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# RESPIRATORY PROPERTIES OF BLOOD AND PATTERN OF GAS EXCHANGE IN THE LUNGFISH *Neoceratodus forsteri* (Kreffft)

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**Abstract.** Blood respiratory properties and gas exchange patterns have been studied in the lungfish *Neoceratodus*. O<sub>2</sub> - Hb dissociation curve reveals a high affinity for O<sub>2</sub> (P<sub>50</sub> of 11 mm Hg at P<sub>CO<sub>2</sub></sub> 3.5 mm Hg). No Root effect but a pronounced Bohr effect was discernible. Temperature exerted only a minor influence on the affinity for O<sub>2</sub>. The CO<sub>2</sub> dissociation curves conform to the shape observed in other classes of vertebrates and show a steep initial portion. The buffering capacity of the blood was low being 13.3 mMol/l/pH. An increased buffering power upon reduction of Hb was apparent.

During rest in well oxygenated water, gill breathing prevailed entirely. Pulmonary arterial blood assumed to represent mixed arterial blood characteristically showed a P<sub>CO<sub>2</sub></sub> averaging 3.5 mm Hg. PaO<sub>2</sub> was 40 mm Hg corresponding to an O<sub>2</sub> saturation of 95 %. The pulmonary venous blood showed gas partial pressures in equilibrium with those in pulmonary arterial blood and air, documenting the minor importance of the lung as a gas exchanger during these conditions. When the fish was artificially exposed to air PaO<sub>2</sub> diminished to 10-15 mm Hg with a concurrent increase in PaCO<sub>2</sub> to 20 mm Hg in 30 min. While the lung performed well as an O<sub>2</sub> absorber during air exposure it was totally inefficient in eliminating CO<sub>2</sub>. The data indicate that *Neoceratodus* is unfit to live out of water for any length of time.

**Keywords:** Air breathing in lungfish, Gas exchange in lungfish, Aquatic respiration, Lungfish *Neoceratodus*, Blood gases, Transition from waterbreathing to airbreathing, Evolution of respiration

The lungfishes represent an important step in the direct transition from waterbreathing to airbreathing vertebrates in that they possess both gills and lungs, the latter being true homologues of higher vertebrate lungs. They are very archaic forms with a history of wide distribution but the three surviving genera are geographically isolated with *Protopterus* living in Africa, *Lepidosiren* in South America, and *Neoceratodus* confined to Australia (SMITH, 1931). The three genera show progressive structural, physiological and behavioral adaptations in the transition from waterbreathing to airbreathing; *Neoceratodus* being predominantly a waterbreather while the others are primarily airbreathers. The lungfishes provide a unique opportunity to study the physiological adaptations correlated with the emergence of airbreathing. The present investigation concerns the respiratory properties of the blood and the normal pattern of gas exchange in the Australian lungfish, *Neoceratodus forsteri*.

## Material and methods

Fifteen specimens of *Neoceratodus forsteri* were netted in the Burnett River in Queensland, Australia, and transported to large holding tanks at the University of Queensland in Brisbane. The fish lived well in captivity and could be retained in good condition for several months. They ranged in weight from 4.2 to 8.8 kg.

Fig. 1 provides a schematic representation of the two respiratory organs in *Neoceratodus*. The blood leaving the heart (Q<sub>T</sub>) is considered to be mixed venous blood (v) which on passage through the branchial vessels of the gills is assumed to be uniformly arterialized (a). Blood leaving the gills is separated into two main circuits, Q<sub>t</sub> indicating the tissue or systemic perfusion, and Q<sub>L</sub> the perfusion of the single lung by the pulmonary arteries (PA). The blood in the latter circuit exchanges gases with the lung and returns separately to the left atrium of the heart through the pulmonary vein (PV). The lung would hence appear to represent an auxiliary gas exchanger with the main respiratory process taking place in the gills.

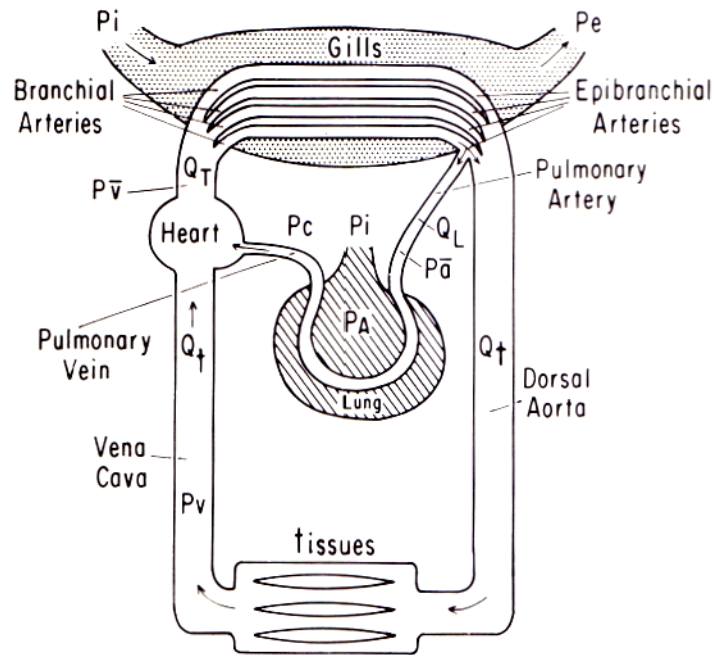


Fig. 1. Schematic representation of the double respiratory apparatus in *Neoceratodus*. Symbols are explained in text.

A greater importance of the lung in gas exchange would depend on a separate conveyance of the most oxygen depleted blood directly to the lungs, while the oxygen rich blood in the pulmonary vein should be distributed to the systemic arteries. This requires a separate return of the pulmonary venous blood to the heart, a feature unquestionably present in all the lungfishes. A selective outflow from the heart, however, is not readily apparent structurally. The development of such a separation in the vertebrates depends on the progressive septation of the heart and the concomitant separation of the outflow arteries from the heart, both concurrent with the gradual reduction of the gills. The status of *Neoceratodus* in this development is commented upon elsewhere (JOHANSEN, LENFANT and GRIGG, in manuscript).

Whenever the subsequent discussion has a bearing on the supposition of a uniformly mixed blood entering and leaving the gills of *Neoceratodus*, due cognizance will be given to the modifying effect of a possible selective passage.

A study of the dynamics of gas exchange in the system represented in fig. 1 required access for repeated sampling of blood returning from the tissues through the large systemic veins (v); blood returning from the lung via the pulmonary vein (c), blood leaving the heart v, and blood leaving the gills a. Samples of inspired (i) and expired (e) water, and gas samples from the lung (A) were also collected.

The animals were anesthetized by being placed in a solution of MS-222 for 10-30 min after which they were removed from the water and placed in a supine position in a wooden trough. During surgery a constant flow of water was passed retrogradely from the opercular openings over the gills and out through the mouth. Attempts to artificially ventilate with water flowing in the normal direction resulted in fatal "drowning" of the animal by filling its lung with water.

One branch of the left pulmonary artery was cannulated 15-20 cm posterior to where the lung changes from a ventral to a dorsal position with reference to the alimentary canal. The artery was exposed close to the midventral stripe of the lung. A polyethylene catheter (PE50) was inserted toward the heart. Since the vessel could not be freed from the pulmonary parenchyma the catheter was secured by passing fine ligatures into the lung and under the vessel. This procedure obstructed the further course of the artery, but extensive anastomosis between the remaining branch of the left pulmonary artery and the right pulmonary artery apparently maintained adequate perfusion of the lung. A pulmonary vein on the ventral aspect of the lung running parallel to the left pulmonary artery was cannulated in a similar fashion with the catheter passed downstream. The lung itself was cannulated through a small incision in the nonvascular midventral connective stripe. Great care was taken to seal any leakage of blood or air from the lung. Systemic venous blood was sampled by means of a catheter passed through and tied to the wall of the large posterior vena cava. The catheter was inserted in the anterior direction and did not obstruct the normal venous return in the vessel. Venous blood from the afferent branchial vessels was very difficult to obtain due to the sharp anterodorsal course of these vessels after leaving the heart. It proved possible in three animals to cannulate the left first afferent artery. All catheters were filled with heparinized

physiological saline solution. After completion of the surgical implantations the fish were allowed to recover 60-90 minutes before any samples or measurements were taken. The chronic implantations provided access to samples for as long as 10-14 days.

Blood, water and gas samples were analyzed for O<sub>2</sub> and CO<sub>2</sub> pressures and/or contents. The pH of selected blood samples was determined. Blood gas content was measured by gas chromatography (LENFANT and AUCUTT, 1966). Partial pressures of O<sub>2</sub> and CO<sub>2</sub> were measured with a Beckman Spinco gas analyzer using the oxygen macro electrode in a special cuvette (0.03 ml) for P<sub>O<sub>2</sub></sub> and the Severinghaus electrode for P<sub>CO<sub>2</sub></sub>. Calibration for P<sub>O<sub>2</sub></sub> was done with known gas mixtures or with blood equilibrated with these. The P<sub>CO<sub>2</sub></sub> electrode was calibrated with known gas mixtures. The instruments were calibrated prior to the measurements and frequently during the course of the experiments. pH was measured by means of a Beckman pH micro electrode and pH meter. All measurements and equilibration of calibrating samples were performed at 18 °C, except when the effect of temperature was specifically studied.

The oxygen capacity of the blood was measured by determining the O<sub>2</sub> content in blood equilibrated with gas having a P<sub>O<sub>2</sub></sub> of approximately 220 mm Hg. The oxyhemoglobin dissociation curves and the CO<sub>2</sub> dissociation curves were established according to a method described earlier (LENFANT and JOHANSEN, 1965). In this paper oxygen affinity is defined by the ratio 1/P<sub>50</sub>, where P<sub>50</sub> is the partial pressure of O<sub>2</sub> in mm Hg which is necessary to produce 50 % saturation when P<sub>CO<sub>2</sub></sub> is equal to the physiological Pa<sub>CO<sub>2</sub></sub>. The magnitude of the Bohr effect is estimated by  $\Delta \log P_{50} / \Delta \text{pH}$ . The Haldane effect is evaluated by the expression  $\Delta C_{\text{CO}_2} / (\text{O}_2 \text{ capacity})$  in which  $\Delta C_{\text{CO}_2}$  is the difference in CO<sub>2</sub> combining power between reduced and oxygenated blood when P<sub>CO<sub>2</sub></sub> is equal to the physiological Pa<sub>CO<sub>2</sub></sub>. The buffering capacity is expressed by  $\Delta \text{HC0}_3 / \Delta \text{pH}$  in which  $\Delta \text{HC0}_3$  represents the change in whole blood bicarbonate concentration in mMol/l when pH changes by 1 unit.

The minute ventilation (Ve) was measured by the method described by OGDEN (1945). Exhaled water was sampled from catheters sutured to the inside of the opercula and protruding out through the opercular openings. The extraction of oxygen from the water passing the gills was calculated from the equation:

$$\text{Extraction (\%)} = \frac{P_{\text{I}_{\text{O}_2}} - P_{\text{E}_{\text{O}_2}}}{P_{\text{I}_{\text{O}_2}}}$$

## Results

### RESPIRATORY PROPERTIES OF BLOOD

Table 1 shows hematocrit, O<sub>2</sub> capacity and CO<sub>2</sub> combining power from eight fish not subjected to previous sampling. Hematocrit and O<sub>2</sub> capacity averaged 31 % and 7.7 vol % respectively. The data reveal a proportionality between hematocrit and O<sub>2</sub> capacity. No significant relationship is discernible between the hematocrit and the CO<sub>2</sub> combining power at a fixed P<sub>CO<sub>2</sub></sub> of 6.0 mm Hg. After repetitive sampling the hematocrit diminished rapidly resulting in a lower oxygen capacity.

TABLE 1  
Hematocrit, oxygen capacity and carbon dioxide combining power<sup>1</sup>

No.	Hematocrit	O <sub>2</sub> vol %	CO <sub>2</sub> vol
1	31	7.8	16.5
5	32	-	-
6	33	8.3	14.7
7	28	7.5	13.4
8	27	6.7	9.5
9	35	9.0	13.0
10	36	8.5	14.0
12	24	6.0	15.5
Means	31	7.7	13.7

<sup>1</sup>All samples were equilibrated with a gas mixture containing O<sub>2</sub> = 29.3 % and CO<sub>2</sub> = 0.84 .

Fig. 2 depicts a series of O<sub>2</sub>-Hb dissociation curves at different P<sub>CO<sub>2</sub></sub>. The oxygen contents correspond to the bound oxygen. The component of dissolved O<sub>2</sub> is subtracted and plotted separately. The dissociation curves are of a typical sigmoid shape and reflect a high affinity of the blood for O<sub>2</sub>. P<sub>50</sub> at P<sub>CO<sub>2</sub></sub> of 3.5 mm

Hg is about 11 mm Hg at 18°C.  $P_{100}$  ( $P_{O_2}$  to reach 100 % oxygen saturation) ranged from 55mm Hg to 75 mm Hg within the range of  $P_{CO_2}$  studied. No Root effect became discernible (ROOT, 1931) but a pronounced Bohr effect was apparent. Fig. 3 demonstrates more specifically the high affinity for  $O_2$  and the magnitude of the Bohr effect.

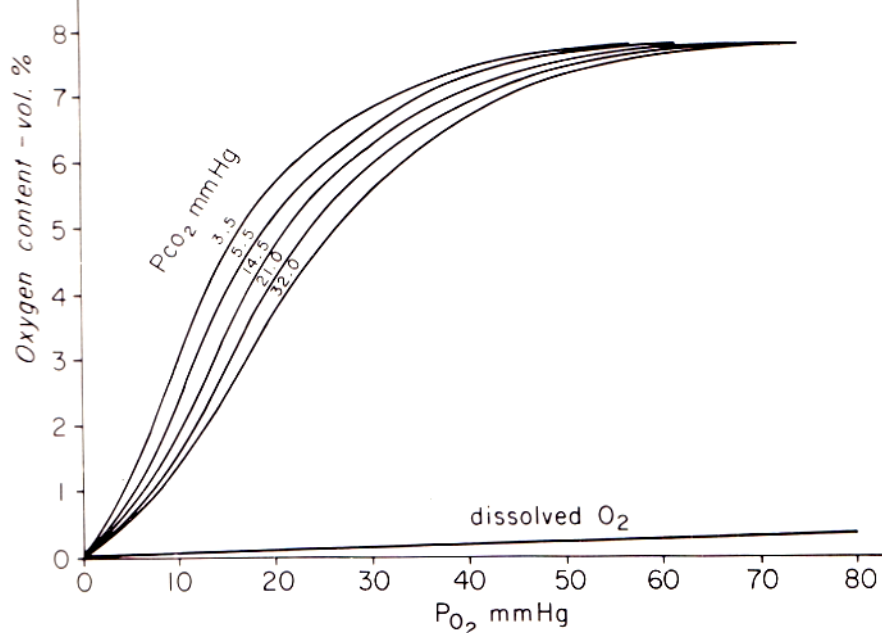


Fig. 2. Typical  $O_2$ -Hb dissociation curves at different  $PCO_2$ . Temperature 18°C. Hematocrit 36%.

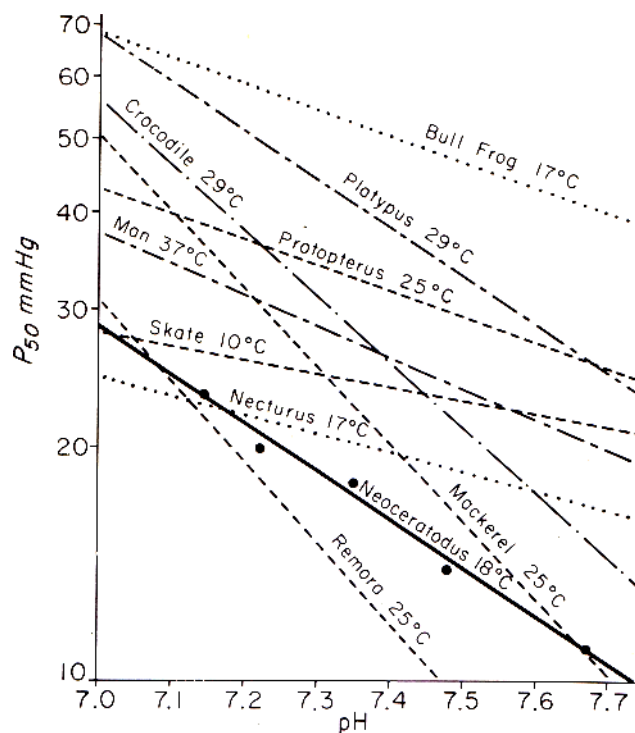


Fig. 3. A comparison of the Bohr effect in *Neoceratodus* (18°C) and various other vertebrates. Man, skate, crocodile (DILL et al., 1931, 1932, 1937) Protopterus (SWAN and HALL, 1966) Platypus (JOHANSEN et al., 1966) Necturus and bullfrog (LENFANT and JOHANSEN, in manuscript) Mackerel and Remora (HALL and McCUTCHEON, 1938).

Comparison with other species demonstrate a larger Bohr effect in *Neoceratodus* than in the African lungfish, Protopterus. The Bohr effect also exceeds that for representatives of elasmobranch fishes (skate), teleosts (Mackerel and Remora), amphibians (Necturus and bullfrog), reptiles (crocodile) and mammals (Man and Platypus). A moderate influence of temperature on the  $O_2$ -Hb dissociation curves is shown in fig. 4. A typical  $CO_2$  dissociation curve is shown in fig. 5. Its shape conforms to that of most other species

by having a steep initial portion. At higher  $PCO_2$  the slope levels off, but is at all times steeper than that for dissolved  $CO_2$  as depicted at the bottom of fig. 5. The effect of oxygenation upon the  $CO_2$  combining power (Haldane effect) is also revealed by this figure.

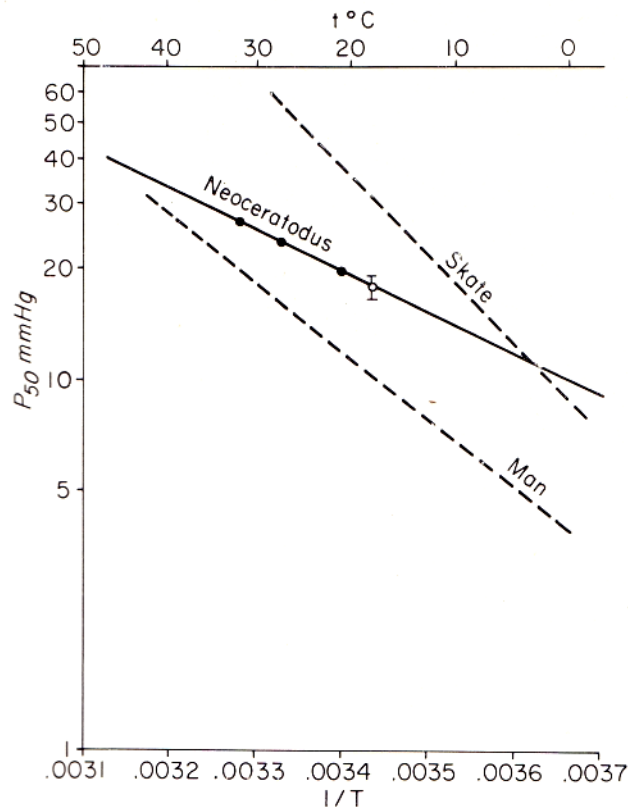


Fig. 4. Effect of temperature on  $P_{50}$  in blood from *Neoceratodus* ( $PCO_2 = 14$  mm Hg). Comparison with skate ( $PCO_2 = 1$  mm Hg) (DILL et al., 1932) and man ( $PCO_2 = 40$  mm Hg) (BROWN and HILL, 1923).

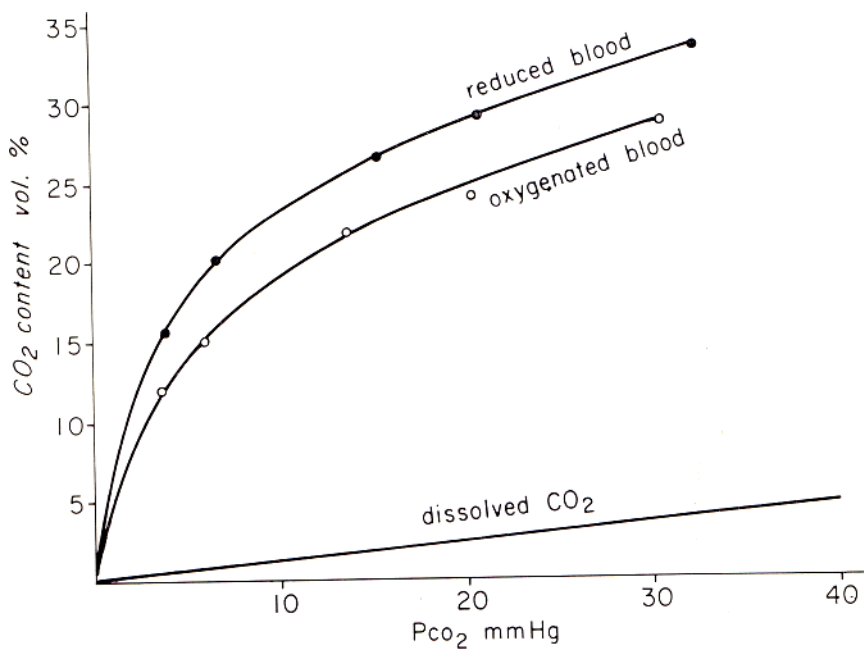


Fig. 5. Typical carbon dioxide dissociation curves in reduced and oxygenated whole blood from *Neoceratodus*. Temperature  $18^\circ C$ . Hematocrit 24 %.

The O<sub>2</sub>-CO<sub>2</sub> diagram (fig. 6) provides a summary of the respiratory properties of blood from *Neoceratodus*. It also shows the limits for normal gas exchange in this lungfish and demonstrates how the Bohr and Haldane effects affect the gas exchange in vivo.

A bicarbonate - pH diagram for lungfish blood is presented in fig. 7. The buffering capacity is moderate being only 13.3 mMol/l/pH. The role of Hb as a buffer becomes apparent from the difference in slope between the whole blood and the separated plasma line. A similar comparison between reduced and oxygenated whole blood reveals an increase in buffering power upon reduction of the Hb. At 18 °C a mean pK value for three animals calculated from the Henderson-Hasselbalch equation was 6.23.

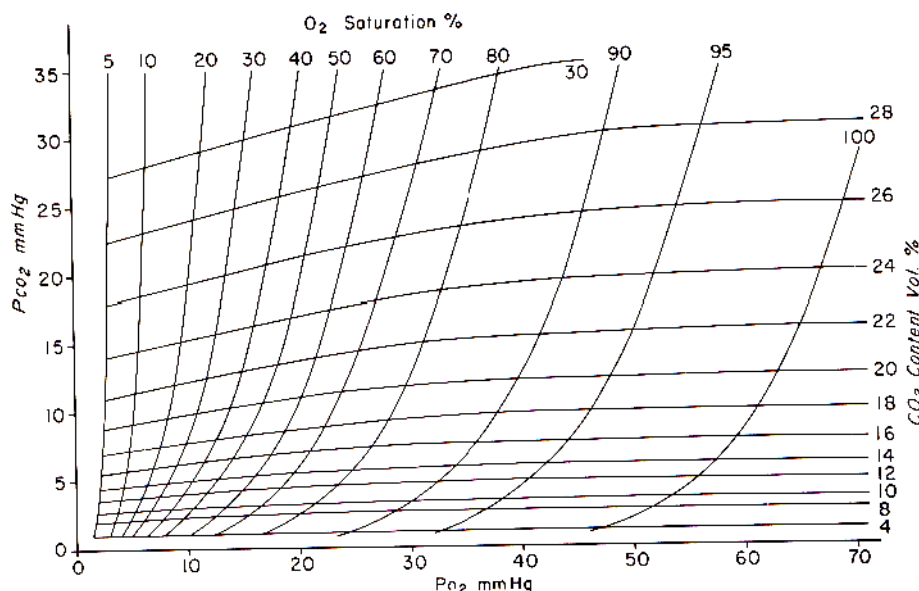


Fig. 6. Oxygen-carbon dioxide diagram for *Neoceratodus* blood. The iso-O<sub>2</sub> saturation and iso-CO<sub>2</sub> content lines were drawn from the mean O<sub>2</sub>-Hb and CO<sub>2</sub> dissociation curves established in blood sampled from three different animals. Temperature 18 °C.

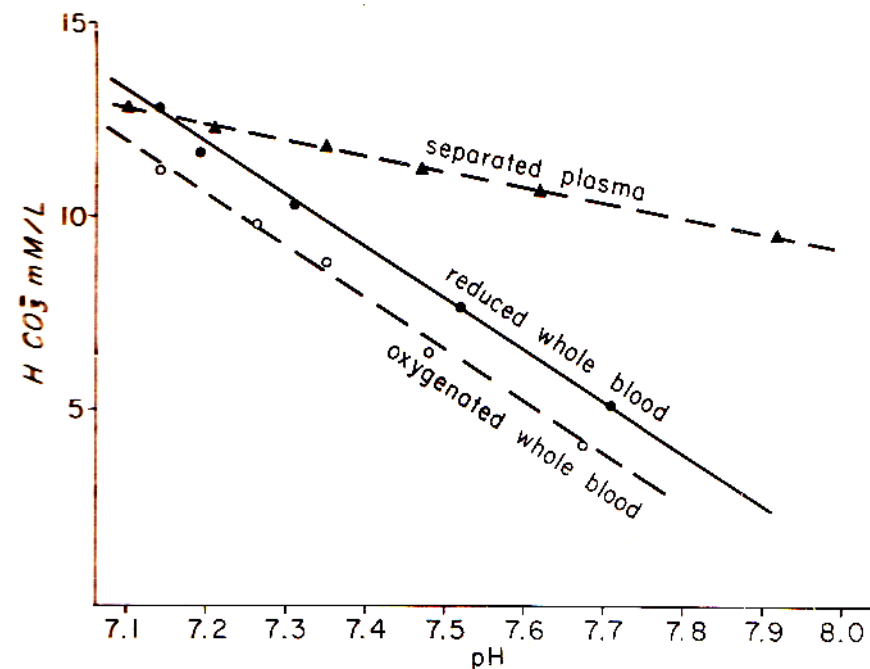


Fig. 7. Buffering capacity in oxygenated and reduced whole blood and in separated plasma from *Neoceratodus*. Mean of three experiments at 18 °C.

#### PATTFRNS OF GAS EXCHANGE

Table 2 is a composite tabulation of the mean values and range of the essential parameters of gas exchange determined in *Neoceratodus*. The samples were obtained from intact fish subsequent to complete recovery from anesthesia. The animals usually were at rest but were free to swim around in aquaria containing essentially air saturated CO<sub>2</sub> free water. No voluntary attempts to breath air took place during the period when the samples were collected. Intervals between voluntary surfacing for air in fish subjected to the above conditions were highly variable, however, in most cases they exceeded one hour. The results present a uniform picture. The arterial blood represented by samples from the pulmonary artery was always highly oxygenated showing an average O<sub>2</sub> saturation of 95 % (table 2 and fig. 6). Although the O<sub>2</sub> absorption on passage through the gills sufficed to oxygenate the blood to almost complete saturation, there always remained a large gradient in O<sub>2</sub> tension between exhaled water and blood from the pulmonary artery. The systemic venous blood sampled from the posterior vena cava had a rather low P<sub>O<sub>2</sub></sub> with an average of 14.3 mm Hg. Due to the high affinity of *Neoceratodus* blood for O<sub>2</sub>, the corresponding saturation was high; 50 % with a range from 20 to 70 %. A comparison between the gas tensions in pulmonary arterial and venous blood and between those in the lung disclosed that the air in the lung is in equilibrium with both the arterial and venous pulmonary blood in the intervals between air breathing. In a few cases when samples of blood from the first afferent branchial artery were obtained, they showed partial pressures of O<sub>2</sub> and CO<sub>2</sub> between those prevailing in the vena cava and the pulmonary vein. Table 2 furthermore reveals low values for PCO<sub>2</sub> in all compartments in accordance with earlier findings from typical waterbreathers. The pH values of blood were within the range normal for poikilothermic vertebrates.

TABLE 2  
Means PO<sub>2</sub>, PCO<sub>2</sub> and pH in fish in oxygenated, CO<sub>2</sub> free water

Fish no.	No. of Experiment	PI <sub>O<sub>2</sub></sub> mmHg	PI <sub>O<sub>2</sub></sub> -Pe <sub>O<sub>2</sub></sub> mmHg	<u>PI<sub>O<sub>2</sub></sub>-Pe<sub>O<sub>2</sub></sub></u> PI <sub>O<sub>2</sub></sub>	Pe <sub>O<sub>2</sub></sub> -Pa <sub>O<sub>2</sub></sub> mmHg	BRR per min	Ve ml/min	VO <sub>2</sub> ml/min
1	3	140	39.6	28.2				
2	1	137	21.3	15.4	70.7	22		
3	5	137	29.8	23.4	49.6	42		
4	3	136	59.3	43.3	36.6	23		
5	2	139	27.5	20	73	34		
6	1	135	50	37	41	28		
7	1	124	55	45.8	22	36		
8	9	125	55.2	44.2	33.6	30		
10	4	121	79.7	65.8		32	275	0.86
11	4	132	41	32.7	51.5	29	328	0.52
12	10	126	61.5	48.8		30	343	0.84
13	3	129	40.7	31.2	54.7	26		
Mean		131	46.7	36.3	48.1	30	315	0.74
	Low	120	13	10	14	22	83	0.19
Range	High	146	103	73.6	80	40	747	1.73

Table 3 summarizes the data concerning the exchange of O<sub>2</sub> between blood and water. The extraction of O<sub>2</sub> from the water on passage past the gills is seen to be highly variable ranging from 10 % to 73.6 %. At the prevailing temperature of 18 °C the branchial respiratory rate averaged 30 per min. The mean value for ventilation was 315 ml/min, but large variations ranging from 83 ml/min to 747 ml/min attested to a striking lability in this parameter. The same was true for the values of O<sub>2</sub> uptake (V<sub>O<sub>2</sub></sub>) through the gills.

TABLE 3  
Parameters concerning the exchange of O<sub>2</sub> between water and blood

Fish no.	No. of Experiment	PI <sub>O2</sub> mmHg	PI <sub>O2</sub> -Pe <sub>O2</sub> mmHg	$\frac{PI_{O_2}-Pe_{O_2}}{PI_{O_2}}$	Pe <sub>O2</sub> -PaO <sub>2</sub> mmHg	BRR per min	Ve ml/min	VO <sub>2</sub> ml/min
1	3	140	39.6	28.2				
2	1	137	21.3	15.4	70.7	22		
3	5	127	29.8	23.4	49.6	42		
4	3	136	59.3	43.3	36.6	23		
5	2	139	27.5	20.0	73.0	34		
6	1	135	50.0	37.0	41.0	28		
7	1	124	55.0	45.8	22.0	36		
8	9	125	55.2	44.2	33.6	30		
10	4	121	79.7	65.8	-	32	275.	0.86
11	4	132	41.0	32.7	51.5	29	328.	0.52
12	10	126	61.5	48.8	-	30	343.	0.84
13	3	129	40.7	31.2	54.7	26		
Mean		131	46.7	36.3	48.1	30	315.	0.74
	Low	120	3.0	10.0	14.0	22	83.	0.19
Range	High	146	103.0	73.6	80.0	40	747.	1.73

A few experiments permitted simultaneous sampling and analysis of the carbon dioxide pressure in exhaled water and blood from the pulmonary artery and vena cava. It is noteworthy that the PCO<sub>2</sub> of the efferent water and blood were near equilibrium at times. This represented a distinct difference from the state of equilibrium in respect to PO<sub>2</sub> (table 4.).

TABLE 4  
Differences between arterial blood, gas from the lung and efferent water

	PO <sub>2</sub> mm Hg				PCO <sub>2</sub> mm Hg				
	I	e	a	A	v	e	a	A	v
8	120	52.0	27.0	29.0	10.	4.10	4.50	4.5	6.6
	124	48.0	34.0	36.0	13.	3.90	3.80	3.9	6.1
	120	48.0	25.0	24.0	10.	4.20	4.60	4.5	6.7
Mean	126	49.3	28.7	29.7	11.	4.06	4.30	4.3	6.4
10	130	90.0	39.0			4.00	4.10		
	140	95.0	34.0			3.60	4.80		
	135	85.0	42.0			4.30	4.80		
Mean	137	90.0	38.3			3.97	4.56		

Fig. 8A and 8B show the response to an artificial inflation of the lung and to spontaneous breathing respectively. There is a prompt increase in PO<sub>2</sub> of the pulmonary venous blood with a concurrent decrease in PCO<sub>2</sub>. The magnitude of the changes bore an obvious relationship to the volume of gas injected or inspired and the extent of the underlying blood flow controls the rate of return to the initial level.



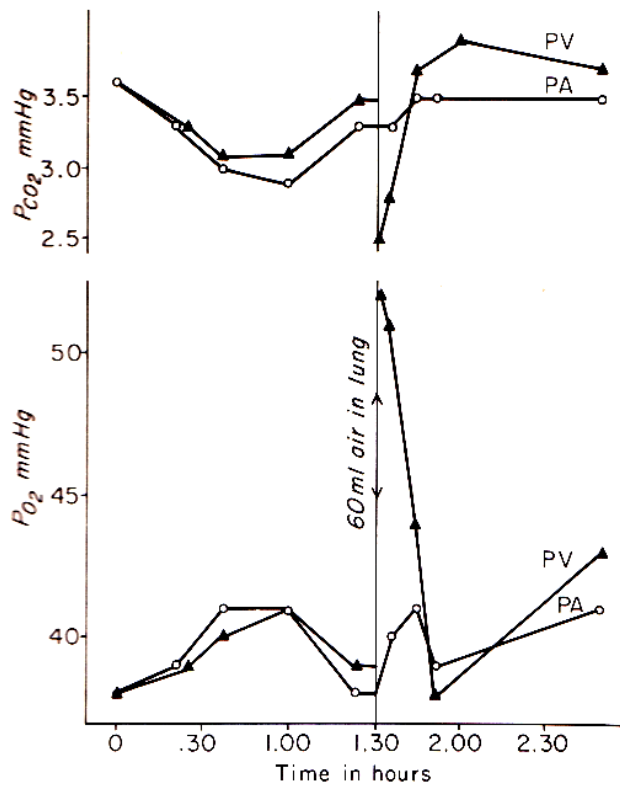


Fig. 8A

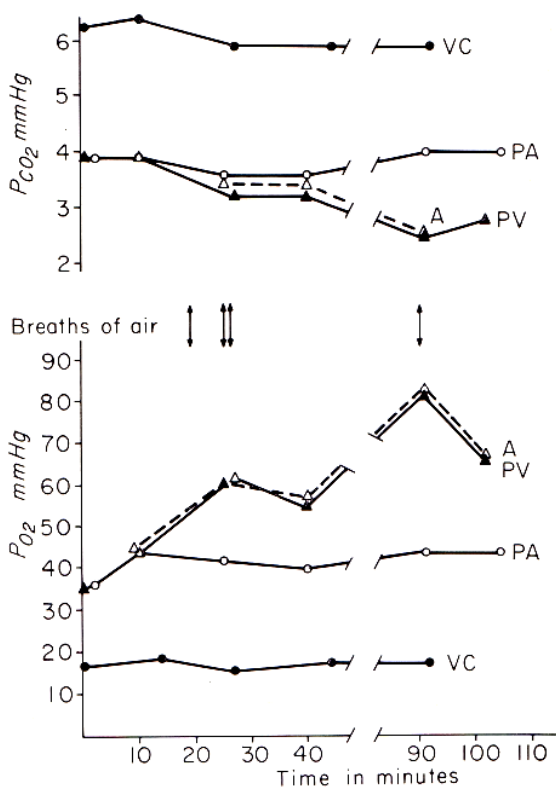


Fig. 8A and B

A. Effects of artificial lung inflation with room air on the  $O_2$  and  $CO_2$  partial pressures in various vessels of *Neoceratodus*.  
 B. Effects of spontaneous breathing of room air on the  $O_2$  and  $CO_2$  partial pressure in various vessels. VC = vena cava; PA = pulmonary artery; PV = pulmonary vein, A = gas from lung.

Although the Australian lungfish normally does not leave the aquatic medium, several specimens were artificially removed from water while the changes in blood gas tensions were monitored. Fig. 9A depicts these changes in pulmonary arterial blood plotted in a  $PO_2$ - $PCO_2$  diagram.  $PCO_2$  accumulated rapidly, demonstrating an interruption of the ability to remove  $CO_2$  through the gills by branchial breathing. Within 39 min the arterial  $PO_2$  dropped from 39 mm Hg to less than 10 mm Hg with a concomitant rise in  $PCO_2$  from approximately 3.5 mm Hg to 19 mm Hg. The corresponding changes in oxygen saturation would be from 97 % to 18 %. On return to water the picture is reversed. Fig. 9B illustrates a similar experiment during which blood was sampled from both the pulmonary artery and vein. The pulmonary artery samples display the same pattern encountered in fig. 9A whereas the pulmonary venous blood increased in  $PO_2$  from 40 mm Hg to 93 mm Hg. The  $PCO_2$  accumulated to about 3/4 of the level reached in the pulmonary artery during the period in air.

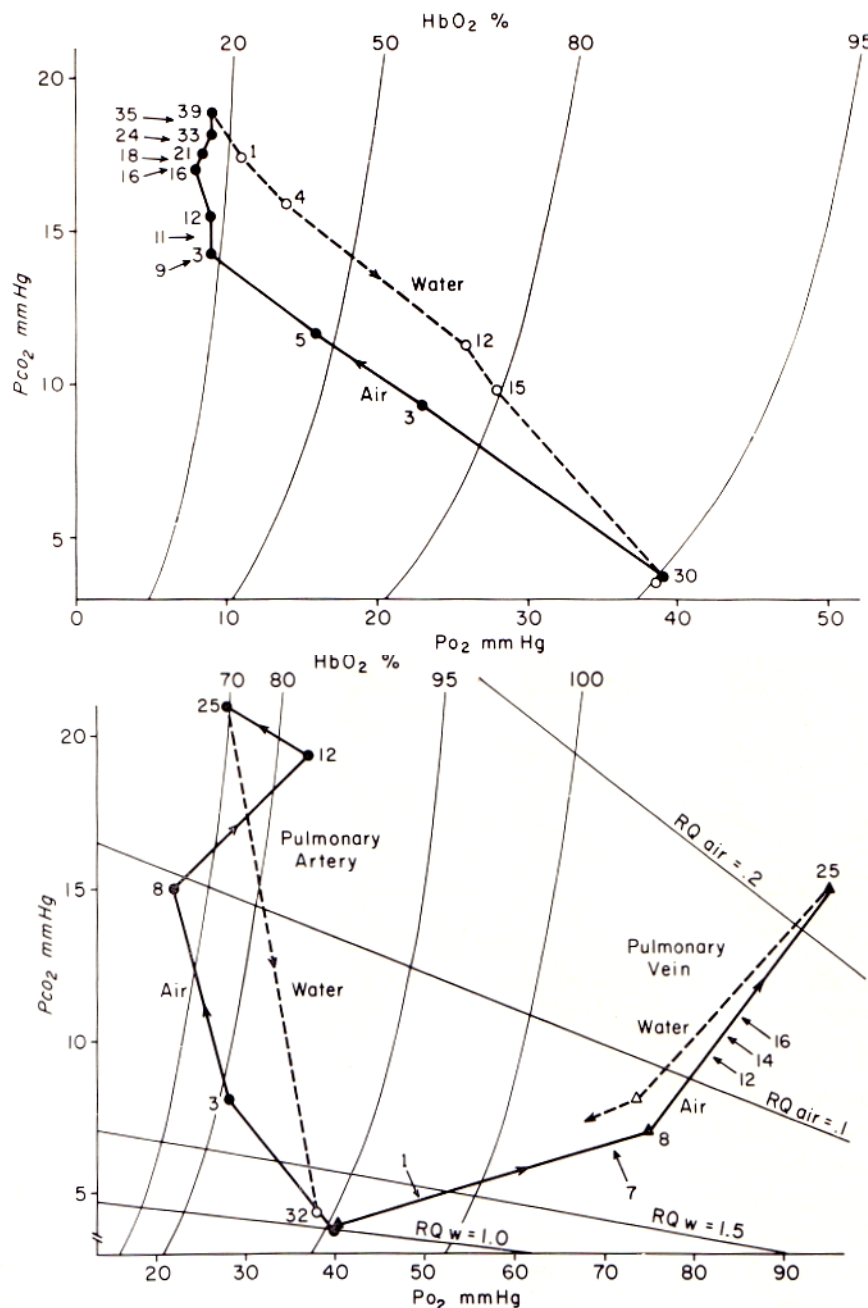


Fig. 9. Effects of artificial exposure to air in two different fish A and B. The iso- $O_2$  saturation lines are reproduced from fig. 6. The iso-RQ lines in air and water were calculated for  $P_{IO_2} = 140$  mm Hg. Each point represents a different sample. The numbers near the points represent the sampling time from start of air exposure (filled circles) or from the return to water (open circles). The arrows with adjacent numbers represent spontaneous air breathings and the time at which they occur. All times in minutes.

The changes induced in the acid-base equilibrium upon artificial exposure to air are plotted in fig. 10. Air exposure invoked an immediate metabolic alkalosis in compensation for the progressive respiratory acidosis, which later becomes inefficient and pH dropped markedly from 7.55 to less than 7.30.

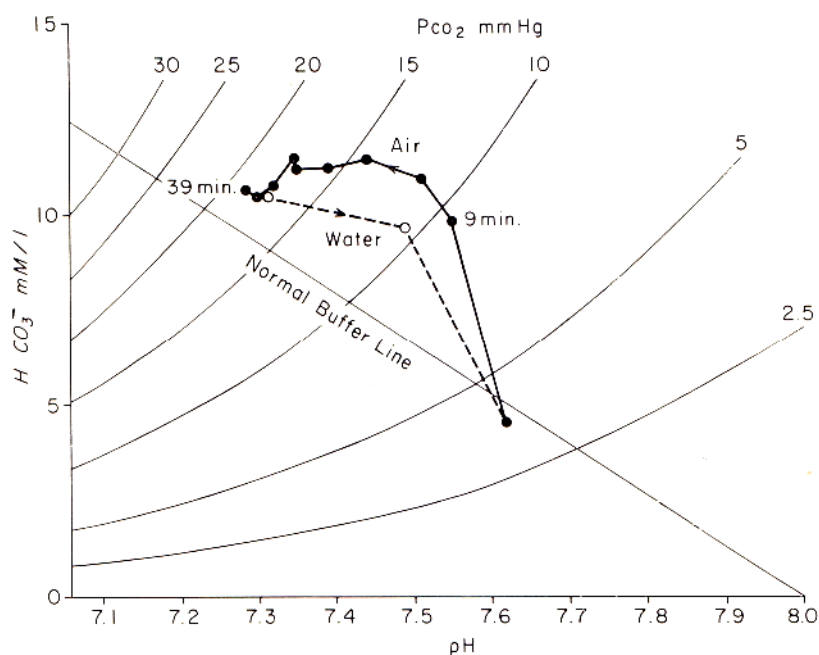


Fig. 10. Acid base changes during exposure to air in fish A of fig. 9. The iso-PCO<sub>2</sub> lines were calculated and the normal buffer line is reproduced from fig. 7. Each point represents an actual sample.

## Discussion

Neoceratodus is regarded as a permanent water dweller that never moves on land or buries itself in mud during seasons of drought. Its habitat however, strongly suggests that the fish at times will encounter water of very low oxygen content.

KROGH and LEITCH (1919) contended that fishes able to survive in oxygen poor water displayed adaptive changes in the respiratory properties of their blood. For instance, the bloods from carp, pike and eel, which live in water poor in O<sub>2</sub>, saturate at much lower O<sub>2</sub> tensions than blood from cod and plaice which are not exposed to low O<sub>2</sub> tensions. WILLMER (1934) opposed the theory of such adaptive change and maintained that differences in the slope and form of O<sub>2</sub>-Hb dissociation curves of animals representing widely different habitats are the result of adaptive changes to the CO<sub>2</sub> contents in the external environment while little or no adaptive significance is referable to the changes in external O<sub>2</sub> availability. Other workers dissent from Willmer's view (FRY and BLACK, 1938; HALL and McCUTCHEON, 1938 and BLACK, 1940) and report marked differences in the P<sub>50</sub> values of a variety of fish at low PCO<sub>2</sub>. The variations can be correlated with the tolerance to low external PO<sub>2</sub>.

Neoceratodus blood shows a high affinity for oxygen at low PCO<sub>2</sub> and differs in this respect from Protopterus (FISH, 1956; SWAN and HALL, 1966). It is reasonable to interpret this difference on the basis that Neoceratodus is only an occasional airbreather while Protopterus habitually breathes air. The oxygen availability within their normal behavior pattern may hence be markedly different for Neoceratodus compared to Protopterus.

Adaptive value also becomes apparent when comparing the magnitude of the Bohr effect between the different species of lungfish. The conspicuously large Bohr effect in Neoceratodus blood is not disadvantageous as long as the animal remains in water and can make use of the high efficiency of the gills in the elimination of CO<sub>2</sub>. Protopterus (FISH, 1956) and Lepidosiren (JOHANSEN and LENFANT, in manuscript) display a considerably smaller Bohr effect in accord with the long periods these forms spend under strictly terrestrial conditions.

WILLMER (1934) maintains that tropical fresh water fish have adapted such that the blood of those species living in stagnant CO<sub>2</sub> rich water shows little effect of CO<sub>2</sub> on oxygen affinity whereas blood from fish in the rivers and open waters is very adversely affected as an O<sub>2</sub> carrier by CO<sub>2</sub>. CARTER (1951) uses Willmer's data to argue that CO<sub>2</sub> insensitive blood must have evolved as a pre-adaptation

before fishes could live as waterbreathers in CO<sub>2</sub> rich environments. He points out the similarity of their blood with that of terrestrial vertebrates which also have their respiratory organs exposed to an elevated CO<sub>2</sub> tension. Carter concludes that a CO<sub>2</sub> insensitive blood is as essential to the evolution of an internal air breathing organ as it is to the invasion of stagnant tropical water. That such teleological arguments are extremely dangerous is demonstrated by the present results involving *Neoceratodus* which possesses a functional lung despite the large Bohr effect of the blood.

Another adaptation in the affinity of the blood for oxygen is expressed in the response to temperature change. Species that live in a thermocline or possess a faculty of internal temperature regulation (homeotherms) show in general a marked influence of temperature change on the O<sub>2</sub>-Hb dissociation curve. *Neoceratodus* is reported to face rather marked annual temperature changes in the water ranging from 11 to 31° (GRIGG, 1965a). Thus, the low sensitivity of the O<sub>2</sub> affinity to temperature changes seems to be of adaptive significance in comparison with blood from species in surroundings of more uniform temperature (fig. 4).

Carbon dioxide is transported in the blood as bicarbonates and carbamino compounds. The ability of any blood to combine with CO<sub>2</sub> may be represented by the "CO<sub>2</sub> combining power" or maximum amount of CO<sub>2</sub> present in a blood sample at a given saturation of O<sub>2</sub>, and at a known PCO<sub>2</sub>. In table 5, this information is presented for *Neoceratodus*, in comparison with other species of fish and representatives of higher vertebrates. In each case the blood was fully saturated with O<sub>2</sub>. *Neoceratodus* has a CO<sub>2</sub> combining power comparative to that of fishes in general which is lower than that of the amphibians which in turn is superseded by that of the mammals. The low CO<sub>2</sub> combining power of fish blood seems to occur in conjunction with a low hemoglobin content and/or a low level of arterial PCO<sub>2</sub> (table 5). The carp and the South-American lungfish (*Lepidosiren*) are seen to have a CO<sub>2</sub> combining power higher than the other fishes tested which correlates with the high hemoglobin content of the carp and the elevated Pa<sub>CO<sub>2</sub></sub> in *Lepidosiren* associated with its airbreathing habit (JOHANSEN and LENFANT, in manuscript). The same situation applies to amphibians, where *Necturus* has the lowest arterial PCO<sub>2</sub> and the lowest CO<sub>2</sub> combining power (LENFANT and JOHANSEN, in manuscript). Variations of CO<sub>2</sub> combining power are more pronounced in animals with a relatively low arterial PCO<sub>2</sub> because of the steep initial portion of their CO<sub>2</sub> dissociation curves. This point is substantiated when comparing variations among the mammals on one hand and fishes and amphibians on the other.

TABLE 5  
CO<sub>2</sub> combining power and Haldane effect in various species

Animal	Reference	Oxygen Capacity <sup>1</sup> vol %	Physiological Pa <sub>CO<sub>2</sub></sub> mm Hg	CO <sub>2</sub> combining power at Pa <sub>CO<sub>2</sub></sub> vol%	Haldane Effect
<i>Neoceratodus</i>	Pres. Exp.	6.67	3.6	11.	-.554
<i>Lepidosiren</i>	JOHANSEN and LENFANT in manuscript	5.72	7.0	21.0	-.263
Toadfish	ROOT, 1931	5.80	3.5	7.5	-.516
Sea Robin	ROOT, 1931	6.80	3.5	8.5	-.662
Dogfish	LENFANT and JOHANSEN, 1966	4.35	2.3	6.8	0.
Carp	FERGUSON and BLACK, 1941	12.50	3.8	21.0	-.659
Bullfrog	LENFANT and JOHANSEN in manuscript	8.02	8.0	32.0	-.238
<i>Necturus</i>	LENFANT and JOHANSEN in manuscript	6.26	5.0	18.7	-.366
<i>Amphiuma</i>	LENFANT and JOHANSEN in manuscript	7.62	7.0	36.0	-.207
Platypus	JOHANSEN <i>et al.</i> , 1966	22.90	28.0	41.0	-.262
Seal	Unpublished	29.30	44.0	45.0	-.128
Man	DILL <i>et al.</i> , 1937	20.00	40.00	48.3	-.325

<sup>1</sup>The values listed in this table correspond to combined O<sub>2</sub> only.

The role of oxygenation is essential in CO<sub>2</sub> transport. Although the Haldane effect is a corollary to the presence of a respiratory pigment, the variations observed between species and larger taxonomic units are

not immediately suggestive of being adaptive to the respiratory habits. Rather, the variations probably reflect fundamental differences in hemoglobin structure. However, the Haldane effect may bear some relationship to the Bohr effect, an idea supported by work on the dogfish (LENFANT and JOHANSEN, 1966) and *Neoceratodus* among the fishes and on the seal (IRVING et al., 1935) and man among mammals.

The buffering capacity in *Neoceratodus* is moderate compared to higher vertebrates but conforms to that of many other species of fish. Fig. 7 reveals that plasma contributes little to the total buffer capacity. This is in accord with the findings for most vertebrates but differs from conditions in the elasmobranch *Squalus suckleyi* (LENFANT and JOHANSEN, 1966) in which the buffers of the plasma predominate. The overall low blood buffering capacity in all waterbreathing vertebrates investigated can be looked upon as corollary to their low levels of  $\text{CO}_2$  and small arterio-venous changes in  $\text{PCO}_2$  of waterbreathing animals which do not require a high buffering capacity to maintain pH within a relatively narrow range.

A typical homeotherm airbreather, on the other hand, shows  $\text{PCO}_2$  variations which require far more potent buffer mechanisms to minimize changes in pH. Thus the lungfishes might provide clues to the factors underlying the phylogenetic appearance of an increase in the buffering capacity of the blood. *Neoceratodus*, which breathes air occasionally, does not show a change from the truly waterbreathing fishes in respect to buffer capacity. *Lepidosiren*, an obligatory air breathing lungfish, however, has a higher buffering capacity which may be accounted for by the larger variations of  $\text{PCO}_2$  (JOHANSEN and LENFANT, in manuscript). A similar comparison can be made between species of amphibians among which the bullfrog, which is mainly terrestrial, has a much higher buffering capacity than *Amphiuma* and *Necturus* which are exclusively aquatic (LENFANT and JOHANSEN, in manuscript).

#### GAS EXCHANGE

The arterial blood sampled from the pulmonary artery remained highly saturated with  $\text{O}_2$  under the conditions of the experiments. The fact that the systemic venous  $\text{O}_2$  saturation stayed high (average 50 %) indicates that the venous blood constitutes a sizeable reservoir of oxygen. In view of the steep  $\text{O}_2$ -Hb dissociation curve, the high saturation of both systemic venous and arterial blood may be necessary to maintain enough driving pressure for the exchange of  $\text{O}_2$  at the tissue level. This hypothesis is supported by the findings of higher partial pressures of  $\text{O}_2$  in other water breathing species, although the  $\text{O}_2$  saturation levels are lower (LENFANT and JOHANSEN, 1966). For instance, if the arterial blood in *Neoceratodus* had been 70 % saturated,  $\text{PaO}_2$  would have been approximately 17 mm Hg. A maintained utilization would yield a  $\text{PO}_2$  in systemic venous blood of approximately 7 mm Hg (fig. 6). This would require that the entire exchange of  $\text{O}_2$  would have occurred within a  $\text{PO}_2$  range of 10 mm Hg as opposed to 25 mm Hg as the average of the actually measured samples. It remains to be explained, however, why the blood after passing the gills shows such a low  $\text{PO}_2$ . In no instance (table 3) did the blood even come close to equilibrium with  $\text{PO}_2$  in efferent water. Shunting of blood past the respiratory surfaces, dead space diffusion and structural diffusion barriers or purely technical contamination of the water samples might account for the large difference ( $\text{PeO}_2$ - $\text{PaO}_2$ ). The same factors would also conceal to what extent a counter current exchange is taking place in the gills. The fact that  $\text{PCO}_2$  measured in efferent water and blood approached equilibrium (table 4) is strongly suggestive that part of the large  $\text{PO}_2$  gradient between blood and water results from a diffusion limitation. The essential point remains that the arterial blood, due to the high  $\text{O}_2$  affinity, is almost completely  $\text{O}_2$  saturated under the specified conditions of rest and high  $\text{PIO}_2$ . Other experiments on *Neoceratodus* have demonstrated a critical dependence of the arterial  $\text{O}_2$  saturation on the oxygen availability in the water (JOHANSEN et al., in manuscript).

It should also be inferred that the low  $\text{O}_2$  tension in the pulmonary artery may result from selective passage through the heart and the gills of oxygen depleted blood from the systemic veins. That a selective passage through the heart is possible in spite of only a partial anatomical separation has been documented for amphibians (JOHANSEN, 1962; HABERICH and EITNER, 1963) arid reptiles (WHITE, 1956, 1959; STEGGERDA and ESSEX, 1957). Fishes with auxiliary respiratory organs often show a marked reduction in the gill surface area (DUBALE, 1951) which could contribute to a nonuniform arterialization of the blood passing the gills. One might anticipate that such a degeneration would initially and primarily occur in the gills which convey the blood to the auxiliary respiratory organ thus increasing the importance of the pulmonary gas exchange. However, *Neoceratodus*, in contrast to the other lungfishes, seems to have uniformly well developed gills, an assumption that should be carefully checked by microscopic examination.

The data demonstrate an essential equilibrium between the air in the lung and the gas tensions in the pulmonary artery and vein indicating that the lung behaves like a highly perfused gas pocket during the long intervals between active airbreathing and that the metabolism of the lung parenchyma is low. In animal 5 a slightly different relationship was present in that  $\text{PCO}_2$  was lower than  $\text{PaO}_2$  whereas  $\text{PcCO}_2$  was higher than  $\text{PaCO}_2$ , a condition indicative of a low perfusion through the lung.

The fact that extraction of oxygen from the water at times reached 70 % suggests that *Neoceratodus* is principally a waterbreather. Low arterial  $P_{CO_2}$  levels support this contention. The data do not warrant a conclusion as to the presence of a counter current exchange in the gills. Conclusive evidence for this must await the analysis of blood selectively sampled from the various epibranchial arteries and information on the degree of shunting, dead space diffusion and inequalities of the water ventilation to blood perfusion ratios.

Figs. 8A and B demonstrate the minor importance of the lung in the overall gas exchange during resting conditions in well oxygenated water. In spite of an efficient exchange in the lung which may be inferred from the near equilibrium in  $PO_2$  between alveolar air and pulmonary venous blood, the  $PO_2$  increase in the latter is hardly perceptible in the pulmonary arterial blood during air breathing. This suggests that the blood flow through the lung is small compared to the total cardiac output, or that the pulmonary venous blood is selectively distributed to branchial arteries other than those giving rise to the pulmonary arteries. The dependence on the gills for gas exchange in *Neoceratodus* was also amply documented by the experiments involving removal of the fish from water. Figs. 9A and B indicate a rapidly developing hypoxia associated with a severe hypercapnia. The pulmonary arterial  $O_2$  saturation dropped from 95 % to less than 20 % in 9 min upon exposure to air (fig. 9A). Much of this reduction may be ascribed to the large Bohr effect when  $CO_2$  elimination by the gills has ceased. During the subsequent 30 minutes  $Pa_{O_2}$  remained at the same level while  $Pa_{CO_2}$  continued to increase. The crucial role of the gills for  $CO_2$  elimination is thus established as is the inadequacy of the lung in this respect despite repeated breathing. Lack of data concerning systemic  $P_{O_2}$  prevents an evaluation of the extent to which a selective distribution of the pulmonary venous blood may reduce the hypoxemia in the systemic arterial blood. It is noteworthy that several teleost fishes variously adapted for auxiliary  $O_2$  absorption are able to sustain air exposure better than *Neoceratodus* (BERG and STEEN, 1965; JOHNSEN, 1966).

In fig. 9B, comparison of the overall gas exchange in the two respiratory organs during water and air breathing confirms the trend expressed in fig. 9A with a less pronounced hypoxia. The gas exchange ratio (RQ) approximates 1 when the animal breathes by means of gills in water. Upon removal of the fish from water RQ drops to nil followed by a gradual increase to 0.2 after 25 min thus demonstrating the inability of the lung to functionally replace the gills. Inefficient  $CO_2$  elimination constitutes the more serious deficiency of the lung, while it performs more adequately as an  $O_2$  absorber. Thus *Neoceratodus* is not adapted to live out of water for a prolonged period of time. However, the lung seems to play a significant role in the adjustments to respiratory distress associated with hypoxic and hypercarbic water and possibly in exercise (JOHANSEN et al., in preparation; GRIGG, 1965b).

The changes in acid-base balance of the blood resulting from air exposure are shown in fig. 10. The respiratory acidosis ensuing from interruption of gill breathing follows a peculiar pattern and the time sequence of the compensatory mechanisms is not readily explainable. Hypercapnia is in general accompanied by an immediate reduction in pH which is minimized by the free buffers of the blood and results in changes following the normal buffer line. In the case of *Neoceratodus*, a large metabolic alkalosis initially prevented a rapid drop in pH. This compensatory response, however, soon became ineffective and pH dropped precipitously with no further increase in bicarbonate concentration. Normally in higher vertebrates a compensatory alkalosis develops slowly but maintains or restores the pH to normal values over longer periods.

We are at present unable to offer any satisfactory explanation for the course of the metabolic alkalosis in *Neoceratodus*. Further experimentation should deal with: the extent of elimination of chlorides from the vascular space, liberation of bicarbonates from the cellular to the extra-cellular compartments and possible water loss from the extra-cellular space. The abrupt alteration in the acid-base balance (at 9 min, fig. 9A) might be associated with a massive release of acid metabolites from hypoxic tissues caused by redistribution of the circulating blood. The removal of teleost fishes from water (air diving) has offered evidence conducive to such reasoning (LEIVESTAD et al., 1957).

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