

## RESPIRATION IN THE AFRICAN LUNGFISH *PROTOPTERUS AETHIOPICUS*

### I. RESPIRATORY PROPERTIES OF BLOOD AND NORMAL PATTERNS OF BREATHING AND GAS EXCHANGE\*

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#### INTRODUCTION

Members of the three existing genera of lungfishes represent different stages in the transition from aquatic to aerial breathing. *Neoceratodus*, the Australian lungfish, is primarily aquatic and utilizes gill breathing, while its lung offers an accessory means for O<sub>2</sub> absorption (Lenfant, Johansen & Grigg, 1966; Johansen, Lenfant & Grigg, 1967). Conversely, the South American lungfish, *Lepidosiren paradoxa*, is a typical air breather and the much reduced gills are of little or no importance for O<sub>2</sub> absorption but may have some functional significance for elimination of CO<sub>2</sub> (Johansen & Lenfant, 1967). The African lungfish, *Protopterus*, resemble *L. paradoxa* in being primarily an air breather. The purpose of the present paper is to evaluate the status of *P. aethiopicus* in the transition from aquatic to aerial breathing by analysis of the respiratory properties of blood and of the normal pattern of breathing and gas exchange.

#### MATERIAL

Ten large specimens of *Protopterus* were transported by aircraft from Lake Victoria, Uganda, East Africa to Seattle, Washington. The fishes arrived in good condition and were kept in aquaria with water temperatures of about 20° C. for several days before the experiments were started. In addition, experiments were performed in the Department of Physiology at the Makerere University College in Kampala, Uganda, on material freshly caught from Lake Victoria.‡ This work not only permitted verification of the data obtained in the U.S.A., but made it possible to study additional problems precluded in the Seattle experiments because of the limited number of fish. All the fishes were fasting during the periods of study and observation.

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## METHODS AND EXPERIMENTAL PROCEDURES

Blood gases and pH were measured by means of a Beckman 160 gas analyser using an oxygen macro-electrode and a Severinghaus-type CO<sub>2</sub> electrode mounted in special micro-cuvettes. Blood pH was measured on a Beckman micro-assembly. Gas contents were measured using gas chromatography according to the method described by Lenfant & Aucutt (1967). The *in vitro* work on the respiratory properties of blood was done according to descriptions by Lenfant & Johansen (1965).

The methods used to measure gas exchange involved a closed temperature-controlled chamber. This chamber, partially filled with water, was used to measure gas exchange in both the water and air phase. Fish placed in the chamber continued their normal aerial and branchial breathing pattern. When without water, the chamber was used to measure gas exchange during air exposure.

In preparation for an experiment the fish was anaesthetized by immersion in MS 222 (Tricaine methane sulphonate, Sandoz). An incision was made along the ventral mid-line slightly posterior to the heart and by careful dissection the large vena cava, the common pulmonary vein, the left pulmonary artery and the coeliac artery were all dissected free. All four vessels were cannulated in the direction of the heart using polyethylene catheters filled with heparinized saline. The catheters entered the main vessels through small side branches or alternatively through holes made in the vessel walls. All cannulations were non-obstructive, allowing continued free passage of blood in the vessels to be recorded or sampled from. Afferent branchial blood samples and blood pressures were obtained by cannulating the 1st or 2nd afferent branchial artery (aortic arches 3 or 4). It is recalled that in *Protopterus* these are direct thoroughfare arteries having no connexion with respiratory exchange circulation. Following cannulation all catheters were anchored in place and the incision was carefully closed. The indwelling catheters were kept in place for variable periods of time extending to more than 10 days. Most fishes were studied over periods lasting several days.

A catheter was also inserted behind an operculum close to the posterior gill arches to permit sampling of expired water, and another catheter was inserted via the mouth through the pneumatic sphincter in the pharyngeal floor into the anterior portion of the lungs to allow sampling of lung gas. Both of these catheters were carefully anchored by means of sutures, taking care not to cause undue mechanical interference with normal branchial breathing movements and the passage of air into and out of the lung.

Following the surgical procedures the fish were permitted to recover from anaesthesia in shallow, well-oxygenated water. This recovery was usually quick and was often aided by artificial inflations via the lung catheter. The fish were allowed to rest in water for several hours before any sampling or recording was done.

Blood for *in vitro* work was obtained early after recovery or from special fish used as blood donors only.

## RESULTS

*Respiratory properties of blood*

The following mean values were determined for three fish used as blood donors: Haematocrit, 25 % Hb concentration, 6.2 g %; mean corpuscular haemoglobin concentration, 24.8 %. The mean O<sub>2</sub> capacity was 6.8 vol %. Figure 1 shows a set of

oxyhaemoglobin dissociation curves. The curves are typically sigmoid and attest to a relatively high affinity of haemoglobin for O<sub>2</sub>. Figure 2A compares the oxyhaemoglobin dissociation in *Protopterus* with earlier published curves for *Neoceratodus* and *Lepidosiren*. At similar P<sub>CO<sub>2</sub></sub>, the O<sub>2</sub> affinity is seen to be slightly higher for *Protopterus* and *Lepidosiren* than for *Neoceratodus*. Figure 2B allows comparison with earlier

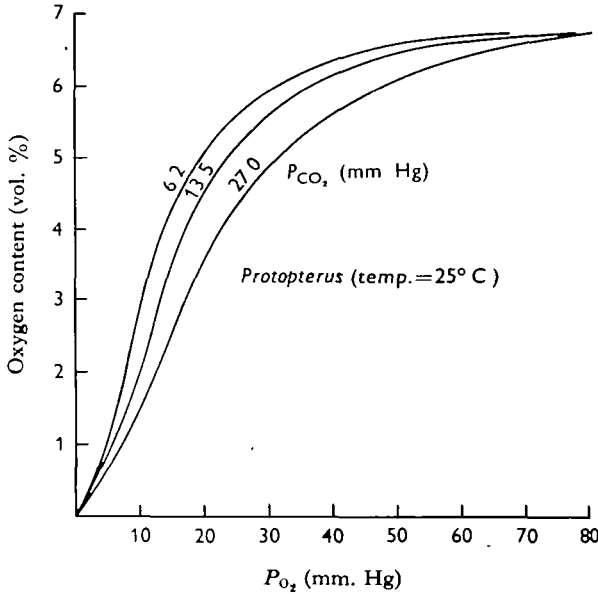


Fig. 1 Oxyhaemoglobin dissociation curves for *Protopterus aethiopicus*.

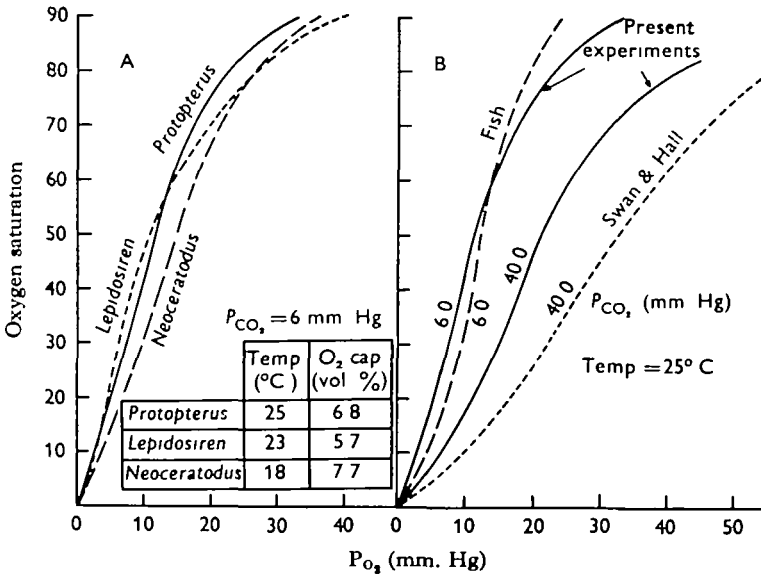


Fig. 2. (A) A comparison of the oxyhaemoglobin dissociation curves in various species of lungfishes. (B) A comparison of the oxyhaemoglobin dissociation curve of *Protopterus* determined by various authors.

published curves for *Protopterus*. The curve reported by Fish (1956) shows a higher affinity at a similar  $P_{CO_2}$ . Data from Swan & Hall (1966) disclose a much lower affinity than we determined. Our curve at  $P_{CO_2}$  40 mm. Hg is extrapolated, since the highest  $P_{CO_2}$  we used was 27 mm. Hg. Figure 3 compares the Bohr effect of *Protopterus* blood with our earlier published data from *Neoceratodus* and *Lepidosiren* and with data from Swan & Hall (1966) on *Protopterus*. The present results show a much higher Bohr

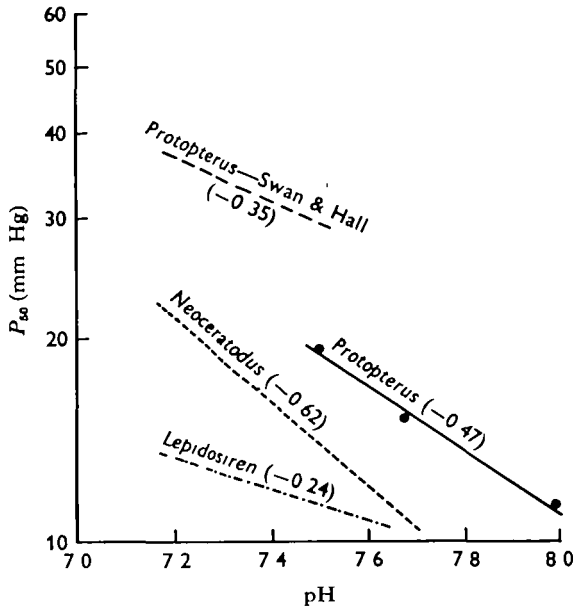


Fig. 3 A comparison of the Bohr effect in lungfishes. The numbers in parentheses indicate the magnitude of the Bohr effect.

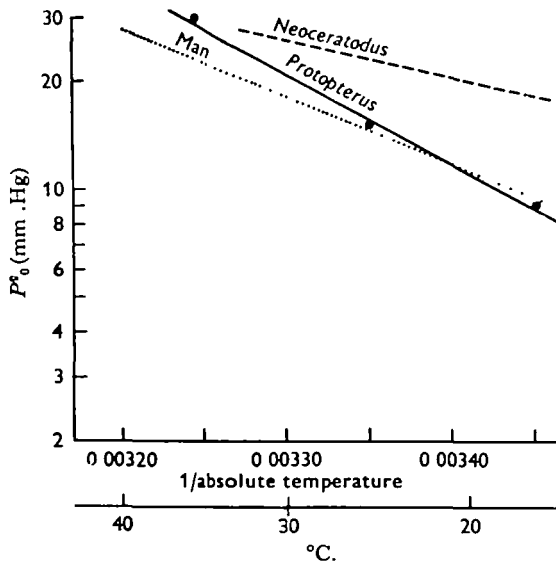


Fig. 4. The effect of temperature on oxygen affinity in blood from *Protopterus* and *Neoceratodus* as compared to man.

effect than reported by these authors. Figure 4 shows the influence of temperature on the oxyhaemoglobin affinity in *Protopterus* and *Neoceratodus* in comparison with human blood. *Protopterus* has a much larger temperature shift than *Neoceratodus*. The annual range of water temperature in Lake Victoria where our animals were obtained is very small (25–28 °C).

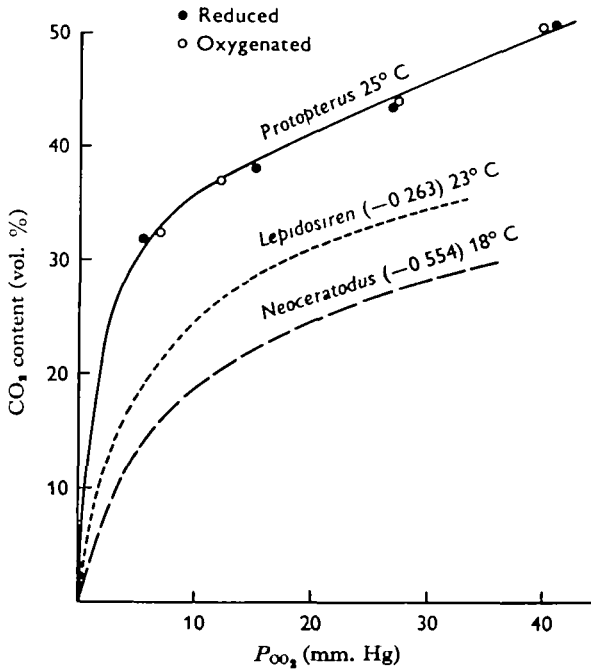


Fig. 5. A comparison of  $\text{CO}_2$  dissociation curves in the lungfishes. The curves of *Lepidosiren* and *Neoceratodus* for oxygenated blood. The numbers in parentheses indicate the magnitude of the Haldane effect in these two last species.

In Fig. 5 the  $\text{CO}_2$  dissociation curve of *Protopterus* is compared with  $\text{CO}_2$  dissociation curves for *Neoceratodus* and *Lepidosiren*. The  $\text{CO}_2$  combining power of *Protopterus* blood is considerably higher than for the other lungfishes. *Protopterus*, moreover, showed no Haldane effect (Fig. 5), whereas the other species, especially *Neoceratodus*, did. In Fig. 6 the buffering capacities of the blood in the three species are compared. The buffering capacity of *Protopterus* blood exceeds that of the other lungfishes.

#### Pattern of branchial and aerial breathing

When *Protopterus* were observed in aquaria it became apparent that the animals normally utilized both branchial and pulmonary breathing. Both means of breathing, however, were quite irregular, although steady rhythms sometimes prevailed for long periods when the fish were kept in well-aerated water in which they were free to swim and surface for air. The activities of the two modes of breathing were clearly inter-related. In particular, the branchial breathing rate increased prior to each air-breath (Johansen & Lenfant, 1968).

Table 1 lists branchial breathing rates recorded for 5 min. intervals several times in

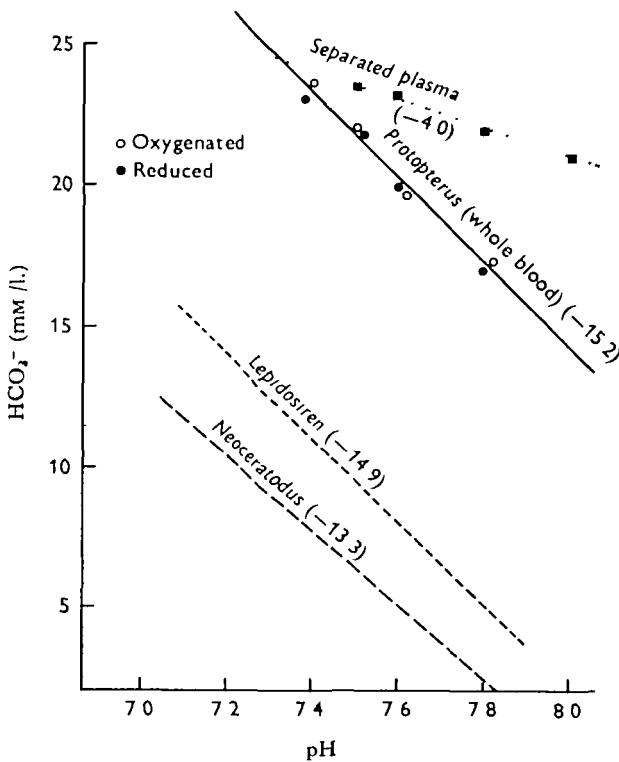


Fig. 6. Buffering capacity of oxygenated and reduced blood and separated plasma from *Protopterus* in comparison with buffering capacities of oxygenated blood from the other lung-fishes. The numbers in parentheses indicate the magnitude of the buffering capacity in mm./l./pH.

Table I. *Branchial respiratory rate and oxygen extraction in gills*

| No. of animals | No. of experiments | BRR per min. (5 min.) | $P_{iO_2}$ (mm. Hg) | $P_{eO_2}$ (mm. Hg) | $O_2$ extraction (%) |
|----------------|--------------------|-----------------------|---------------------|---------------------|----------------------|
| 1              | 1                  | 4.1                   | 88                  | 78                  | 11                   |
|                | 2                  | —                     | 120                 | 92                  | 23                   |
|                | 3                  | 3.8                   | 115                 | 75                  | 35                   |
|                | 4                  | 1.2                   | 125                 | 80                  | 36                   |
|                | 5                  | 10.0                  | 117                 | 76                  | 35                   |
| 4              | 1                  | 2.0                   | 80                  | 61                  | 24                   |
|                | 2                  | 1.4                   | 81                  | 66                  | 18                   |
| 6              | 1                  | 4.5                   | 90                  | 60                  | 33                   |
|                | 2                  | 3.7                   | 104                 | 70                  | 33                   |

each of the three specimens. The  $P_{O_2}$  of external water, exhaled water and the extraction of  $O_2$  from the branchial water current are also tabulated. Ideally the percentage extraction of  $O_2$  from the water should have been correlated with actual ventilation of water, but such ventilation measurements were not technically feasible. Four measurements on two different specimens showed  $CO_2$  tensions in exhaled water ranging from 5.4 to 10.6 mm. Hg, when the fish was in aerated water.

Figure 7 shows the branchial breathing rate for one lungfish plotted against time during continuous recording for 25 min. A great variability is evident. Exhaled water samples taken at 5 min. intervals for calculation of percentage extraction of  $O_2$  from the water also showed a large variability.

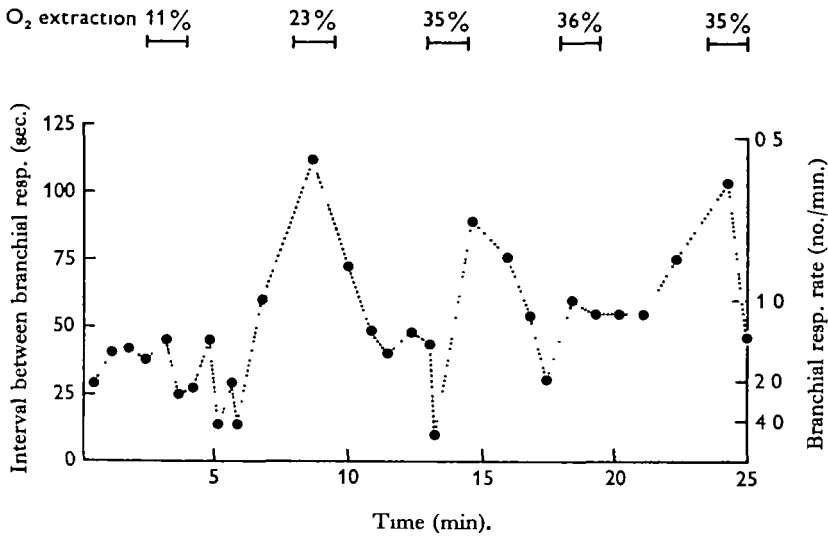


Fig. 7. Frequency of branchial breathing and percentage extraction of  $O_2$  from the water during a 25 min. period of normal undisturbed breathing.

Table 2. *Interval between breaths (min.)*

| No. of animals | No. of experiments | Normal water |      |    | Air  |      |    |
|----------------|--------------------|--------------|------|----|------|------|----|
|                |                    | Mean         | s.d. | N  | Mean | s.d. | N  |
| 1              | 1                  | 4.8          | 1.0  | 9  | 1.7  | 0.9  | 17 |
|                | 2                  | 5.5          | 2.5  | 9  | —    | —    | —  |
| 2              | 1                  | 2.50         | 1.0  | 24 | —    | —    | —  |
|                | 2                  | 3.7          | 1.2  | 12 | —    | —    | —  |
|                | 3                  | 2.8          | 1.0  | 7  | —    | —    | —  |
|                | 4                  | 3.6          | 1.3  | 12 | —    | —    | —  |
| 4              | 1                  | 7.5          | 4.2  | 14 | —    | —    | —  |
|                | 2                  | 7.2          | 2.0  | 13 | —    | —    | —  |
| 6              | 1                  | 7.5          | 2.6  | 12 | —    | —    | —  |
|                | 2                  | 6.7          | 2.4  | 17 | —    | —    | —  |
| 7              | 1                  | 4.5          | 2.2  | 14 | 2.1  | 1.3  | 13 |
| 8              | 1                  | 3.4          | 1.26 | 6  | 2.1  | 1.6  | 14 |

Table 2 shows average intervals between air breaths recorded several times for each individual specimen listed. The table includes fish in which a fairly regular air-breathing rhythm prevailed. Air exposure of the fish by slowly draining the tank water elicited a drastic increase in the rate of air breathing. This increase could clearly be dissociated from excitement or arousal as the fish rarely responded by violent behaviour to air exposure. When air-exposed the fish usually started slow searching movements and after variable times attempted to dig with their noses, seemingly

seeking to bury in the substratum. Fish air-exposed on a muddy surface where burrowing was possible did actually bury themselves, slowly but methodically. Not only did air exposure elicit an increased rate of air-breathing, it also changed the pattern of breathing into groups of several breaths at shortened intervals.

Table 3. *Rate of gas exchange in gills and in lung*

| Conditions            | $V_{O_2}$ (ml./min./kg.) |       |       |       | $V_{CO_2}$ (ml./min./kg.) |       |       |       | $R_g$ |
|-----------------------|--------------------------|-------|-------|-------|---------------------------|-------|-------|-------|-------|
|                       | Water                    | Gas   | Water |       | Water                     | Gas   | Water |       |       |
|                       |                          |       | Gas   | Total |                           |       | Gas   | Total |       |
| In water              | 0.036                    | 0.294 | 0.122 | 0.33  | —                         | —     | —     | —     | —     |
| with access<br>to air | 0.024                    | 0.191 | 0.125 | 0.21  | 0.110                     | 0.041 | 2.68  | 0.15  | 0.715 |
|                       | 0.022                    | 0.186 | 0.118 | 0.21  | 0.102                     | 0.038 | 2.68  | 0.14  | 0.666 |
|                       | 0.016                    | 0.131 | 0.122 | 0.15  | 0.092                     | 0.051 | 1.80  | 0.14  | 0.936 |
| In air                | —                        | —     | —     | 0.24  | —                         | —     | —     | 0.13  | 0.542 |
|                       |                          |       |       | 0.22  |                           |       |       | 0.11  | 0.500 |

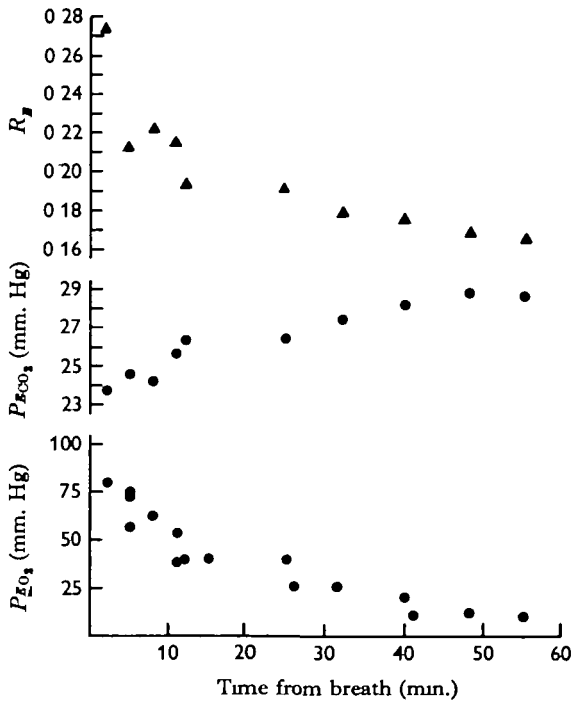


Fig. 8. Time course of change in  $P_{O_2}$  and  $P_{CO_2}$  in the pulmonary air of *Protopterus* between successive air breaths. The gas samples were collected during five breath intervals of various duration.

The relative contribution of gills and lungs in  $O_2$  absorption and  $CO_2$  elimination are indicated in Table 3. Gas exchange ratios have been calculated for aquatic versus aerial gas exchange as well as for over-all gas exchange when the fish is in water. The lower horizontal column lists  $O_2$  absorption,  $CO_2$  production and over-all gas-exchange ratios during air exposure. The data disclose that aquatic absorption of  $O_2$  constitutes only between 10 and 12% of the entire  $O_2$  uptake. Conversely, aquatic



gas exchange is on the average 2.5 times more effective than the pulmonary gas exchange in eliminating CO<sub>2</sub> when the fish is in water. The over-all gas exchange ratio is quite variable. All data tabulated on gas exchange represent average values from readings every hour for 6–10 hr. The reliability of the absolute values suffers from the inevitable exchange between the gas phase and the water phase in the

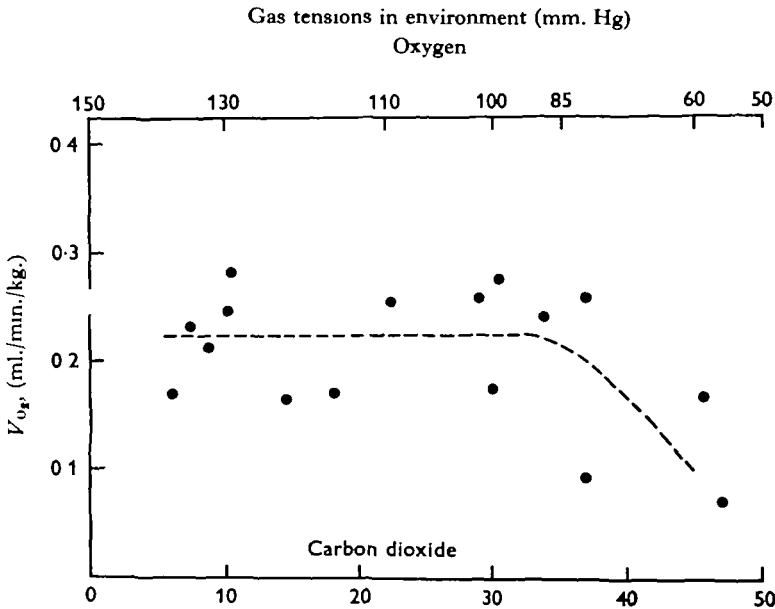


Fig. 9. Relationship between oxygen uptake in *Protopterus* and gas composition in the ambient environment during air exposure in a closed respiration chamber.

Table 4. Blood gas tensions

| No. | In water with access to air |      |      |                     |      |      | In air             |      |      |                     |      |      |
|-----|-----------------------------|------|------|---------------------|------|------|--------------------|------|------|---------------------|------|------|
|     | $P_{O_2}$ (mm. Hg)          |      |      | $P_{CO_2}$ (mm. Hg) |      |      | $P_{O_2}$ (mm. Hg) |      |      | $P_{CO_2}$ (mm. Hg) |      |      |
|     | DAo                         | PV   | PA   | DAo                 | PV   | PA   | DAo                | PV   | PA   | DAo                 | PV   | PA   |
| 1   | 27.6                        | 36.3 | 24.2 | 26.4                | 21.2 | 26.1 | 36.7               | 40.3 | 29.0 | 35.5                | 32.2 | 45.3 |
| 2   | 18.3                        | 25.6 | 14.6 | 29.7                | 26.4 | 28.2 | 20.0               | 25.0 | 12.0 | 36.8                | 28.5 | 47.0 |
| 3   | 25.7                        | 30.5 | 19.5 | 24.6                | 20.1 | 25.3 | 32.5               | 38.5 | 24.5 | 30.6                | 28.0 | 36.2 |
| 4   | 36.5                        | 67.0 | 23.3 | 22.2                | 19.1 | 22.6 | —                  | —    | —    | —                   | —    | —    |

Each value is the mean of at least eight consecutive measurements for  $P_{O_2}$  and at least four for  $P_{CO_2}$ . DAo = dorsal aorta, PV = pulmonary vein and PA = pulmonary artery.

metabolism chambers. However, this exchange would tend to minimize the role of aquatic exchange for CO<sub>2</sub> elimination and the aerial exchange for O<sub>2</sub> absorption. It is notable that air exposure did not promptly reduce the over-all oxygen uptake. The apparent reduction in the gas-exchange ratio represented the only real change during the relatively short periods of observation.

Figure 8 illustrates the time course of changes in the partial pressures of pulmonary gases in the interval between air breaths. The time course of the gas exchange ratio is

also plotted. This specimen and other fish used for these measurements were free to swim in large tanks and had not been subjected to any surgical treatment. The expired gas was collected by trapping the released gas into water-filled funnels suspended above the heads of the fishes.

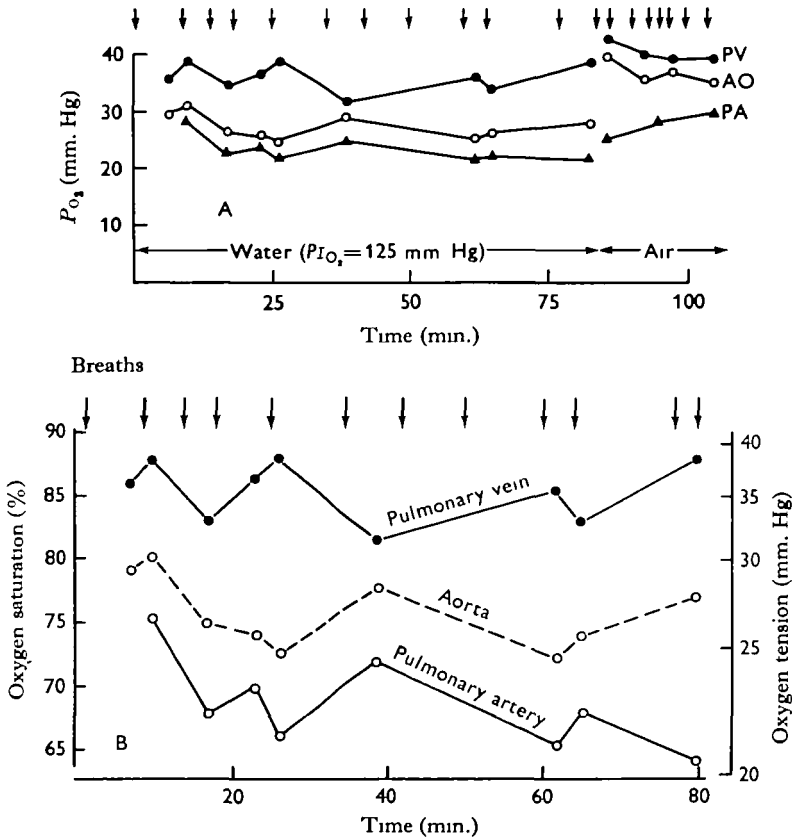


Fig. 10. (A) Repetitive blood gas analysis during undisturbed breathing in *Protopterus*. To the right are plotted blood gas values during air exposure. Arrows mark the time of air breaths. PV, Pulmonary vein; PA, pulmonary artery; AO, dorsal aorta. (B) Blood gas values expressed in partial pressure and  $O_2$  saturation during undisturbed breathing in aerated water. Arrows mark the time of air breaths.

Figure 9 shows the relationship between oxygen uptake ( $\dot{V}_{O_2}$ ) and the gas composition in the ambient environment. In this typical experiment the fish is seen to maintain his  $\dot{V}_{O_2}$  down to a  $P_{I_{O_2}}$  of approximately 85 mm. Hg with a corresponding  $P_{CO_2}$  increase in the metabolism chamber of about 35 mm. Hg. A further reduction in  $P_{I_{O_2}}$  was accompanied by an abrupt fall in  $\dot{V}_{O_2}$ . The experimental arrangement did not make it possible to decide whether the  $P_{I_{O_2}}$  or the  $P_{I_{CO_2}}$  or both are instrumental in bringing about this change. The experiments underlying Fig. 9 lasted for variable periods up to 8 hr. All fish survived the experiments. Consistently, prolonged air exposure was accompanied by a progressive vasodilation of the skin.

Table 4 shows the blood gas tensions in undisturbed fish resting in well aerated

water as well as during air exposure. Figure 10A, B emphasizes the difference in blood gas tensions borne out in the table and shows the time course of blood gas changes occurring between consecutive breaths. Note that blood from the dorsal aorta shows a consistently higher oxygen tension than pulmonary arterial blood. Expressed in percentage oxygen saturation (Fig. 10B), the pulmonary arterial blood is seen to be about 65–70% saturated against more than 75% average saturation in systemic arterial blood from the dorsal aorta. Finally, pulmonary venous blood is about 85% saturated with oxygen. To the right in Fig. 10A are plotted the blood oxygen tensions following air exposure. The arrows indicate the number of air breaths and draw attention to a conspicuous stimulation of air breathing associated with air exposure. All blood gas values are increased and the gradient between systemic arterial blood and pulmonary arterial blood has become larger.

#### DISCUSSION

The lungfishes occupy habitats similar to those of many other air-breathing fishes, yet the  $O_2$  capacity of their blood seems to be generally much lower. Thus, the blood of *Protopterus* compares well with values reported for the South American *Lepidosiren* and the Australian *Neoceratodus* (Johansen & Lenfant, 1967; Lenfant *et al.* 1966). In contrast, three South American air-breathing fishes—the electric eel, *Electrophorus electricus* (Johansen, Lenfant, Schmidt-Nielsen & Petersen, 1968); the hassa, *Hoplosternum littorale* (Willmer, 1934) and *Symbranchus marmoratus* (Johansen, 1966)—showed average  $O_2$  capacities of 12.3, 18.1 and 14.7 vol. % respectively. Since tropical air-breathing fishes largely occupy similar habitats, the marked difference in  $O_2$  capacity may be related to the efficiency in  $O_2$  transport. Many air-breathing fishes have a permanently low arterial  $O_2$  saturation because the blood draining the aerial gas-exchange organ is mixed with the general systemic venous blood before it perfuses the arterial circulation. In the lungfishes such extensive shunting of oxygenated blood to the venous circulation is prevented by a separate pulmonary vascular circuit and a partial division of the heart and its outflow channels (Johansen, Lenfant & Hanson, 1968).

The oxyhaemoglobin dissociation curves (Figs. 1, 2) conform in shape to those reported for lungfishes earlier but the actual affinity for  $O_2$  shows definite variations and was higher in our experiments. Fish (1956), working on haemoglobin solutions, which in general have higher affinity than whole blood, reported a  $P_{50}$  value of about 11 mm. Hg at  $P_{CO_2}$  6 mm. Hg for *P. aethiopicus*. Swan & Hall (1966), working with whole blood from *Protopterus*, found an  $O_2$  affinity much lower than our experiments revealed (Fig. 2B).

It has been suggested that the  $O_2$  affinity of haemoglobin generally decreases with increasing dependence on aerial gas exchange (McCutcheon & Hall, 1937). This tendency should reflect an adjustment to the higher  $O_2$  availability in air than water. More recent data from transitional forms among amphibians substantiate this hypothesis (Lenfant & Johansen, 1967). In regard to fishes, most workers endorse the early suggestion by Krogh & Leitch (1919) that higher  $O_2$  affinity of haemoglobin correlates with the ability of a species to survive in an  $O_2$  poor medium. Among air-breathing fishes, those showing accessory air breathing with a dominance of aquatic gas exchange

may show adaptation to conditions in the water whereas obligatory air-breathing fishes, like *Lepidosiren* and *Protopterus*, should show adjustments towards atmospheric conditions.

Willmer (1934) offered convincing data demonstrating that blood from freshwater fish inhabiting well-aerated water of low CO<sub>2</sub> content displays a marked sensitivity to CO<sub>2</sub> changes (Bohr and Root's shifts), whereas blood from fish in stagnant swamps and muddy creeks with a high CO<sub>2</sub> content was practically insensitive to CO<sub>2</sub>. Later workers, notably Carter (1951, 1962) and Fish (1956), used such data to generalize that a transition from aquatic to aerial gas exchange is accompanied by a reduction in the CO<sub>2</sub> sensitivity of the blood.

Carter's generalization on the evolution of the Bohr shift receives support from a comparison of blood from *Neoceratodus* (Lenfant *et al.* 1966) and *Lepidosiren* (Johansen & Lenfant, 1967). *Lepidosiren*, which is decidedly more of an air breather, shows a much smaller Bohr shift than *Neoceratodus*. An extension of this comparison to *Protopterus*, based on the present results or those of Swan & Hall (1966), does not support such a trend. A comparison of Bohr shifts in selected amphibians showing increasing dependence on air breathing similarly does not provide support for Carter's hypothesis (Lenfant & Johansen, 1967). The latter comparison, however, is complicated by neoteny of the species of urodeles studied. Further work is obviously desirable to resolve the role of adaptive changes in the CO<sub>2</sub> sensitivity of the blood in the evolution of air breathing.

The effect of a temperature change on the O<sub>2</sub> affinity of haemoglobin is different in blood from the Australian and African lungfishes. *Neoceratodus*, which occupies waters that may show large annual and diurnal changes in temperature (up to 20 °C.), has blood that is notably insensitive to temperature changes. Conversely, *Protopterus*, with very slight temperature variations in its habitat, possesses blood which is much more sensitive to temperature changes.

The CO<sub>2</sub> combining power and buffering capacity of blood from the lungfishes and other forms in the transition to air breathing reveals some consistent trends. Figure 5 shows an increasing CO<sub>2</sub> combining power from *Neoceratodus* through *Lepidosiren* to *Protopterus*. This tendency substantiates predictions made by Lenfant *et al.* (1966) that a greater dependence on air breathing is correlated with an increasing CO<sub>2</sub> combining power.

The role of the gills and skin in O<sub>2</sub> uptake in *Protopterus* was found to be about 10% of the total uptake when the fish rests in aerated water (Table 3). In *Lepidosiren* the importance of the gills for O<sub>2</sub> absorption is even less and reported to be only 2% of the total O<sub>2</sub> uptake (Sawaya, 1946). The relatively high extraction of O<sub>2</sub> from the water found presently (Table 1) was of little importance to oxygen uptake because of extremely low values of ventilation. On the other hand, aquatic exchange by gills and skin was 2.5 times as efficient as the pulmonary exchange for CO<sub>2</sub> elimination. The role of the skin could be important in this comparison since Cunningham (1934) reports that the skin in *Lepidosiren* has a gas-exchange ratio of more than 10 when the fish is in water. The progressive vasodilation of the skin in *Protopterus* during air exposure attests to an active role in gas exchange. The present results for *Protopterus* are in accord with the tendency for animals having bimodal, aquatic and aerial, gas exchange to show a low gas-exchange ratio for the air-breathing organ, and a gas-

exchange ratio greater than unity for the water-breathing organ (Lenfant & Johansen, 1967).

Figure 9 demonstrates that *Protopterus* while in air retains respiratory independence down to environmental  $O_2$  tensions of about 85 mm. Hg correlated with an external  $P_{CO_2}$  of 30–35 mm. Hg. Most aquatic vertebrates show critical  $O_2$  tensions as low as 30 mm. Hg. Hall (1929) compared factors limiting oxygen uptake in active and sluggish species of fishes. His data suggest that active fishes have a greater tolerance to variations in external oxygen tension. Similarly, fishes with a higher haemoglobin content were more capable of maintaining a steady oxygen uptake in spite of reduced external oxygen availability. If *Protopterus* was primarily a water breather, Hall's generalizations would be applicable to explain the narrow range of  $O_2$  independence, but being

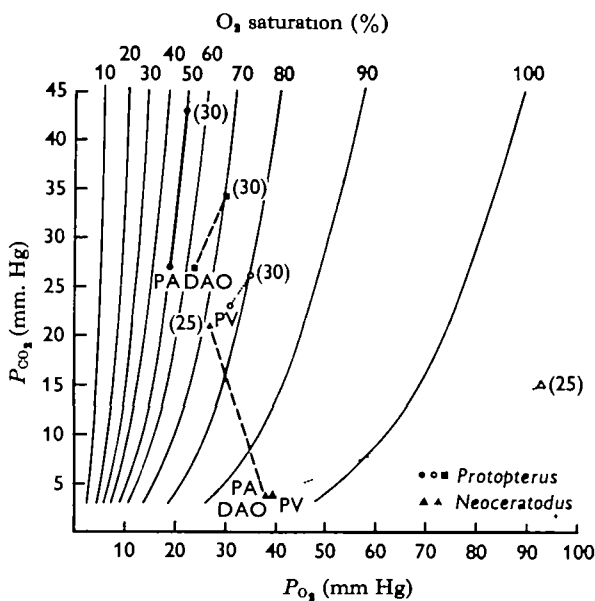


Fig. 11. Nomogram comparing blood gas values in *Neoceratodus* and *Protopterus* during undisturbed breathing in water (low  $P_{O_2}$ ) and following air exposure (duration of air exposure is indicated by the numbers in parentheses). PA, pulmonary artery; PV, pulmonary vein; DAO, dorsal aorta.

primarily an air breather, *Protopterus* should be more tolerant to external  $P_{O_2}$  changes than water breathers. The profound metabolic adjustments occurring during fasting and aestivation (Smith, 1935) may be related to the unusual metabolic responses of *Protopterus* to changes in ambient gas composition.

The nomogram (Fig. 11) provides a basis for discussing differences in gas exchange between *Neoceratodus*, in which the lung functions as an auxiliary gas exchanger and *Protopterus* which depends predominantly on pulmonary breathing. When resting in well-aerated water *Neoceratodus* employs air breathing very infrequently. During such conditions the lung is of no consequence to gas exchange, and similar blood gas values prevail in systemic arterial, pulmonary arterial and pulmonary venous blood. Efficient branchial gas exchange maintains the arterial blood more than 90% oxygen saturated and arterial  $P_{CO_2}$  values are extremely low, a character shared with typical aquatic

breathers. *Protopterus*, on the other hand, practises active, frequent air breathing and the lungs play a major role in gas exchange. The importance of the lungs is apparent in the differences in oxygen saturation as well as  $P_{CO_2}$  values of pulmonary arterial and pulmonary venous blood. The intermediate saturation value of systemic arterial blood sampled from the dorsal aorta testifies that pulmonary venous blood is channelled

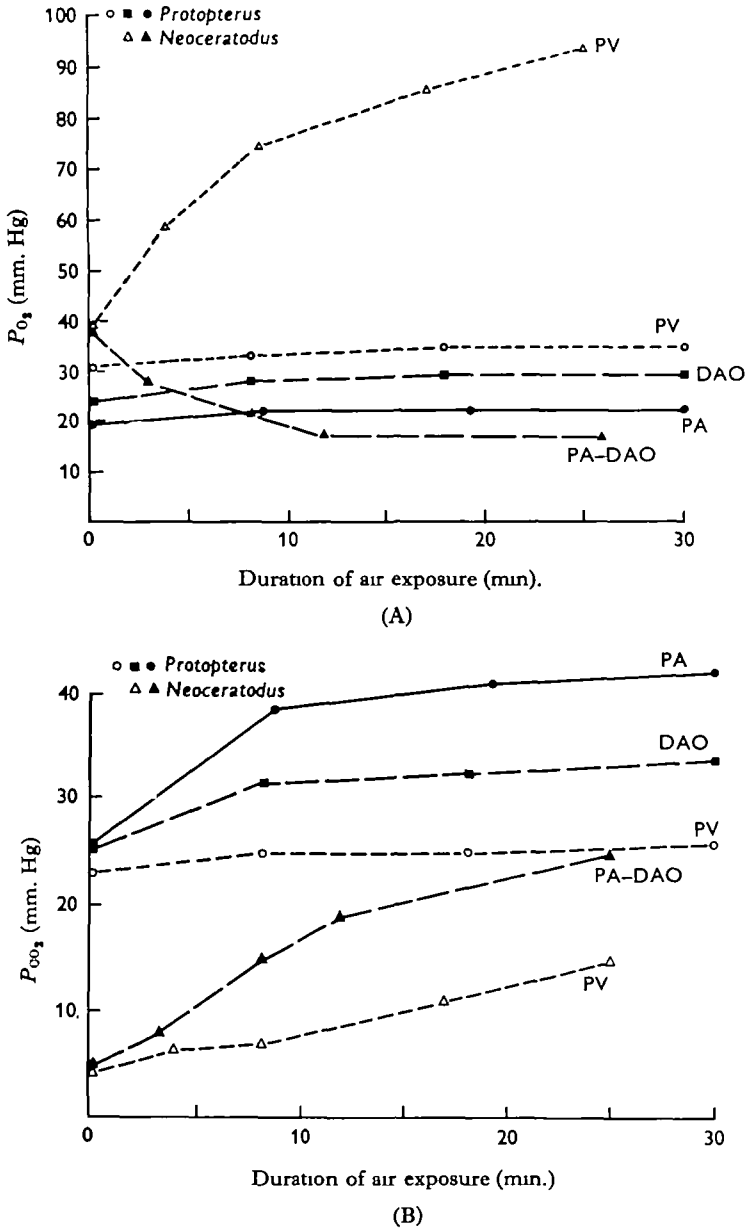


Fig. 12. Time course of changes in blood gas composition during air exposure of *Neoceratodus* and *Protopterus*. (A) Changes in blood oxygen tensions; (B) changes in blood carbon dioxide tensions. PA, pulmonary artery; PV, pulmonary vein; DAO dorsal aorta.

selectively into the cardiac outflow channels giving rise to the systemic circulation. The emphasis on air breathing in *Protopterus* is also borne out by the much higher blood  $P_{\text{CO}_2}$  values, being on the average more than 20 mm. Hg above the values in *Neoceratodus*.

The limitations of pulmonary air breathing in the bimodal gas exchange of lungfishes were tested by exposing the fish to the air and thus interrupting aquatic gas exchange altogether. Unlike *Neoceratodus*, *Protopterus* never reacted violently or became restless and excited when removed from water. Air-exposure accelerated air breathing in both species. In *Neoceratodus* this became manifest in a conspicuous increase in oxygen tension of pulmonary venous blood. However, systemic arterial oxygen tension dropped sharply and  $\text{CO}_2$  tensions increased markedly in all blood samples. In *Protopterus* air exposure increased the oxygen saturation in both pulmonary venous and systemic arterial blood, while pulmonary arterial blood maintained the same saturation level. The increase in blood  $\text{CO}_2$  tensions was much less marked than in *Neoceratodus*. Figure 12A and B show the time course of blood gas tensions following air exposure. The inability of the lung of *Neoceratodus* to keep up the oxygen saturation of arterial blood seems attributable to a low pulmonary blood flow rather than to the efficiency of gas exchange in the lung. In contrast, the pulmonary blood flow in *Protopterus* is high enough to maintain the arterial oxygen tension when gill breathing is suspended (Fig. 12A). In *Neoceratodus* the arterial  $\text{CO}_2$  tensions were increased more than 4 times during 30 min. exposure to air. *Protopterus* blood showed a corresponding increase of about 30%. In *Protopterus*, however,  $\text{CO}_2$  elimination represents the most severe limitation of pulmonary breathing. The ability of *Protopterus* to survive several months of terrestrial existence while aestivating during periodic droughts is most probably related to the moist conditions in the cocoon allowing some  $\text{CO}_2$  to escape through the skin. Also the markedly reduced over-all metabolism during aestivation will reduce the requirements for gas exchange.

#### SUMMARY

1. Respiratory properties of blood and pattern of aerial and aquatic breathing and gas exchange have been studied in the African lungfish, *Protopterus aethiopicus*.
2. The mean value for haematocrit was 25%. Haemoglobin concentration was 6.2 g% and  $\text{O}_2$  capacity 6.8 vol. %.
3. The affinity of haemoglobin for  $\text{O}_2$  was high.  $P_{50}$  was 10 mm. Hg at  $P_{\text{CO}_2}$  6 mm. Hg and 25 °C. The Bohr effect was smaller than for the Australian lungfish, *Neoceratodus*, but exceeded that for the South American lungfish, *Lepidosiren*. The  $\text{O}_2$  affinity showed a larger temperature shift in *Protopterus* than *Neoceratodus*.
4. The  $\text{CO}_2$  combining power and the over-all buffering capacity of the blood exceeded values for the other lungfishes.
5. Both aerial and aquatic breathing showed a labile frequency. Air exposure elicited a marked increase in the rate of air breathing.
6. When resting in aerated water, air breathing accounted for about 90% of the  $\text{O}_2$  absorption. Aquatic gas exchange with gills and skin was 2.5 times more effective than pulmonary gas exchange in removing  $\text{CO}_2$ . The low gas-exchange ratio for the lung diminished further in the interval between breaths.