RESPIRATORY FUNCTION IN THE SOUTH AMERICAN LUNGFISH, *LEPIDOSIREN PARADOXA* (FITZ)*

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Few animal species have aroused as much excitement upon their discovery as the South American lungfish. Its possession of distinct piscine as well as amphibian characters made the animal a link in the transition of vertebrate life from water breathing to air breathing. Its special attributes are clearly reflected in its name, *Lepidosiren paradoxa.*

Physiological experimentation on respiratory function in this fish is very scarce (Cunningham, 1934; Carter & Beadle, 1930; Carter & Beadle, 1931; Cunningham & Reid, 1932; Sawaya, 1946). No measurements of pulmonary-gas concentration and blood–gas tensions have come to our notice. The present investigation concerns the respiratory properties of the blood and the dynamics of gas exchange as well as the relative importance of branchial and aerial respiration. The experiments were also designed to elucidate possible regulatory mechanisms in the breathing pattern of *Lepidosiren paradoxa.*

MATERIAL AND METHODS

Twelve specimens of *Lepidosiren paradoxa* ranging in weight from 104 to 212 g. were used in the investigation. Although the animals were juvenile, only non-functional traces of external gills were still present.

Respiratory behaviour was studied in intact specimens free to breathe air, while the gas composition in the ambient water (temp. *ca.* 20° C.) was changed. Thus water was equilibrated with room air or with nitrogen or oxygen to give hypoxic water (Pi_{O_2} < 80 mm. Hg) or hyperoxic water (Pi_{O_2} > 200 mm. Hg). In other cases, fish were exposed to normoxic but hypercarbic water (Pi_{CO_2} > 5 mm. Hg). Finally, fish were placed in normoxic water and made to breathe pure oxygen. The frequencies of air breathing and branchial respiratory rate were recorded during these procedures. Expired gas was collected by inverting a water-filled stoppered funnel over the animal's head, trapping the expired gas as it was voluntarily released. Following an expiration, the exhaled gas was immediately drawn into a glass syringe by needle puncture through the stoppered end of the funnel. The dead space of the syringe was filled with saturated lithium chloride solution. Exhaled water was sampled via a

catheter attached to the inside of one operculum. Pressure changes caused by the branchial respiratory movement were also recorded via this catheter.

Oxygen uptake ($\dot{V}_{O_2}$) was estimated for fish kept in a closed container initially filled with air by measuring the changes in P_{O_2} in the gas phase. The container was immersed in water for temperature stabilization. $\dot{V}_{O_2}$ was calculated as follows:

$$\dot{V}_{O_2}(\text{STPD}) = \frac{\Delta P_{O_2} \times V \times f}{(P - P_{H_2O}) \times t};$$

ΔP_{O_2} = change in partial pressure during time interval t (in minutes) between
    two measurements;

P = barometric pressure;

P_{H_2O} = water vapour pressure at temperature of water;

V = volume of chamber;

f = correction factor (ATPS to STPD).

$\dot{V}_{O_2}$ was also studied in fish kept in water with access to air. A closed container was used following a method slightly modified from the description of Lenfant (1961). The water was stirred by magnetic stirrers and equilibrium was maintained between the gas and liquid phases. Samples for analyses were drawn from the liquid phase.

In some animals it was possible to extend the experimental approach by implanting polyethylene catheters in the pulmonary artery and vein and/or a branch of the dorsal aorta permitting blood samples to be drawn. Anaesthesia was induced by immersing the fish in a solution of MS 222 (tricaine methane sulphonate) in concentration 1:1000. A ventral incision 5–7 cm. anterior to the cloaca permitted access to a branch of one pulmonary artery. The pulmonary arteries in lungfishes arise from the most posterior pair of epibranchial arteries. Cannulation with a small-bore polyethylene catheter (PE 50) was possible in the terminal course of the artery, minimizing the effects of obstructing the vessel. A pulmonary vein draining a different portion of the lung was cannulated in a similar way. Systemic arterial blood was obtained through cannulation of a branch of the dorsal aorta. The fish were allowed to recover from anaesthesia and all samples and measurements were taken from fish free to move about in the aquaria.

By reason of the small size of the fish repeated blood sampling and duplicate analyses were limited. For the same reason pH measurements were not feasible; instead, values of log HCO_3^-/H_2CO_3 were used to assess the Bohr effect and the buffering capacity. Blood-gas contents (C_{O_2} and C_{CO_2}) were measured by gas chromatography (Lenfant & Aucutt, 1966). Partial pressures of O_2 and CO_2 in gas, water and blood samples were measured with a Beckman Spinco gas analyser using the oxygen electrode and a special micro-cuvette (0·03 ml. samples) for P_{O_2} and the Severinghaus electrode for P_{CO_2}, calibrating with known gas mixtures. All measurements and calibrations were performed at room temperature (18–20° C.)

Work on respiratory properties of blood *in vitro* was done on blood stored 48 hr. or more. Oxygen capacity was evaluated from measurement of O_2 contents in blood samples equilibrated with gas containing 20 % O_2 and 0·8 % CO_2. Other details of the techniques used have been described by Lenfant & Johansen (1965).

RESULTS

Respiratory properties of the blood

Haematocrit from three different specimens yielded values of 19, 14 and 14%
while the corresponding oxygen capacities were 6·8, 5·4 and 4·9 vol. % respectively.
Fig. 1 shows the O_2–Hb dissociation curve based on measurements using mixed
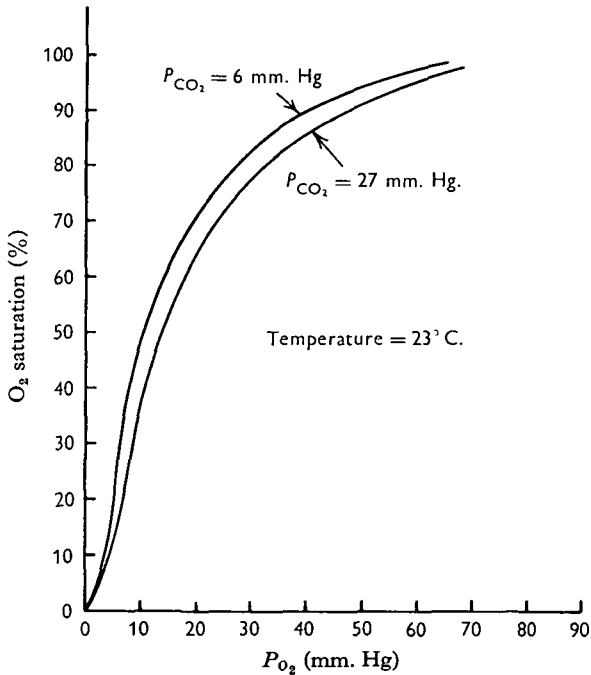

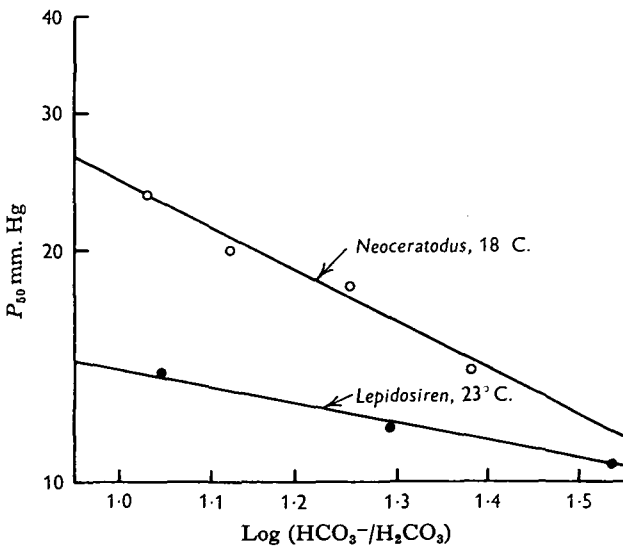Fig. 1. O_2–Hb dissociation curves for *Lepidosiren* blood.

Fig. 2. Relationship between O_2–Hb affinity and Log HCO_3^-/H_2CO_3 in the blood (Bohr effect)
of *Lepidosiren* and *Neoceratodus*. Data for *Neoceratodus* taken from Lenfant *et al.* (1966).

blood from these specimens. A high affinity for O_2 is apparent. The P_{50} value was 10·5 mm. Hg at P_{CO_2} 6 and 23° C. Fig. 2 compares the O_2–Hb affinity and its dependence on pH changes (Bohr effect) in two species of lung-fishes. In *Lepidosiren* the affinity is markedly higher while the Bohr effect is much less than in *Neoceratodus*.
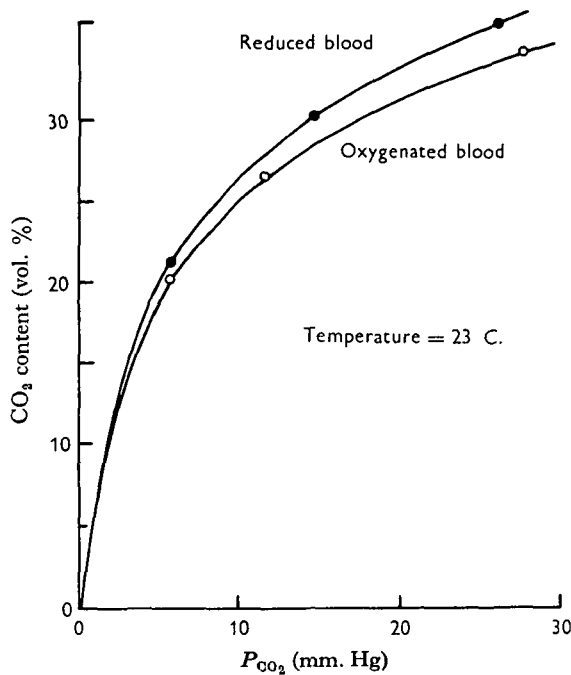
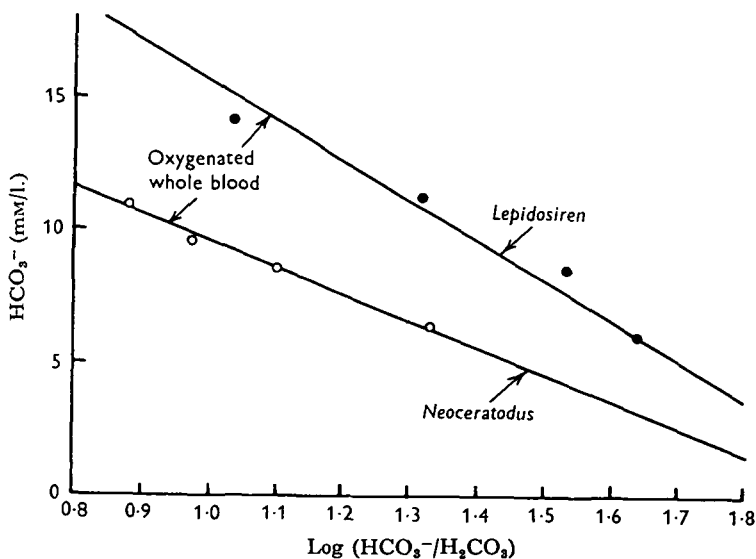Fig. 3. CO_2 dissociation curves for reduced and oxygenated blood of *Lepidosiren*.

Fig. 4. A comparison of the buffering capacity of oxygenated whole blood of *Lepidosiren* and *Neoceratodus*. Data for *Neoceratodus* taken from Lenfant *et al.* (1966).

Fig. 3 shows the CO_2 dissociation curves for reduced and oxygenated blood. The initial ascending parts of the curves are very steep and indicate a high CO_2 combining power. The Haldane effect is small in keeping with the moderate Bohr effect. Fig. 4 displays the buffering capacity of oxygenated whole blood of *Lepidosiren* in comparison with that of *Neoceratodus*.

Respiratory behaviour

When resting in well-aerated water *Lepidosiren* displayed marked variations in both aerial and branchial respiration. The interval between air breaths varied from 3 min. to about 10 min., with 3–4 min. as the most common. Table 1 shows the average time interval in minutes from observations on five different individuals. Each inspiration was normally followed by several expirations. Part of the expired gas was simply excess of that taken into the pharyngeal cavity during inspiration.

Table 1. *Interval in minutes between air breaths in* Lepidosiren

Exp. no.	Normal Pi_{O_2} >90	Low Pi_{O_2} <80	High Pi_{O_2} >200	High Pi_{CO_2} >5
1. Mean	8·8	—	—	—
n	12	—	—	—
S.D.	5·6	—	—	—
2. Mean	8·2	—	—	—
n	8	—	—	—
S.D.	4·9	—	—	—
3. Mean	3·1	—	—	—
n	7	—	—	—
S.D.	1·8	—	—	—
Mean	6·7	—	—	—
n	3	—	—	—
S.D.	1·5	—	—	—
4. Mean	5·1	2·8	—	—
n	7	9	—	—
S.D.	3·4	1·4	—	—
Mean	4·4	1·8	4·4	2·5
n	4	4	9	5
S.D.	1·9	1·5	3·2	1
5. Mean	5·9	—	—	—
n	5	—	—	—
S.D.	5·7	—	—	—

n = number of observations.
S.D. = standard deviation.

The branchial respiratory movements were extremely shallow and almost imperceptible when the fish was resting quietly. The frequency was widely variable ranging from 1/min. (Fig. 5) to about 20/min. In conjunction with a slight motor movement, the breathing rate showed a sudden acceleration and the branchial movements became more forceful. When active periods alternated with rest the branchial breathing rate showed acceleration and deceleration corresponding with activity and rest.

The extreme lability of the branchial respiratory rate prompted experiments as to its underlying mechanism. It was found that slight agitation of the aquarium water by gentle stirring, or simple tapping on the outside wall of the aquarium, elicited a prompt acceleration of the branchial respiratory rate. This response was caused by the

mechanical waves resulting from the stirring or tapping, since great care was taken to exclude the possibility that excitement caused by visual stimulation was influencing the breathing rate. Fig. 5 demonstrates a direct recording of branchial respiratory rate before, during and subsequent to slight mechanical agitation of the aquarium water. A sudden change of both the frequency and depth of breathing is readily apparent.

Attempts to influence the breathing pattern by changing the quality of the external water gave rather inconclusive results. In two specimens, however, a clear increase in the frequency of air breathing resulted when the fish was exposed to hypoxic water ($Pi_{O_2} < 80$ mm. Hg); similarly one of these fish increased the rate of air breathing when transferred to hypercarbic water ($Pi_{CO_2} < 5$ mm. Hg). Exposure to hyperoxygenated water ($Pi_{O_2} > 200$ mm. Hg) had no significant effect on the aerial breathing pattern (Table 1). Possible effects of changing the external conditions on the
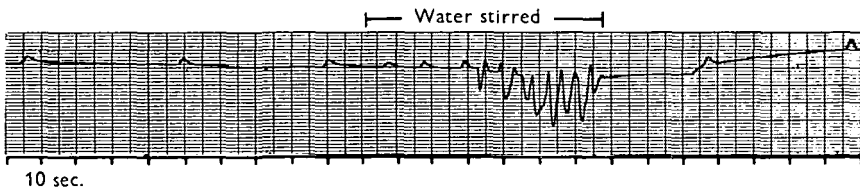

Fig. 5. Direct recording of branchial respiratory rate in *Lepidosiren* showing the influence of mechanical stirring of the water.

branchial breathing pattern were difficult to assess because of the above mentioned sensitivity to mechanical agitation of the water. Slight bubbling of gases through water, or even gentle transfer to previously equilibrated water, caused an increased branchial respiratory rate obviously referable to the mechanical agitation. A possible influence of the external gas composition on the branchial breathing pattern is hence probably of less importance than the mechanosensitive response.

Exposure of the fishes to air by drainage of the aquarium water was invariably accompanied by an increased frequency of air breaths. The fishes did not become hyperexcited or restless upon exposure to air.

Gas exchange

Branchial respiration

The gills of *Lepidosiren* are much reduced compared to those of gill-breathing aquatic vertebrates in general. Gill arches 1 and 2 are almost devoid of gill filaments, while the other arches show filaments much reduced in surface area compared to teleost gills in general. Table 2 shows evidence that some degree of gas exchange takes place in the gills. The extraction of O_2 from the water ($Pi_{O_2}-Pe_{O_2}/Pi_{O_2}$) varied and was generally less than 30%. Attempts to measure water ventilation were, however, completely unsuccessful by reason of its exceedingly low values. Oxygen uptake through the gills must hence represent a very small fraction of the total oxygen consumption. A comparison of oxygen tension in blood from the pulmonary artery and from the dorsal aorta in normally aerated and in hyperoxygenated water, as well as subsequent to inhalation of oxygen, is presented in Table 3.

Aerial respiration

Fig. 6 shows a composite plot of the rate of O_2 depletion in the expired gas subsequent to spontaneous air breathing. There is a rapid drop initially which later changes slope at a P_{O_2} of about 60–70 mm. Hg. Fig. 7 shows the course of the P_{O_2}

Table 2. *Percentage O_2 extracted from the respiratory water current in various external conditions*

Exp. no.	Pi_{CO_2} (mm. Hg)	Pi_{O_2} (mm. Hg)	Pe_{O_2} (mm. Hg)	$\dfrac{Pi_{O_2} - Pe_{O_2}}{Pi_{O_2}} \times 100$
1	0	127	115	9·4
	0	127	103	18·9
	0	118	96	18·6
2	0	139	126	9·4
	0	124	106	14·5
3	0	139	118	15·1
4	0	133	81	39·1
	0	127	80	37·0
	0	131	82	37·4
	0	131	84	35·9
	0	132	98	25·8
	0	132	93	29·5
	0	85	72	15·3
5	15	150	113	24·7
	15	145	114	21·4
	15	144	106	26·4
	15	144	104	27·8
	15	137	107	21·9
	15	137	112	18·2
	15	144	108	25·0
6	0	663	483	27·1
	0	655	270	58·8
	0	640	540	15·6

Table 3. *Comparison of oxygen tension (mm. Hg) in blood from the dorsal aorta and pulmonary artery sampled during various external conditions*

Normoxic water Pi_{O_2}: 120 to 140 mm. Hg		Hyperoxic water $P_{O_2}i > 200$ mm. Hg		After inhalation of pure O_2	
Dorsal aorta	Pulmonary artery	Dorsal aorta	Pulmonary artery	Dorsal aorta	Pulmonary artery
—	8·5	—	30	79	24·5
—	7·5	—	26	138	70
—	10	10	10	—	—
—	11	61	—	—	—
—	11	—	—	—	—
10	5	—	—	—	—
37	—	—	—	—	—
40·5	—	—	—	—	—
21	11	—	—	—	—
31	16	—	—	—	—
37	14	—	—	—	—
43	14·5	—	—	—	—
33	—	—	—	—	—

14

changes in the pulmonary venous blood and in the expired gas after an air breath. In the first 4 min. after the breath P_{O_2} remains relatively unchanged in the pulmonary venous blood after which it falls off in parallel with the rate of change in the expired gas.
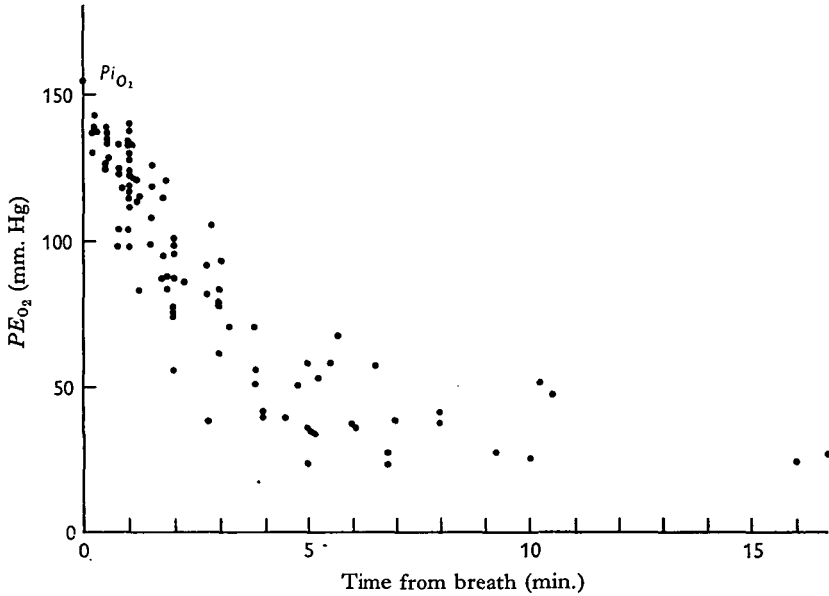

Fig. 6. A composite plot of the depletion rate of oxygen from the lungs of *Lepidosiren* after spontaneous breaths of air.
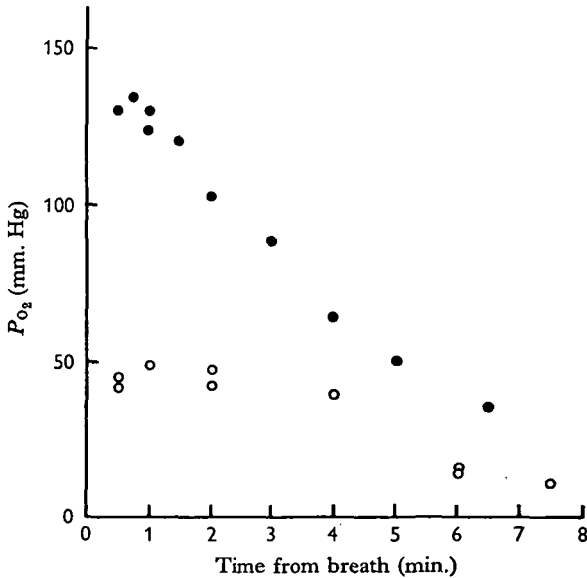

Fig. 7. Changes in oxygen tension of pulmonary venous blood (open circles) and pulmonary gas (filled circles) in *Lepidosiren* after a spontaneous breath.

Oxygen uptake

Oxygen uptake ($\dot{V}_{O_2}$) when the fish were resting in water was measured twice over a 5 hr. period. No significant changes with time were observed. Table 4 gives the mean values for two fish. Fig. 8 shows as a function of time the oxygen uptake of three specimens exposed to air. $\dot{V}_{O_2}$ is seen to decrease with the duration of exposure to air. The decrease is not referable to a reduced oxygen availability (respiratory dependence) since the Pi_{O_2} dropped only to 125 mm. Hg.
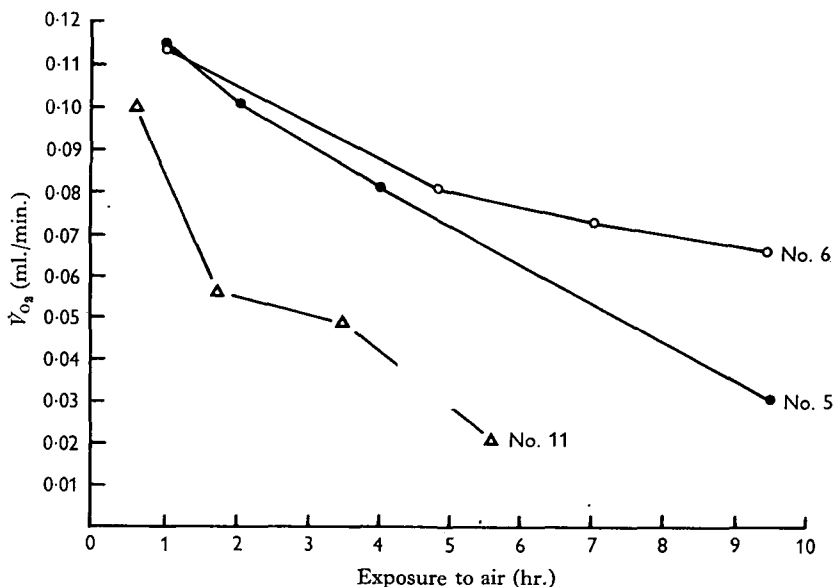

Fig. 8. Oxygen uptake versus time for three specimens of *Lepidosiren* exposed to air.

Table 4. *Mean values of oxygen uptake for two specimens of* Lepidosiren *resting in water with access to air*

Exp. no.	Weight (g.)	$\dot{V}_{O_2}$ (ml./min.)	$\dot{V}_{O_2}$ (ml./min./g.)	$\dot{V}_{O_2}$ (ml./hr./kg.)
1	204	0·159	$0·78 \times 10^{-3}$	46·8
2	104	0·116	$1·01 \times 10^{-3}$	60·8

Fig. 9 shows the increase in P_{CO_2} in the closed respiration chamber during the air breathing (re-breathing). There was no apparent response to the increased P_{CO_2} in terms of restlessness and frequency of air breathing. However, upon return to water the fishes started intense branchial breathing with high frequency and forceful branchial movements but without any immediate attempts to surface for air.

It was possible in a few cases to analyse the P_{CO_2} in expired air and pulmonary arterial blood. In expired air P_{CO_2} varied from 5 to 7 mm. Hg while two measurements revealed a P_{CO_2} in pulmonary arterial blood of 6 mm. Hg during the time the fishes remained in normal aerated water.

DISCUSSION

The advent of air breathing in vertebrates instigated major changes in the structure of the organs for gas exchange and the supporting vascular apparatus. The Australian lungfish, *Neoceratodus forsteri*, represents an early stage in this transition with gill ·breathing still responsible for the major part of the gas exchange (Lenfant, Johansen & Grigg, 1966; Johansen, Lenfant & Grigg, 1967). *Neoceratodus* is unable to live for any extended length of time out of water. The African and South American lungfishes (*Protopterus* and *Lepidosiren*) on the other hand are primarily air breathers and tolerate months of exclusively terrestrial life during their periods of aestivation (Smith, 1931; Carter & Beadle, 1930; Sawaya, 1946).
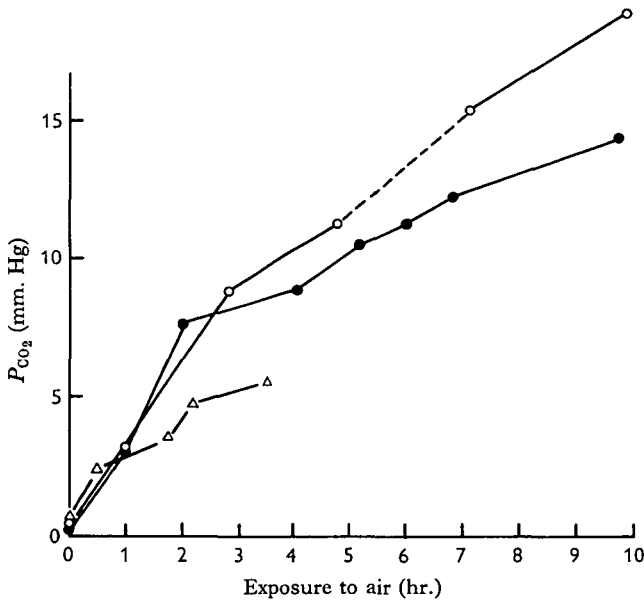

Fig. 9. Increase of CO_2 tension with time for three specimens of *Lepidosiren* re-breathing air in a closed respiration chamber.

In discussing the data obtained in the course of the present investigation it should be borne in mind that the specimens studied were young and of small size, which severely restricted the number of blood vessels that could be cannulated for sampling. Furthermore, blood sampling had to be restricted to retain conditions as normal as possible. The *in vitro* work was done on pooled blood from three different specimens. The O_2–Hb dissociation curve of *Lepidosiren* blood revealed a very high affinity for oxygen exceeding that for *Neoceratodus*. Several possibilities may be invoked to explain this difference: the affinity for oxygen may have been affected by the age of the animals and by the storage of the blood for 48 hr. The hypoxic water of the swamps representing the normal habitat of *Lepidosiren* (Carter & Beadle, 1931) may well have provided the necessary selection pressure for development of a high affinity for oxygen. This view, however, opposes that of Willmer (1931) who was unable to demonstrate such a correlation in the tropical swamp-water fishes he studied. Finally,

the deep burrows used for aestivation during seasons of drought may present an hypoxic environment that could lead to the development of a high-affinity haemoglobin.

The presence of a small Bohr effect is in keeping with the high prevailing CO_2 tensions in the swamp water (Carter & Beadle, 1931) and also with the argument developed by Carter (1951) that the transition from water breathing to air breathing correlates with a decrease in the influence of hydrogen ions on the O_2–Hb affinity.

The higher buffering capacity of the blood of *Lepidosiren* as compared with that of *Neoceratodus* (Fig. 4) may reflect the different stages the two species occupy in the transition from water breathing to air breathing. One can trace an increased buffering capacity of the blood correlated with an elevation of the arterial P_{CO_2} tension in more typical air breathers. This line of reasoning receives support from recent work on amphibians (Lenfant & Johansen, 1966).

The breathing pattern as well as the data obtained on gas exchange show that *Lepidosiren* is primarily an air breather. The extensive reduction in the branchial respiratory circulation is itself suggestive that aerial respiration is responsible for the major part of the oxygen uptake in *Lepidosiren*. In *Neoceratodus* at rest the pulmonary arterial blood was fully saturated when the fish was in normal aerated water (Lenfant *et al.* 1966). Thus there was little or no opportunity for the lung to contribute to gas exchange. The data listed in Table 3 indicate that in *Lepidosiren* the pulmonary arterial blood is normally less than 40–50 % saturated with oxygen. The active role of the lung in gas exchange is also readily apparent from Figs. 6 and 7. It is interesting that the air taken in at one breath by an animal resting in aerated water suffices to maintain nearly full O_2 saturation of pulmonary venous blood through most of the interval between breaths.

The functional value of gills and lungs present simultaneously in an animal is closely tied in with the quality of the blood perfusing the two gas-exchange systems. The lungfishes present a crucial transition in the development from the single vascular circuit in the typical fishes to the completely separated systemic and pulmonary circuits in birds and mammals. It has been alleged on anatomical evidence that the partitioning of the heart into right and left parts among the lungfishes is most complete in *Lepidosiren* and least complete in *Neoceratodus*, with *Protopterus* occupying an intermediate position (Robertson, 1913). In *Lepidosiren* there is a separate return of the pulmonary venous blood to the left atrium; the reduced gill filaments are sparsely vascularized (Fullerton, 1931), and all the arterial arches can be traced uninterrupted from the ventral to the dorsal side of the branchial arches (Foxon, 1950) making it possible for blood to by-pass the respiratory portions of the branchial circulation. The present data testify that a clear selective passage of blood must take place through the heart of *Lepidosiren* with a preferential flow of the pulmonary venous blood to the most anterior branchial arches contributing most to the systemic circulation, while most of the systemic venous blood must be conveyed to the most posterior branchial arteries giving rise to the pulmonary arteries. Such selective passage is documented by the finding of consistently higher P_{O_2} values in the dorsal aorta than in the pulmonary artery (Table 3). The fact that this gradient increased following inhalation of pure oxygen substantiates the selective passage of the pulmonary venous blood to the systemic arteries.

The data justify the conclusion that hypoxic as well as hypercarbic water stimulates

air breathing, while excess oxygen in the water is without any effect. The chemorecep-tors responsive to hypoxic stimulation must be located on the exterior of the fish facing the main body of water or in the efferent branchial arteries. The latter seems less probable because the gills are poor exchangers for oxygen and because ventilation often ceased altogether, resulting in a slow depletion of oxygen in the water enclosed in the branchial chambers.

Johansen et al. (1966) have recently analysed the influence of chemoreceptors on respiration in Neoceratodus and discussed the possible location of the chemoreceptors.

The role of the gills in gas exchange of Lepidosiren is difficult to assess fully. They certainly played a minor role in oxygen absorption and our data are not complete enough to evaluate their ability to eliminate CO_2.

Sawaya (1946) measured oxygen uptake of Lepidosiren when the animals were in water with free access to atmospheric air. His data demonstrate that only 2 % of the oxygen uptake takes place via the gills. The few measurements of P_{CO2} in expired gas and pulmonary arterial blood seem, however, to indicate that they are contributing to CO_2 elimination. It seems justifiable to predict that the gas-exchange ratio (R_E) in the gills of Lepidosiren is above unity. Cunningham (1934) demonstrated that the skin in Lepidosiren functions as a gas exchanger predominantly in the elimination of carbon dioxide with a gas-exchange ratio exceeding 10. One should expect that all transitional forms utilizing both water and air as respiratory media will have an R_E above unity for exchange organs facing the water and less than unity for lungs and other accessory air-breathing organs. The applicability of this concept to respiration in amphibians was demonstrated by Krogh (1904). The gills of Lepidosiren are few and have very irregular filaments. The diffusion distance between water and blood is also extremely long, on the average much above 10 μ (Fullarton, 1931). Among teleosts studied the longest distance measured was found in catfish (bullhead) with 10 μ against less than 5 μ for most teleosts and less than 1 μ for active fish like mackerel and herring (Steen & Berg, 1966). In the present context it should be remembered that our specimens were juvenile. Fullarton (1931) has clearly demonstrated the number of gill filaments to increase with age up to the adult stage in Lepidosiren.

It is of considerable interest that the rate and depth of branchial breathing were stimulated by mechanical disturbance in the water (Fig. 5). This seems to be the first report of a mechano-sensitive influence on the breathing pattern of a fish. This is of particular significance in view of the habitat of Lepidosiren, which is usually shallow stagnant swamp lakes extremely low in oxygen and rich in CO_2. Stirring or agitation of these waters will improve their quality for respiratory purposes. The coupling of a mechanoreceptive sensory system with the regulation of breathing seems to be a very specialized adaptation which should be carefully looked for in other fishes living in stagnant tropical waters.

The values obtained for the oxygen uptake, $\dot{V}_{O_2}$, at 22° C. when the fish was in water with access to air, are very similar to those reported by Sawaya (1946) also working on Lepidosiren. The present study gave 53·4 ml. O_2/kg./hr. as an average of four runs on two specimens compared with 41·9 ml. O_2/kg./hr. for seventeen specimens studied by Sawaya. These values also compared well with those given by Smith (1930) working on metabolism of Protopterus. The oxygen uptake when the fish were confined in air (Fig. 8) showed a drop, reducing the $\dot{V}_{O_2}$ in one case to 20 % of the initial value in

$5\frac{1}{2}$ hr. (fish 11). The other fish showed a similar but less pronounced trend. It merits considerable interest that this reponse cannot be the result of a reduced O_2 availability in the air since Pi_{O_2} only fell to 125 mm. Hg. Rather it seems that exposure to air as such brings about a reduction in metabolic rate. Smith (1930), in his studies on metabolism of *Protopterus*, measured an oxygen consumption during aestivation (average duration of aestivation was 350 days) about one-third of that during active life. Our data tentatively suggest that *Lepidosiren* can adjust very promptly to the aestivating level of metabolism. Fig. 9 demonstrates a pronounced increase in external P_{CO_2} during the measurements of oxygen uptake in air in a closed system. This increase may be one factor related to the depressed oxygen uptake.

SUMMARY

1. Respiratory properties of blood and pattern of branchial and pulmonary gas exchange have been studied in twelve specimens of the South American lungfish, *Lepidosiren paradoxa* (Fitz).

2. Haematocrit ranged from 14 to 19 % and blood oxygen capacity from 4·9 to 6·8 vol. %. The blood had a high affinity for O_2 with a P_{50} value of 10·5 mm. Hg at P_{CO_2} 6 mm. Hg and temperature 23° C. The Bohr effect was low.

3. The CO_2 dissociation curves show a steep ascending slope resulting in a relatively high CO_2 combining power at physiological values of blood P_{CO_2}. The Haldane effect was small. Buffering capacity of oxygenated whole blood was high and exceeded that in typical water breathers.

4. Air breathing was prominent and intervals between air breaths varied from 3 to 10 min. Branchial respiratory movements were extremely shallow and showed a labile frequency. Air breathing was stimulated by hypoxic and hypercarbic water while hyperoxygenated water had no effect. Branchial respiratory rate showed a marked acceleration in response to mechanical agitation of the water.

5. Gas exchange was predominantly carried out by pulmonary breathing. In less than 10 min. the P_{O_2} of expired gas dropped from 150 mm. Hg to less than 30 mm. Hg. The shallow branchial breathing with very low ventilation values resulted in a low O_2 uptake via the gills.

6. Blood-gas analysis documented a clear selective passage of blood through the only partially divided heart. A consistently higher P_{O_2} in dorsal aortic than in pulmonary arterial blood indicates a preferential passage of pulmonary venous blood to the anterior branchial arteries giving rise to most of the systemic circulation while systemic venous blood was largely conveyed to the most posterior branchial arteries giving rise to the pulmonary arteries.

7. The oxygen uptake for fish resting in water with access to air averaged 53·4 ml./hr./kg. Exposure to air lowered the O_2 uptake markedly.

8. The increased importance of pulmonary breathing in *Lepidosiren* is discussed in relation to the transition from water breathing to air breathing.

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