blood velocity in *Protopterus* before and during bubbling of 5% CO_2 in air through the water.

Pharmacological stimulation of breathing

Studies of mammalian chemoreceptors have shown that chemicals known to stimulate sympathetic ganglion cells by a nicotine-like action also exert a stimulatory effect on peripheral chemoreceptor cells involved in respiratory control. There is no previous data to indicate whether chemoreceptors of lower vertebrates possess similar sensory qualities, but it was decided to test the effect of nicotine on the breathing pattern in *Protopterus*. These experiments revealed that nicotine, in the form of Nicetamide, elicited an increase in both aerial and branchial breathing. The response occurred whether the drug was administered intravenously or introduced beneath the

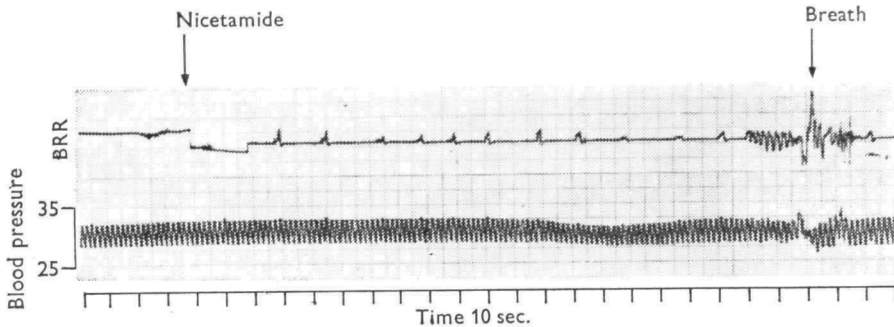


Fig. 11. Change in branchial breathing rate (BRR) when Nicetamide was introduced beneath an operculum in *Protopterus*. The lower tracing represents systemic arterial blood pressure. Prior to application of the drug, branchial breathing rate was shallow and infrequent (less than 1 per min.).

opercula in the water bathing the gills. In addition, the drug had a hypotensive effect. When administered intravenously the fall in arterial blood pressure more or less coincided with the appearance of the branchial breathing response. However, if the drug was introduced beneath the opercula in the water close to the gills, the accelerated branchial breathing appeared long before the blood-pressure drop (Fig. 11). If chemoreceptors in respiratory control have homologous response mechanisms in all vertebrates the results indicate that such receptors do exist in *Protopterus*. The location of these receptors in *Protopterus* is not known, but it merits attention that the response is evoked sooner from administration of the drug in the water close to the gills than from giving it intravenously.

Histamine is not known to interfere directly with chemoreceptor stimulation. The drug, however, exerted a marked stimulatory effect on air breathing in *Protopterus*, when administered either intravascularly or externally in the water. Histamine had the additional effect of producing restlessness and coughing. The response may well be related to a general constriction of vascular smooth muscle in the gills, or to a constrictive effect on the profusely distributed smooth musculature in the lung parenchyma of *Protopterus* (Johansen & Reite, 1967).

Coupling of respiratory and circulatory events

It was consistently observed that spontaneous breathing caused marked changes in heart rate, blood pressure and blood flow. Figure 12 shows blood velocity tracings from

the vena cava (top) and the pulmonary artery (bottom) in conjunction with a spontaneous air breath. Note the marked increase in blood flow associated with the breath. The bottom tracing from the pulmonary artery shows that the flow increase could occur in anticipation of the actual breathing act. The blood flow increase in the pulmonary artery occurred largely in the form of an increased diastolic flow component while the peak systolic velocity remained essentially unaltered. If long intervals prevailed between air breaths there was often a marked slowing of the heart rate with reduced flow towards the end of a breathing cycle.

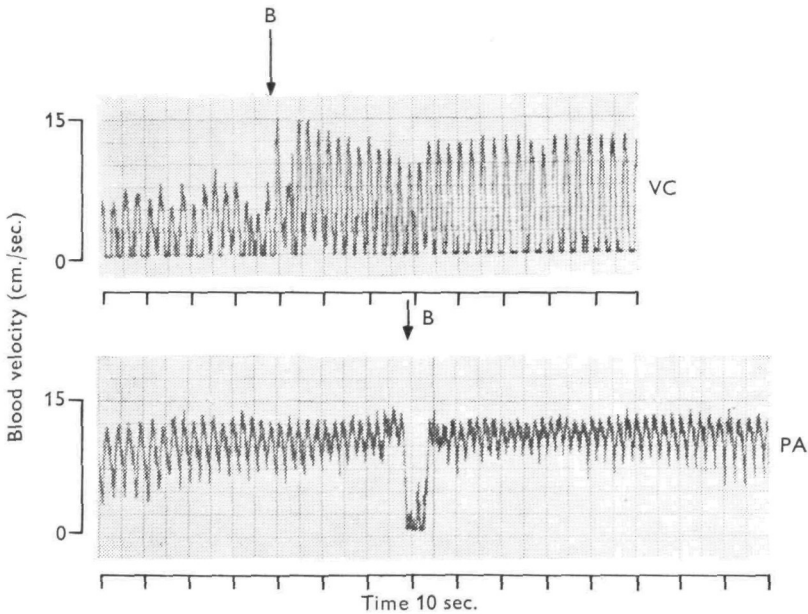


Fig. 12. Blood-velocity tracings from the vena cava and pulmonary artery in two different *Protopterus*. Both records demonstrate an increase in blood velocity associated with an air breath. B.

DISCUSSION

In an earlier paper it was shown that the African lungfish employs a bimodal gas exchange. The fish depends almost exclusively on its lungs for O_2 absorption, while the coarse, atrophied gills and skin remain responsible for the major part of CO_2 elimination (Lenfant & Johansen, 1968). It is to be expected that such bimodal gas exchange is reflected in the respiratory control system of the fish. On this assumption it becomes imperative to distinguish between external stimuli in the water and in the atmosphere (gas phase) surrounding the fish, when attempting to evaluate its respiratory control.

The remarkable variability in both branchial and aerial breathing rates was striking. Without apparent changes in external conditions the time interval between spontaneous air breaths could vary from 30 sec. to more than 1 hr. Figures 4 and 5 demonstrate that the variability of intervals between air breaths shows no correlation with the arterial O_2 tension at which another breath is taken. The variability was large enough to suggest that the actual level of arterial blood gas tensions or intrapulmonary

gas tensions played no prominent role in setting the breathing rhythm. It appears that the fish relies more on tolerance to large internal variations in gas tensions than on regulations against them.

The most powerful stimulus to increased air breathing in *Protopterus* was air exposure itself. The persistence of this response in fish recovering from anaesthesia while still unresponsive to tactile or painful stimuli served to emphasize that this apparent reflex has a marked degree of structural and functional autonomy from higher integrative activities of the nervous system. All indications are that the sensory input resulting in this response is related to the removal of the head from the water, and possibly related to the sudden influence of net gravitational forces acting on the fish in air.

A remote parallel to this response in the lungfish is present in the mammalian foetus when emerging from aquatic foetal life to a terrestrial existence based on air breathing. Controlled experiments have indicated that the crucial initiation of air breathing in the newborn mammal cannot be directly triggered by internal chemoreceptor stimulation but may be related to the stimulus of exposure to air.

The large literature on breathing responses to changes in external gas composition in teleost fishes is practically without exception in attributing a marked stimulatory effect on ventilation from both hypoxic water and CO₂-rich water (Hughes & Shelton, 1962). *Protopterus* differs from all other fishes studied by being unresponsive to deoxygenated water. Since the gills in the adult fish are of very little consequence in oxygen absorption (Lenfant & Johansen, 1968), a change in O₂ tension of the ambient water will only slowly become manifest internally. A reduction in gill surface area as well as increased diffusion resistance across the gas-exchanging surfaces of the gills are common in air-breathing fishes with accessory or alternative air-breathing organs. In essence there exists no effective, functional linkage between internally located chemoreceptors responsive to hypoxic stimuli and the external water.

After this paper was ready for submission an investigation on lung and gill ventilation in the African lungfish by Jesse, Shub & Fishman (1968) appeared. Their results differ in important respects from ours. The fishes used by Jesse *et al.* responded to decreased oxygen tensions in the water by augmentation of both branchial and pulmonary breathing. The juvenile status of their fish weighing on the average 65 g. against an average of 3 kg. for our fish may explain some of the differences in the results. Juvenile specimens of lungfish are known to depend more on gill breathing than adults. The survival of their fishes for several days without access to air substantiates this contention, as numerous reports from studies of adult fish claim that they succumb rapidly if prevented access to the atmosphere.

Further comparison with the work by Jesse *et al.* is also limited by their experimental procedures which do not allow a distinction between external stimuli in the water and in the ambient gas phase.

Neoceratodus, the Australian lungfish, lives in water having a generally high but somewhat variable O₂ level in water. This lungfish has well-developed gills and utilizes them effectively in O₂ absorption (Lenfant *et al.* 1966). *Neoceratodus* promptly responded to deoxygenated water by intensified breathing (Johansen *et al.* 1967).

For *Protopterus* conditions are markedly different. Its habitat is much less conducive to oxygen absorption and the fish is structurally better adapted for oxygen absorption

from the atmosphere than from the water. When two sources of oxygen can be used simple consideration of energetics suggests that the most accessible source and the method of breathing requiring the least amount of energy be utilized.

The large spontaneous variability in the normal undisturbed breathing rhythm of *Protopterus*, apparently uncorrelated with internal gas tensions and pH, seems to preclude any great importance in the level of these parameters for the regulation of breathing. Yet it was quite evident that inspiration or injection of hypoxic gas mixtures into the lung prompted an increased frequency of air breathing. It appears possible that the receptors triggering air breathing are exposed to stimuli other than the P_{O_2} level in the arterial blood (Figs. 4, 5 and 9).

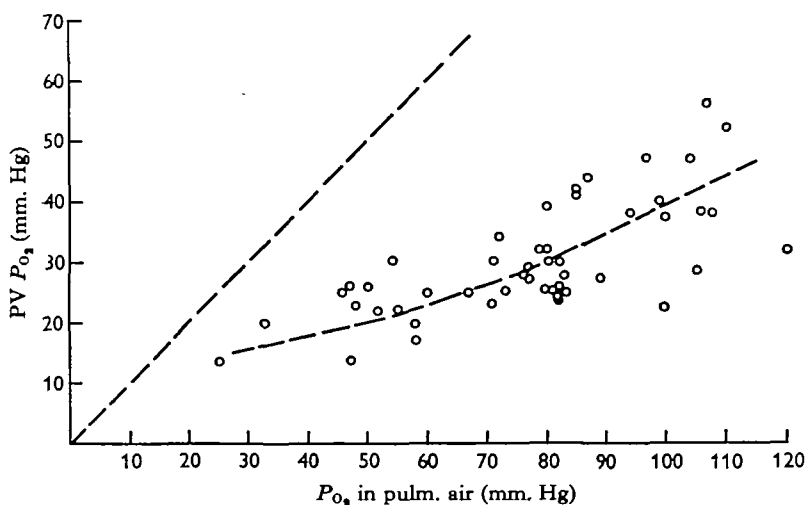


Fig. 13. Composite plot of the relationship between O_2 tension in pulmonary gas and in the pulmonary venous blood (PV).

It seems appropriate to comment briefly on the low sensitivity of chemoreceptors responding to arterial conditions in *Protopterus* in relation to the structural development of the lung as a gas exchanger. In higher air breathing vertebrates there are normally small gradients in O_2 and CO_2 tensions between the alveolar gas and the arterial blood. In *Protopterus* large gradients exist between pulmonary gas and blood leaving the lung and even steeper gradients between the pulmonary gas and the systemic arterial blood. The long intervals between breaths result in a wide range in composition of pulmonary gas between breaths. The large gradients between gas and blood will tend to minimize the changes in the blood against the changes in pulmonary gas. Figure 13 demonstrates an average plot of the gradients in O_2 tension between pulmonary gas and pulmonary venous blood in *Protopterus*. While the pulmonary gas ranges from 120 to 30 mm. Hg in oxygen tension the pulmonary venous blood has a corresponding range between 55 and 17 mm. Hg. A smaller gradient between gas and blood would have resulted in larger cyclic variations in the latter. The large gradients may result from a crude structure of the lung with large diffusion resistance and long diffusion paths or they can result from an uneven matching of blood and gas in the lung.

Fish living in temperate, well aerated waters have very low values of internal CO₂ tensions because of the relative ease by which CO₂ is eliminated in aquatic gas exchange. It has been argued (Hughes & Shelton, 1962) that the only logical advantage a fish can expect from a sensitivity to CO₂ tensions is as a measure of oxygen deficiency in arterial blood. These authors saw, however, a conflict in their statement, since increased ventilation of fish in CO₂-rich waters seems inappropriate in as much as CO₂ then will accumulate rather than being eliminated from the fish.

In *Protopterus* the gills are much more effective than the lungs for CO₂ elimination while the fish stays in water (Lenfant & Johansen, 1968). In spite of their crude and degenerated form the gills hence constitute a functional linkage for CO₂ between the internal environment and the ambient water. On that basis it seems a significant finding that increased CO₂ tension in the ambient water evokes a breathing response, whereas hypoxic water has no effect on the breathing pattern. It is noteworthy that the depressive effect on gill breathing elicited by bubbling 5% CO₂ in the water was evoked before the arterial CO₂ tensions had changed appreciably (Fig. 7). *Symbranchus marmoratus*, a facultative air-breathing fish inhabiting tropical freshwater swamps in South America, and the Australian lungfish, show a similar inhibition of branchial breathing in CO₂-rich water (Johansen, 1966; Johansen *et al.* 1967). The inhibition was not removed in spite of increasing internal CO₂ tensions until the ambient CO₂ tension was again lowered. When this was done, however, a high frequency of forceful branchial movements was established.

Hypothetically the sensory mechanism for CO₂ will distinguish between the ambient and internal CO₂ tensions and drive the effectors to keep the internal CO₂ tensions as low as possible. However, it is obvious that when increased external CO₂ exerts a depression on branchial breathing, internal hypoxia will ensue unless other corrective measures are mobilized. On that account it is of interest that the response has only been described for fish capable of aerial breathing. Typically in the Australian lungfish, *Neoceratodus* (Johansen *et al.* 1967), as well as shown presently for *Protopterus*, depression of branchial breathing will stimulate aerial breathing. It should be noted that our experiments do not exclude that a different response might occur to lower CO₂ concentrations (Willmer, 1934; Jesse *et al.* 1968).

Fishes in general show a close correlation between respiratory and circulatory events (Satchell, 1960). A functional coupling between ventilation and perfusion also appears to exist in lungfishes (Johansen *et al.* 1968). The evidence suggests that such coupling may be important for the efficiency of the matching process between blood and the respiratory media. It has been shown for both *Protopterus* and *Neoceratodus* that lung inflation will elicit a tachycardia and conversely lung deflation will produce a bradycardia. These responses were clearly dependent upon the prevailing heart rate and did not occur if the rate was high prior to lung inflation. In addition to the heart-rate change there was typically an increase in total cardiac output and sometimes in the ratio of pulmonary flow to total flow. In all spontaneously occurring events showing a coupling of respiration and circulation, it was a change in respiration that instigated a circulatory change.

SUMMARY

1. Factors controlling aerial and aquatic breathing have been studied in the intact, free-swimming African lungfish, *Protopterus aethiopicus*. Frequencies of aerial and branchial breathing were correlated with gas tensions in the lung and in blood from pulmonary and systemic arteries and veins.
2. Studies were made on fish resting in aerated water, during exposure to air and during hypoxic and hypercarbic conditions in the environment.
3. Both branchial and pulmonary breathing were irregular during rest in aerated water. The rate of branchial breathing normally exceeded that of air breathing. An increased rate and vigour of branchial breathing commonly preceded an air breath. There was no indication that a new air breath was related to the values of arterial P_{O_2} and pH.
4. Air exposure elicited a marked and immediate increase in the rate of air breathing. Hypoxic water never evoked any compensatory breathing responses while breathing from a hypoxic gas mixture quickly increased the rate of air breathing. Bubbling 5% CO_2 in air into the aquarium caused a reduction in branchial breathing while the rate of air breathing increased.
5. Nicotine injected intravenously or in the water close to the gills elicited an increase in both aerial and branchial breathing.
6. Respiratory and circulatory events were correlated during undisturbed breathing and during artificial lung inflation. Increased cardiac output and a shift in regional blood flow to a higher pulmonary flow occurred with air breaths.

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