Comp. Biochem. Physiol., 1967, Vol. 20, pp. 835-854

RESPIRATORY CONTROL IN THE LUNGFISH, *NEOCERATODUS FORSTERI* (KREFT)

KJELL JOHANSEN, CLAUDE LENFANT and GORDON C. GRIGG

Abstract-1. Respiratory control has been studied in the lungfish, *Neoceratodus forsteri* by measuring ventilation (V_e), oxygen uptake (VO_2), per cent O_2 extraction from water, breathing rates of branchial and aerial respiration and changes in blood gas and pulmonary gas composition during exposure to hypoxia and hypercarbia.

2. Hypoxic water represents a strong stimulus for compensatory increase in both branchial and aerial respiration. Water ventilation increases by a factor of 3 or 4 primarily as a result of increased depth of breathing.

3. The ventilation perfusion ratio decreased during hypoxia because of a marked increase in cardiac output. Hypoxia also increased the fraction of total blood flow perfusing the lung. Injection of nitrogen into the lung evoked no compensatory changes.

4. It is concluded that the chemoreceptors eliciting the compensatory changes are located on the external side facing the ambient water or in the efferent branchial blood vessels.

5. Elevated pCO_2 in the ambient water depressed the branchial respiration but stimulated aerial respiration.

6. It is suggested that the primary regulatory effect of the response to increased ambient pCO_2 is to prevent CO_2 from entering the animal, while the secondary stimulation of air breathing is caused by hypoxic stimulation of chemoreceptors located in the efferent branchial vessels.

INTRODUCTION

It is generally accepted that vertebrates acquired functional lungs before they possessed a locomotor apparatus for invasion of a terrestrial environment. Shortage of oxygen in the environment is thought to have been the primary driving force behind the development of auxiliary air breathing. Paleontological and geological evidence indicate that such development took place in shallow fresh-water pools with a high temperature and water of low O_2 content often considerably enriched in CO_2 (Barrell, 1916). The surviving lungfishes are considered to have changed remarkably little since they developed in the late Silurian and Devonian periods, and the adaptations they display and their physiological regulation must reflect closely those of the contemporary vertebrates responsible for the permanent invasion of the terrestrial environment.

The experiments reported here were designed to study the regulation of breathing in the Australian lungfish *Neoceratodus forsteri*.

MATERIALS AND METHODS

Fifteen specimens of *Neoceratodus forsteri* ranging in weight from 4.2 to 8.8 kg were used in the present investigation. In preparation for an experiment each animal was anesthetized and intravascular catheters were chronically implanted according to the procedure described elsewhere (Lenfant *et al.*, 196b). Figure 1 depicts schematically the anatomical arrangement of the major blood vessels in *Neoceratodus.* The posterior vena cava (VC), the pulmonary artery (PA), the pulmonary vein (PV) and, in some experiments, the first afferent branchial artery were cannulated. In addition, a catheter was inserted and attached inside the operculum for collection of exhaled water and for direct recording of branchial breathing rate. Another catheter was placed in the lung for collection of pulmonary air and measurement of intrapulmonary pressure. Ventilation of water across the gills was measured by the method described by Ogden (1945).

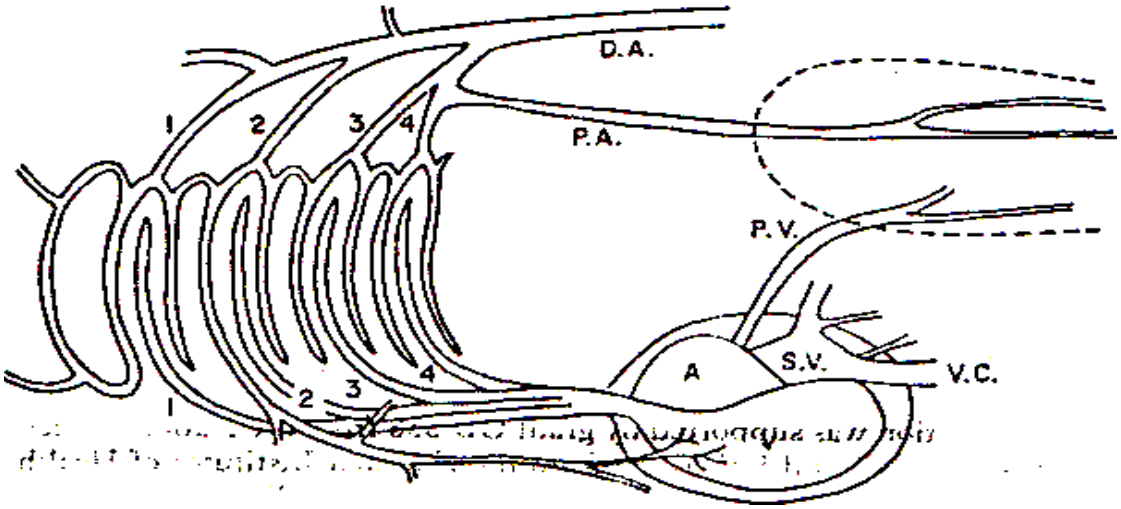


FIG. 1. Schematic drawing of the heart and major blood vessels in *Neoceratodus forsteri*. SV, sinus venosus; A, atrium; V, ventricle. The numbers designate the afferent and efferent branchial vessels. DA, dorsal aorta; PA, pulmonary vein; VC, vena cava.

The animals were allowed to recover fully from anesthesia before any sampling of blood and expired water was done. In general the animals showed no signs of deterioration from surgical intervention throughout periods up to 10-14 days. Commonly, however, the experiments were terminated 2-3 days after cannulation. The regulation of aerial and aquatic breathing was studied by changing the external environment and by introducing selected gas mixtures into the lung. Responses to hypoxia were investigated by equilibrating the aquarium water with nitrogen. Responses to introduction of nitrogen directly into the lung were also studied. The effects of elevated pCO_2 were similarly studied by equilibrating the water with 2% or 3 % CO_2 in 23% O_2 and nitrogen or by adding CO_2 directly into the lung. The blood gas composition in all cannulated vessels as well as CO_2 and O_2 pressures in aquarium water, exhaled water and pulmonary gas were monitored by means of successive sampling during these experimental procedures. The water ventilation V_e and branchial and pulmonary breathing rates were also recorded. A continuous record of V_e was obtained by recording the pressure rise in a volumetric cylinder receiving the exhaled water. Blood gas contents were measured by gas chromatography (Lenfant & Aucutt, 1966). Partial pressures of O_2 and CO_2 were measured with a Beckman Spinco model 160 gas analyzer using the oxygen macro-electrode in a special cuvette (0.03 ml) and the Severinghaus pCO_2 electrode. Calibration for pO_2 was done with known gas mixtures or with blood equilibrated with these. The pCO_2 electrode was calibrated with known gas mixtures. The instruments were calibrated prior to the measurements and frequently during the course of the experiments. All measurements were taken at 18˚C. Intrapulmonary pressure was measured via the implanted catheter using a Statham strain gauge transducer (P23bb) and a Beckman-Offner system of amplifiers and recorder. Symbols not explained in the text are in standard use in respiratory physiology.

RESULTS

Mechanics of air breathing

During resting conditions in well-oxygenated water, air breathing was very irregular and intervals of 1 hr or more often passed without attempts to breath air. Several authors have commented on the air breathing pattern of *Neoceratodus* (Semon, 1899; Dean, 1906; Atz, 1952; Grigg, 1965a). Inspiration is compared to the positive pressure buccal compression and swallowing described for amphibia. The present measurements of intrapulmonary pressure during breathing show that air is initially taken into the oral cavity while communication to the lung is closed by the contracted pneumatic duct. A few seconds later the duct opens and air is pressed into the lung by the buccal force pump supported by hyoid musculature. This phase is discernible in the pressure record as a positive wave superposed on the positive baseline pressure. The release of gas from the lung is similarly accomplished in two steps. A contraction of the smooth musculature in the lung parenchyma is instrumental in the expulsion of air from the lung to the oral cavity. The pressure records reveal that the lung is not at any time communicating directly with the ambient air, since the intrapulmonary pressure remains positive throughout a breathing cycle.

The basis for the presence of a maintained positive pressure inside the lung of *Neoceratodus* resides in the abundance of smooth muscle throughout the pulmonary parenchyma. Apart from its role in the mechanics of inspiration and expiration, the contraction of the pulmonary smooth muscle provides a means of regulating the buoyancy of the animal.

Response to hypoxia

Figure 2 demonstrates the changes in oxygen tensions of the blood and water, oxygen uptake through the gills as well as changes in water ventilation and O_2 extraction, before, during and after hypoxia. Information concerning the branchial respiratory rate (BRR) and frequency of air breathing are also given. Prior to hypoxia with no breaths of air in the 50 min preceding the hypoxia, blood from the pulmonary artery and vein was in equilibrium with respect to pO2. The vena cava pO_2 was low and blood from the first afferent branchial artery had a pO2 between the vena cava and the pulmonary vessels. The ventilation of water was about 300 ml/min and the extraction of O_2 from the water about 30 per cent. At the onset of nitrogen bubbling a marked increase in water ventilation took place.

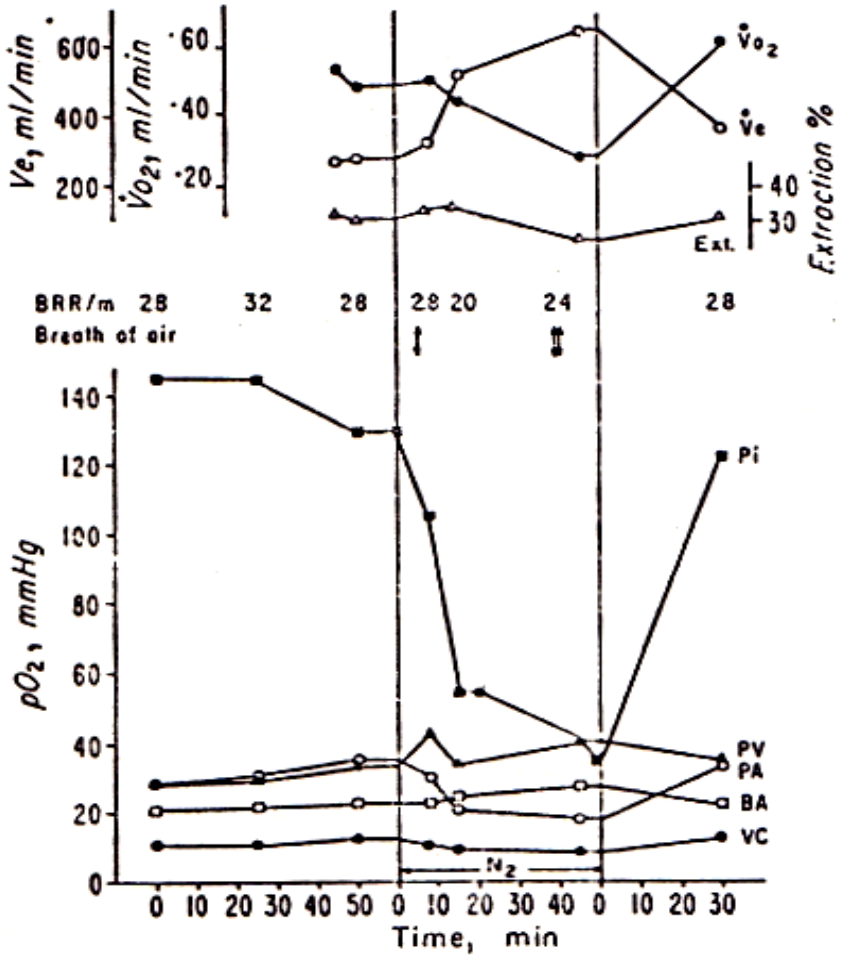


FIG. 2. Water ventilation (V_e), oxygen uptake through the gills (VO_2), extraction of O_2 from the water, breathing rates and pO_2 in external water (p), pulmonary vein (PV), pulmonary artery (PA), first afferent branchial artery (BA) and vena cava (VC) before, during and after exposure to progressive hypoxia.

The ventilation increase was commonly not associated with an increase in the branchial respiratory rate (Figs. 2, 3). The results expressed in Fig. 3 show water ventilation and branchial respiratory rate plotted against the external $p0_2$. More than a doubling in ventilation occurred with hardly any change in tile branchial breathing rate.

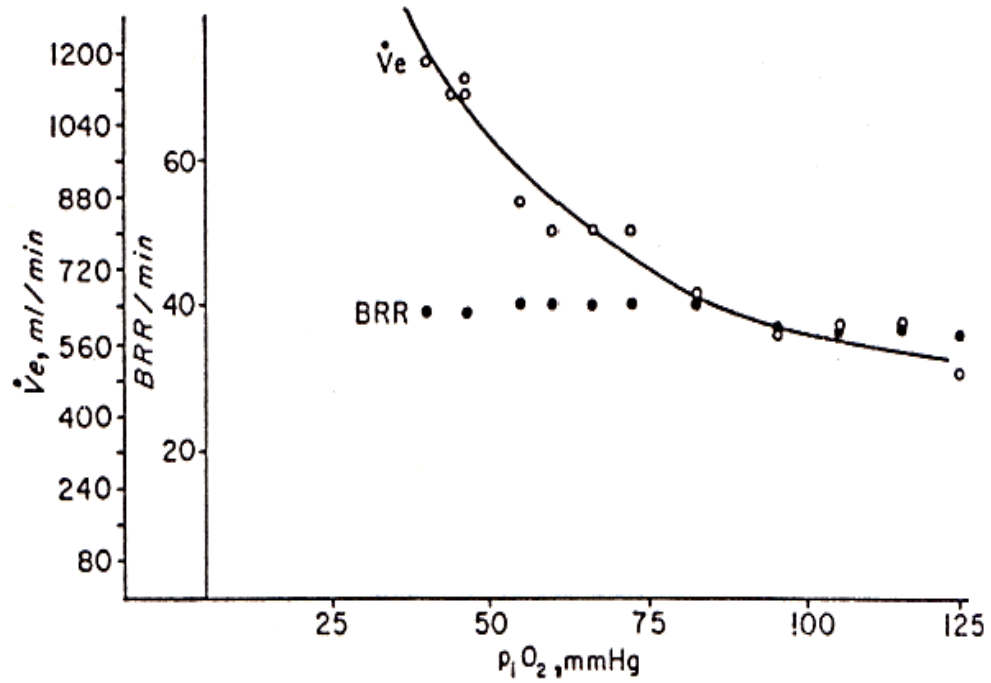


FIG. 3. Relationship between water ventilation (V_e), branchial respiratory rate (BRR) and pO_2 of the ambient water (PO_2).

The O_2 uptake by the gills (VO_2) showed a steady drop throughout the period of hypoxia, declining from 0.45 ml O_2/min to 0.30 ml O_2/min. Other experiments followed the same trend with one exception (see later). The extraction of 0_2 from the water on passage by the gills showed a net decline (Fig. 2). Most other experiments showed a much more pronounced trend in that direction.

Aerial respiration, like water breathing, was stimulated by hypoxia. As a general rule it occurred after the onset of compensatory changes in the branchial respiration. In Fig. 2 aerial respiration occurred when p_iO_2 had dropped to approximately 115 mm Hg. Most fish, however, showed an even more delayed response and air breathing was not commonly observed until the surrounding water O_2 tension had dropped below 85 mm Hg (Figs. 4, 5, 6). Figure 2 also shows how the blood picture was modified by the progressive hypoxia. The most conspicuous changes were a drop in the pO_2 of pulmonary arterial blood from 36 to 18 mm Hg, while blood in the first branchial artery showed a concurrent rise from 34 to 41 mm Hg. The vena cava blood showed a steady decline in pO_2 during hypoxia.

Figure 4 depicts the results of another hypoxia experiment indicating typical changes of blood pCO_2. Again hypoxia stimulated air breathing. The first breath of air was taken at a P_iO_2 of 70 mm Hg. The pulmonary arterial pO_2 was then 36 mm Hg and dropped only moderately throughout the remainder of the hypoxia period. The pulmonary venous blood showed a marked increase in pO_2 from 25 mm Hg to 65 mm Hg in response to four successive breaths of air.

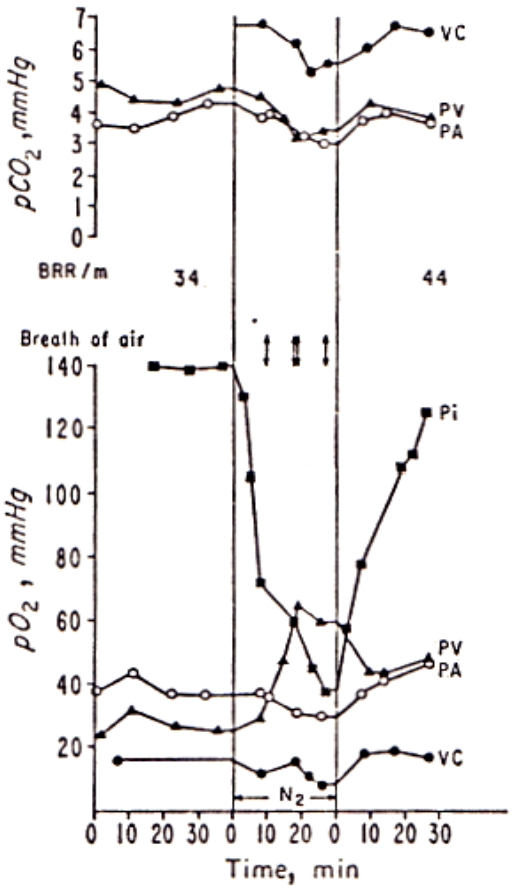


FIG. 4

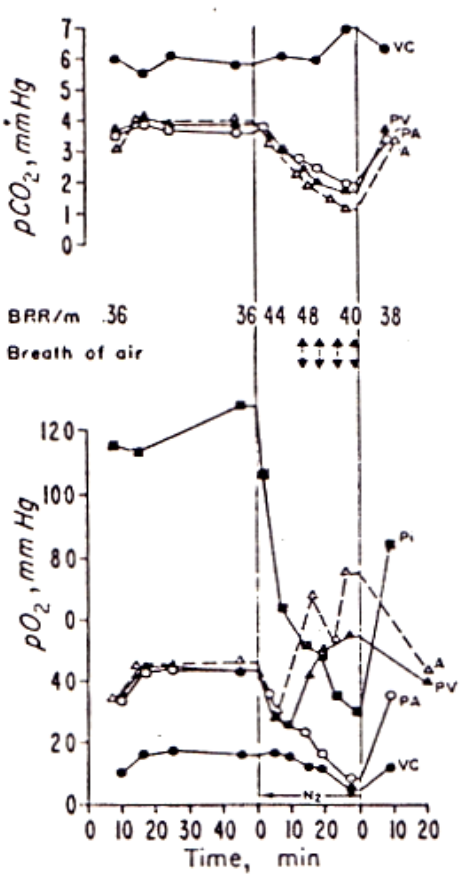


FIG. 5

FIG. 4. Blood pO_2 and pCO_2 and breathing rates before, during and after exposure to progressive hypoxia.
FIG. 5. Blood gases and pulmonary gas during progressive hypoxia.

Figure 5 is similar to Fig. 4 but contains additional information about pulmonary gas composition. The first breath of air was slow to occur and took place when P_iO_2 and pulmonary artery pO_2 had dropped to 53 mm Hg and 23 mm Hg respectively. Three additional breaths occurred in close succession after the first. The pO_2 in pulmonary gas more than doubled upon the first breath of air. The pulmonary venous pO_2 followed quite closely the changes in pulmonary gas pO_2, whereas the pulmonary arterial pO_2 dropped drastically and reached 8 mm Hg after 30 min hypoxia. The pCO_2 dropped precipitously in pulmonary arterial and venous blood as well as in pulmonary gas.

Figure 6 illustrates the responses to hypoxia in terms of changes in O_2 content of the blood samples. From the start of hypoxia until the first breath of air the O_2 content dropped steadily in all samples, but more in the pulmonary vessels than in the first afferent branchial vessel. The first breath increased the O_2 content in the pulmonary venous blood and afferent branchial blood, the latter again exceeding the pulmonary arterial O_2 content. The branchial respiration responded as in earlier experiments. The extraction of O_2 from the water dropped from 70 per cent to 27 per cent during the progressive hypoxia while water ventilation increased nearly three times.

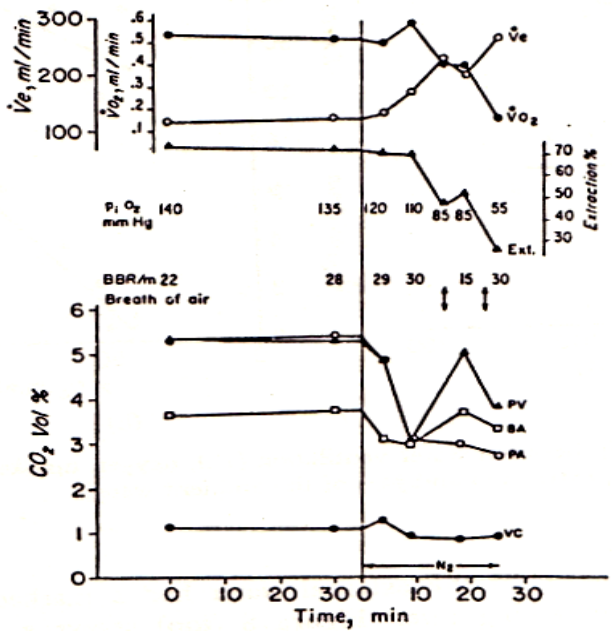


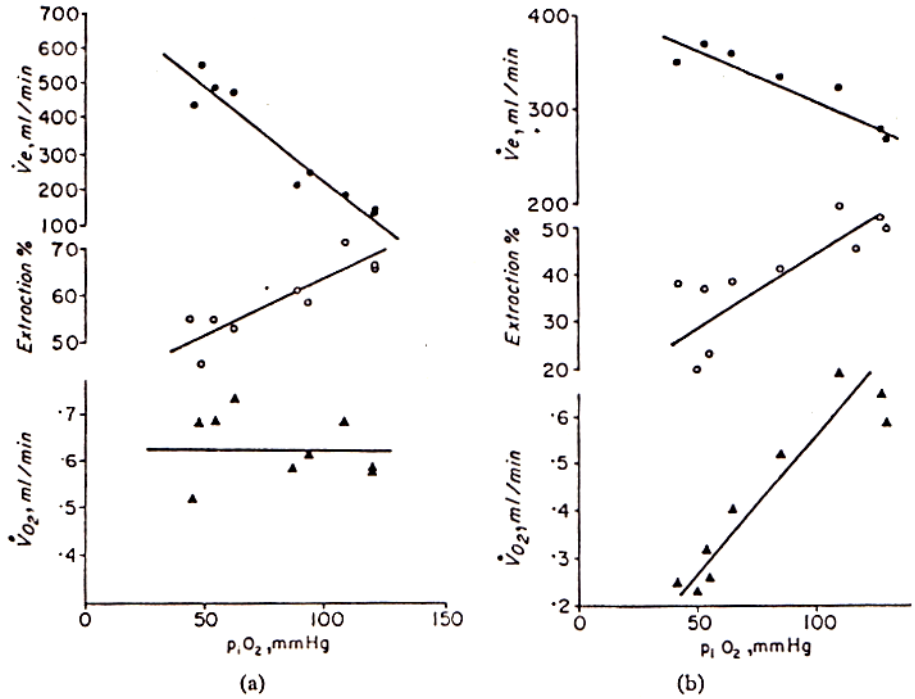
FIG. 6. Water ventilation (V_e), oxygen uptake (VO_2), per cent extraction of O_2 from water and O_2 content in blood from various vessels during exposure to progressive hypoxia.

The extraction of O_2 from the water is related to the volume of water ventilated and to changes in the underlying circulation. Figures 7a and b represent examples of this relationship in the present studies.

Figure 7a illustrates a case in which the ventilation increase was particularly prominent, resulting in a maintained O_2 uptake through the gills in spite of the reduced O_2 availability in the external water. Figure 7b, however, showing a net drop in O_2 uptake was by far the more common response.

In every experiment all changes occurring during hypoxia were completely reversible within 30 min after cessation of hypoxia.

Injection of nitrogen into the lung evoked no discernible compensatory response in branchial nor in aerial respiration.



FIGS. 7a, b. Relationship between water ventilation (V_e), oxygen uptake (VO_2), per cent O_2, extraction and pO_2 *of* the ambient water.

Responses to increased pCO_2

A gradual increase in pCO_2 of the external water had a marked effect on ventilation and branchial breathing rate. Figure 8 (top) shows a continuous recording of branchial respiratory rate and ventilation when a mixture of 2% CO_2 in 23% O_2 and nitrogen was bubbled into the aquarium water. A progressive reduction in breathing rate and ventilation is readily apparent and was observed in all experiments of this nature. Upon reversing the external changes in pCO_2 by bubbling in CO_2-free air the phenomenon was completely reversed (Fig. 8, bottom). Atropinization of the fish prevented the response to external pCO_2 changes. Figure 9 illustrates the relationship between breathing rate, breath volume and the total ventilation changes during the respiratory inhibition evoked by increased pCO_2. The average breath volume increases because the total ventilation declines more rapidly than the breathing rate. A drop in breathing rate from 28 to 6/min is hence accompanied by a doubling in the breath volume from 15 ml to 29 ml. With elimination of CO_2 from the water, V_e, BRR and breath volume returned to their original values.

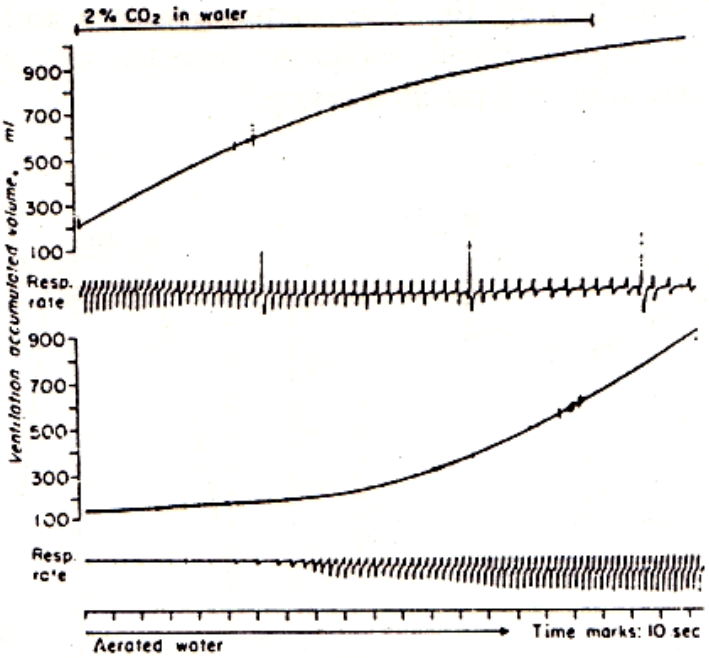


FIG. 8. Continuous recording of water ventilation (V_e), and branchial respiratory rate (BRR) during (top) and after (bottom) an increase in pCO_2 of the ambient water (p_iCO_2).

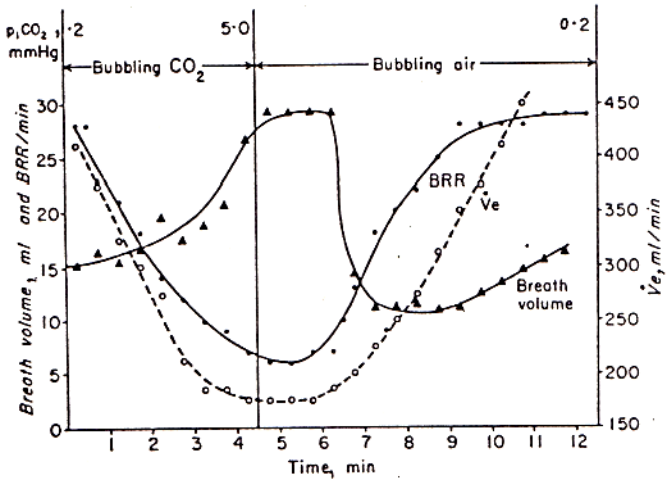


FIG. 9. Water ventilation (V_e), branchial respiratory rate (BRR) and breath volume during and after exposure to elevated p_iCO_2.

Figure 10 provides information on the changes in blood gas composition during increase in the external pCO2. Air breathing was stimulated by the elevated external pCO_2 while BRR, water ventilation and O_2 uptake through the gills dropped abruptly. The extraction of O_2 from the water was maintained. The changes in O_2 content and pO_2 in blood sampled from the various vessels are plotted in the lower half of Fig. 10. The partial pressure and content of O_2 declined in pulmonary arterial blood, while an increase was apparent in the pulmonary vein and first afferent branchial artery.

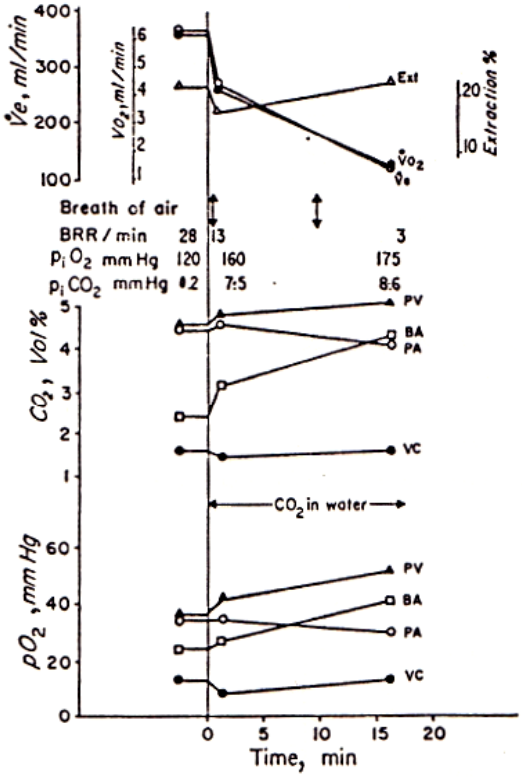


FIG. 10

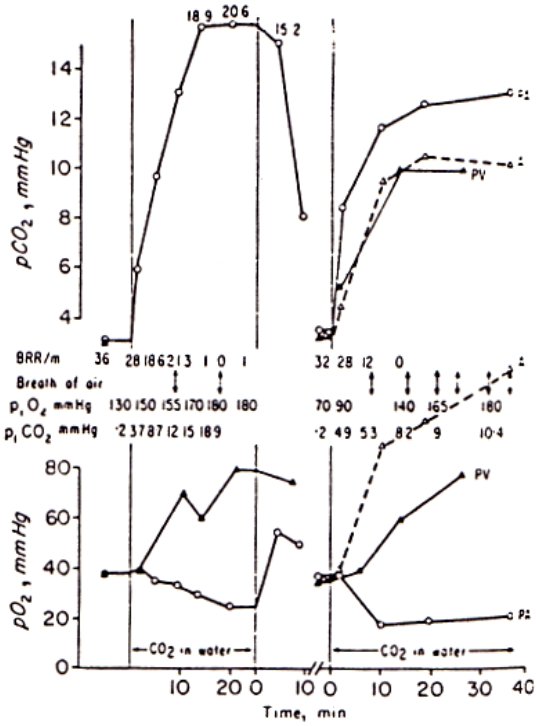


FIG. 11

FIG. 10. Water ventilation (V_e), oxygen uptake (VO_2), per cent O_2 extraction from water and changes in blood gas composition before and during increased pCO_2 in ambient water.
FIG. 11. Two consecutive runs showing effects of increasing pCO_2 in the ambient water on blood gas composition and breathing rates.

Figure 11 shows two consecutive experiments of similar nature. Note the increase in pCO_2 of both pulmonary arterial and venous blood and pulmonary gas despite the intensified air breathing. There is an apparent relationship between the increase in pCO_2 of pulmonary arterial blood and the pCO_2 of the external water, the former being 21 mm Hg in the first run compared to 13 mm Hg in the second run.

An entirely different response ensued when the CO_2 was introduced directly into the lung rather than into the aquarium water. No change in the branchial breathing rate occurred and air breathing was not induced. Meanwhile, profound changes took place in the blood gas composition. Figure 12 illustrates the changes in pCO_2 of blood and pulmonary gas when 10 ml 100% CO_2 was added to the lung.

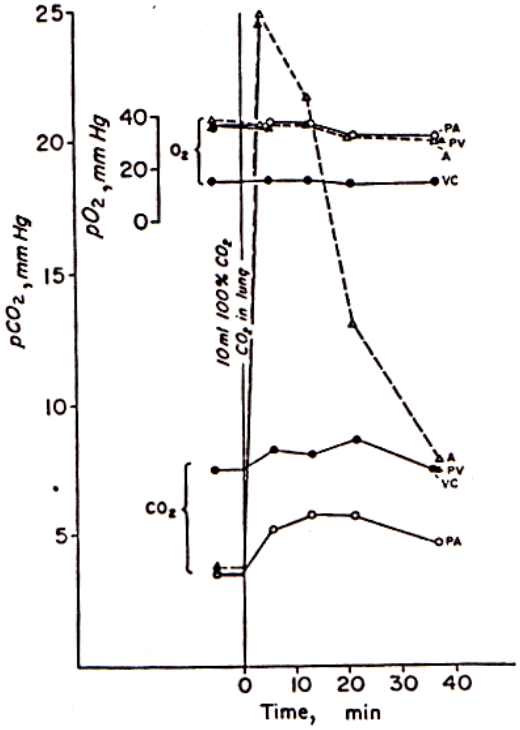


FIG. 12

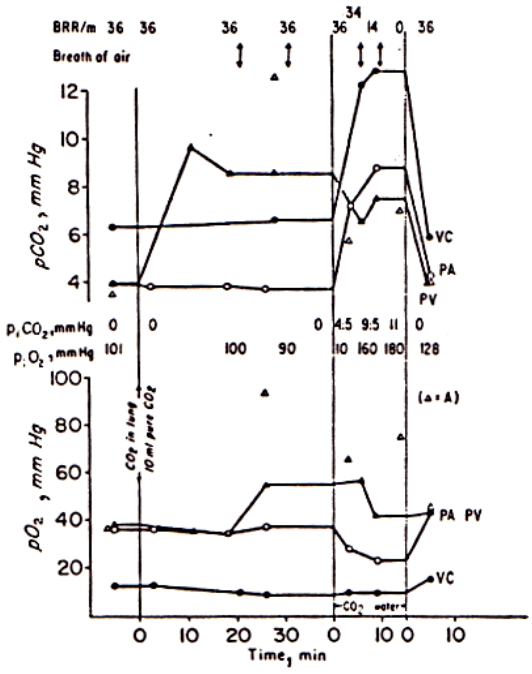


FIG. 13

FIG. 12. Changes in blood pO_2 and PCO_2 following injection of 10 ml 100% CO_2 into the lung.
FIG. 13. Blood gas composition and breathing rates following injection of CO_2 into the lung (first part), and during exposure to increased pCO_2 in the ambient water.

The pulmonary gas and pulmonary venous blood showed a conspicuous increase in PCO_2 reaching values of 25 mm Hg in less than 5 min, while the PCO_2 of the pulmonary arterial blood showed only a very modest increase. This fact substantiates the contention that the gills -possess a remarkable ability to eliminate CO_2. Figure 13 shows the results of a sequence of events starting with adding CO_2 to the lung followed by bubbling CO_2 into the external water. Respiratory inhibition is not apparent until PCO2 in the external water is increased, nor is there an increased PCO2 of pulmonary arterial blood before that time. The ability of the gills to eliminate CO_2 is again demonstrated and the stimulating effect of external PCO_2 on air breathing confirmed.

DISCUSSION

Water-breathing vertebrates in comparison with terrestrial vertebrates characteristically show extremely low values of arterial pCO_2 and their ventilation is considerably larger than that of an air breather with the same O_2 consumption.

These distinctions between water breathers and air breathers are referable to the solubility properties of the respiratory gases in water. Rahn (1966) uses these facts to generalize that the external respiration of water breathers is primarily concerned with maintaining proper oxygenation as opposed to the air breathers whose greatest concern is maintenance of an optimal pCO_2.

Important implications in the comparison of water breathers and air breathers are also referable to the confinement of the lung to the interior of the organism. This enables the animal, within limits, to control the pCO_2 and pO_2 of the alveolar environment by the rate and depth of breathing. The gills of an exclusive water breather are, on the other hand, generally exposed to the conditions of the external water and this affords very limited opportunity for control of the gas composition at the external side of the exchange organ. Respiratory control in the water breather is also complicated by the fact that pCO_2 and pO_2 in the water at times can change independently of each other. Conspicuous differences in specific weight and viscosity of air and water in addition to the differences in diffusibility of the gases in these two media are other important factors that emphasize the paramount distinction between water breathers and air breathers.

The surviving lungfishes provide an unexcelled opportunity to evaluate respiratory control in an organism possessing both gills and lungs, where the latter are true homologues of the higher vertebrate lungs.

The Australian lungfish is the most primitive and archaic of the living Dipnoans (Dean, 1906). It is decidedly more aquatic than the African and South American lungfishes and is incapable of estivating out of water. Its gills are well developed and all the afferent branchial arteries subdivide into gill capillaries. In *Protopterus* and *Lepidosiren* the gills are much reduced and some arches completely lack gill filaments. Every structural and behavioral indication is hence suggestive that *Neoceratodus is* primarily a water breather. This contention has recently been attested to from physiological experimentation by Lenfant *et al.* (1966) who demonstrated that O_2 absorption as well as CO_2 elimination in *Neoceratodus* when resting in well-oxygenated water was a function almost exclusively of the gills. Attempts to breathe air during these conditions were sporadic and often several hours passed without air breathing. The fishes were docile and rested quietly in the tanks. No voluntary exercise was displayed during day time. In the long intervals between air breathing, pO_2 and pCO_2 in pulmonary arterial and venous blood and pulmonary gas were nearly uniform; the lung hence was reduced in importance to a richly perfused gas pocket. The pulmonary arterial blood was almost fully saturated and little or no oxygenation could be gained from the lung.

The present paper provides evidence that the relative importance of the gills and lung is subject to marked changes during hypoxia and hypercapnia. Indirect evidence (Ramsay, 1876; Dean, 1906; Grigg, 1965b) also suggests an increased dependence upon pulmonary respiration in *Neoceratodus* during voluntary exercise. Before attempting to discuss in detail the relative importance of the double respiratory exchange system in *Neoceratodus* during respiratory stress one must assess to what extent the vascular system is able to support an optimal operation of the two gas exchange systems.

The advent of lungs in Dipnoans required major changes in the piscine pattern of circulation before the lungs could gain functional importance. Ideally, the lung should be coupled into a separate vascular circuit similar to that existing in birds and mammals. However, the phylogenetic development of a double circulation is gradual, and the living lungfishes, amphibians and reptiles represent distinct stages in this development. The first structural modification toward a double circuit was the development of a separate return of the pulmonary venous blood to the heart. This condition is present in the lungfishes. Next the undivided heart had to modify for the accomodation of two separate blood streams. The lungfishes have separated right and left atria, but the septation of the ventricle is only partial and there are structurally no discrete outflow channels directing the blood into a systemic and a pulmonary circuit. However, recent work on amphibians (Simons, 1959; Johansen, 1962; Haberich, 1965) and reptiles (White, 1956; Steggerda & Essex, 1957) has proven that a complete anatomical separation of the pulmonary and systemic circuits is not a requisite for functional separation.

Unlike most amphibians and all reptiles the problem of a selective distribution of blood in the lungfishes is further complicated by the presence of gills. Functional lungs and gills existing simultaneously in an animal confined to an aquatic medium require special regulation of the blood perfusion to circumvent a detrimental effect of one on the other in special environmental conditions. If air breathing is intensified in hypoxic water, the animal risks losing the O_Z absorbed by the lung to the surrounding water when the blood passes through the gills. To prevent such loss of efficiency two alternatives seem possible. One is to abandon the use of the gills which by gradual degeneration divert the total gas exchange to the lungs. This is the way vertebrate evolution progressed when aquatic life was given up altogether. Even in the African and South American lungfishes which seasonally, during drought, are exposed to long periods of strictly terrestrial conditions we find marked degeneration of the gills and reduction in gill surface area (Kerr, 1902; Parker, 1892). A number of teleost fishes variously adapted for auxiliary oxygen absorption through accessory respiratory organs show a similar degeneration of the gills (Dubale, 1951). However, *Neoceratodus* never leaves the water and its gills appear to be well developed. In this case an optimal use of the lung in O_2-deficient water must depend on a specialized vascular perfusion through the gills, that can allow the bulk of the blood pumped by the heart to bypass selectively the respiratory capillaries of the gills. The danger of losing chemically bound O_2 to the surrounding water can also be minimized if the blood has a very high affinity to oxygen. Recent work by Lenfant *et al.* (1966) has demonstrated this to be the case in *Neoceratodus.* Conversely in well oxygenated water, when the lungs are not engaged in respiratory exchange, a maximum perfusion through the gill capillaries and a reduced pulmonary perfusion are desirable. The present data provide evidence that a certain degree of selective passage of blood through the heart of *Neoceratodus* occurs and that the mode of breathing and the relative size of the blood flow through the lung are modifying factors in this selective passage. Upon comparison of blood from the first afferent branchial artery and blood from the pulmonary artery, one has a manifest documentation of selective passage in those cases where the oxygen content of the former surpasses that of blood already past the exchange vessels of the gills. This phenomenon showed up invariably during the gradual exposure to hypoxia and elevated water pCO_2 (Figs. 2, 6, 10).

Whether selective passage occurs during rest in well-oxygenated water is more uncertain. The relatively high oxygen values of blood from the first afferent branchial artery compared to the vena cava are suggestive of some degree of selective passage. If this were not so, the blood flow through the pulmonary circuit would almost have to match the systemic in size, a requirement hardly compatible with the anatomical conditions.

With due regard for its approximate value, the data acquired in the present study will in certain respects be treated as though the blood distributed to the afferent branchial vessels were uniform in O_2 content and as though the pulmonary artery is carrying mixed arterial blood. It is also presumed that the oxygen uptake occurs predominantly in the gills. These assumptions permit the use of Fick's principle for the estimation of blood flow in the various vascular circuits. The following equations pertain to the calculation of blood flow under these specified conditions.

Blood flow through the gills or the total cardiac output, QT:

$$QT = (VO_2)/[C_aO_2-C_vO_2]. \quad (1)$$

Ratio of the pulmonary blood flow Q_L to total blood flow O_T:

$$QL/QT = [C_vO_2-C_vO_2]/C_cO_2-C_vO_2] \quad (2)$$

Responses to hypoxia

The most consistent and immediate response to gradual hypoxia was an increase in the branchial respiration. Ventilation increased sharply in some cases by a factor of 3 or 4 depending on the initial level, while the branchial breathing rate showed no change or only moderate increase. The increased ventilation was hence primarily the result of an increased volume of individual breaths. No earlier reports on the effects of hypoxia on respiration in lungfishes have come to our notice. Several workers have, however, dealt with similar problems in teleost and elasmobranch fishes, although direct measurements of ventilation in combination with information on blood gas composition across the gills are extremely scarce.

In accordance with most earlier reports (Winterstein, 1908; van Dam, 1938; Fry, 1957; Saunders, 1962) dealing with several species of teleosts, a conspicuous increase of the ventilation in non-swimming fish in response to hypoxia has been recorded. Elasmobranch fishes, on the other hand, are not reported to show a similar compensatory increase in ventilation (Ogden, 1945; Johansen & Hanson, 1967).

In consideration of the high energy requirements of ventilatory movements in fishes, the present finding of an increase in V_e primarily as a result of an increased depth of breathing rather than an increased frequency seems to be of significance in reducing the energy cost. Shuman & Piiper (1966) have recently found that the cost of ventilation reaches 70 per cent of the total resting metabolism at high ventilation rates in teleosts. This clearly suggests a limit in the capacity for compensatory increase.

The present data confirm earlier findings on teleosts (van Dam, 1938; Saunders, 1962) of an inverse relationship between ventilation and extraction of O_2 from the water. The absolute values for extraction were smaller than earlier reported values for teleosts (Hall, 1930; Hazelhoff, 1938; Saunders, 1962).

Our data from *Neoceratodus* as well as findings on elasmobranchs (Lenfant & Johansen, 1966) suggest marked changes in cardiac output with consequent alterations in the ventilation-perfusion relationships. Such changes have recently been directly measured in elasmobranch fishes (Johansen & Hanson, 19G7). The application of Fick's principle to data shown in Fig. 2 indicates a total output increase of more than seven times after 45 min of hypoxia. The ventilation perfusion ratio in the gills changed from 12 before the hypoxia to 4 in the terminal phase. Half an hour into the recovery the ventilation perfusion ratio was 17. These calculations suggest that the circulatory changes evoked by hypoxia exceed the compensatory changes in external respiration. A decrease in O_2 uptake when the ambient oxygen pressure decreases, referred to as respiratory dependence, is of common occurrence in fishes (Fry, 1957). The present study provides evidence that *Neoceratodus* shows respiratory dependence throughout the entire range of ambient water pO_2. It is generally advocated that the presence of respiratory dependence is related to limitations imposed by the maximum amount of water which can be pumped past the gills (Fry, 1957; Hughes & Shelton, 1962). However, the fact that respiratory dependence occurs at oxygen tensions in the water well above those required for complete saturation of the blood in *vitro* does not eliminate the possibility that a reduced saturation of the arterial blood (in vivo) can be a primary factor in the elicitation of respiratory dependence. Recent work has amply demonstrated the presence of large gradients in pO_2 between arterial blood and ambient water (Steen & Kruysse, 1964; Lenfant & Johansen, 1966; Johansen & Lenfant, 1966). Furthermore, the present data document fluctuations of arterial pO_2 with external pO_2 even at very high ambient O_2 tensions.

The air breathing elicited by hypoxia was much slower in appearance than the changes in branchial respiration and was as a rule not observed before marked changes were established in the blood gas

tensions. No distinct threshold in the latter is suggested but the external p_iO_2 had commonly dropped to values around 85 mm Hg or below before air breathing was stimulated.

The data strongly indicate that an intensitified use of the lung is associated with marked changes in relative blood flow in the systemic and pulmonary circuit. By applying equation (2) and using the oxygen contents in Fig. 6, it can be calculated that the fraction of the total blood flow perfusing the lung increases 25 per cent after 25 min of progressive hypoxia. Similar increases in Q_L/Q_T were observed in all experiments but the magnitude varied considerably.

The changes in blood gas composition offer a measure of the efficiency of the compensatory changes to hypoxia. Most experiments disclosed a marked drop in pO_2 of pulmonary arterial blood. The O_2 content of vena cava blood always diminished indicating a potential to unload practically all the bound oxygen. The changes in pO_2 of pulmonary venous blood demonstrate the role of the lung as an O_2 exchanger. Prior to the first breath, pO_2 always declined, but rose rapidly subsequent to the first breath and always increased to a pO_2 value exceeding that needed for complete saturation of the blood. There was always a gradient in pO_2 between pulmonary gas and pulmonary venous blood which consistently increased during and shortly after inspirations. The overall importance of the lung for total gas exchange varied. Figure 4 represents an example of maximum importance. In this case PO_2 in pulmonary venous blood increased very steeply from 25 mm Hg to 65 mm Hg within a period of 20 min while p_iO_2 decreased to about 50 mm Hg. The pulmonary arterial pO_2 is seen to be only moderately reduced. This strongly suggests a selective passage of pulmonary venous blood to the systemic arteries which hence maintain a relatively high arterial O_2 content in spite of the severe hypoxia in the ambient water.

The CO_2 tension in blood sampled from all vessels showed most commonly a decline during the hypoxia. This is attributable to the hyperventilation stimulated by the hypoxia. The general decrease of pCO_2 in the blood helps to maintain a high O_2 saturation in the presence of the large Bohr effect in *Neoceratodus* blood.

The experiments involving a partial filling of nitrogen directly into the lung show that the receptor mechanisms eliciting the compensatory responses to hypoxia are not located internally in the pulmonary venous vessels or vessels afferent to the gills. Thus the chemoreceptors involved in compensation for hypoxia must be located on the efferent vascular side (the arterial side) or on the external side facing the ambient water (p_iO_2). An external receptor mechanism for elicitation of compensatory water breathing seems useful in that the animal may escape the unfavorable external condition before any internal changes due to the hypoxic water have occurred. Such a mechanism may be particularly significant in the shallow, often stagnant fresh-water habitat of the lungfishes where large gradients in pO_2 prevail. The receptors should accordingly face the main body of water (inhaled water) rather than the exhaled water since the latter shows large variations in pO_2 due to spontaneous changes in extraction. Conversely it seems logical for chemoreceptors eliciting compensatory air breathing to be located intravascularly on the arterial side of the circulation.

It can be concluded that hypoxic water represents a strong stimulus for compensatory increase in external respiration of *Neoceratodus,* whereas changes in CO_2 tension have no stimulatory effect. Schmidt & Comroe (1940) carried this theme to comparison with the air-breathing mammal and postulated that the organization of the chemoreflexes in air breathers represents a Survival of a system of utmost significance to the water breather. A chemoreflex system with direct contact with the external environment can be envisaged of particular value to the water breather, whereas in the air breather it more logically should be protected from direct external contact while sensing the quality of the arterial blood. An important link in such a phylogenetic development of the chemoreflexes seems from the present work to be represented by the surviving lungfishes.

Responses to increased pCO2 in ambient water

The reflex slowing of the branchial respiration in response to elevated pCO_2 in the ambient water seemed at first paradoxical, since pCO_2 is considered a primary respiratory stimulant in vertebrates. It stands to reason, however, that in water-breathing species an elevated ambient pCO_2 might cause undesirable internal changes if allowed to leak into the animal. The exceedingly low internal pCO_2 values normally occurring in water breathing species Suggest that internal pCO_2 changes caused by metabolic activities are not a stimulus likely to alter the respiratory pattern. On this basis it seems useful for maintenance of relative internal stability that external increase in pCO_2 causes branchial respiratory depression. When branchial respiration, however, also represents the primary mode for oxygen absorption it follows that a depression of the branchial respiration might represent a stimulus to increased air breathing as was indeed observed in *Neoceratodus.*

The mechanism for this may reside in hypoxic stimulation of chemoreceptors located in the efferent branchial vessels, a possibility invoked above to explain the stimulation of air breathing when the fish

was in hypoxic water. Our data reveal a consistent drop in pulmonary arterial pO_2 during elevated ambient pCO_2 in spite of a maintained or increased p_iO_2. Additional support for this view became available from the fact that injection of acetylcholine (2-10 μg intravenously) was invariably followed by a breath of air. Acetylcholine is known to have a dichotomous effect on the vascular resistance in fishes (Ostlund & Fange, 1962). The drug dilates the systemic vascular bed while the branchial vessels are selectively constricted. The branchial vasoconstriction would in turn result in hypoxia of the efferent branchial blood.

The data show that if the external pCO_2 rose to high levels a substantial amount of CO_2 leaked into the blood stream in spite of the branchial respiratory depression. This resulted in an elevated pCO_2 in the pulmonary gas which was not efficiently removed by repeated air breathings. This finding substantiates the contention advanced by Lenfant *et al.* (1966) that the lung in *Neoceratodus is* a very inefficient CO_2 eliminator.

The branchial respiratory depression following increased external pCO_2 was associated with marked changes in branchial blood flow. Calculations show a threefold increase after 12.5 min of CO_2 bubbling into the water (Fig. 10). This must be looked upon as a useful compensation for the loss in affinity of the blood for O_2 due to the large Bohr effect. As in response to hypoxia the augmented air breathing resulting from the elevated external pCO_2 was also associated with a marked increase in the perfusion of blood through the lung.

The oxygen uptake through the gills dropped sharply while the ambient pCO_2 was increased. The literature contains very conflicting data on the effect of pCO_2 on O_2 uptake in fish. Pry (1957) and Basu (1959), working with several species of teleosts, noted a marked decline in (VO_2). The experiments involving filling of the lung with CO_2 when the fish was in CO_2-free water showed that internal CO_2 accumulation did not represent an important respiratory stimulus. \o change in the breathing pattern ensued in spite of an enormous increase in pCO_2 of pulmonary venous blood and blood from the first afferent branchial artery (Fig. 12). After passage through the gills, however, blood from the pulmonary artery attested to a remarkable efficiency of the gills to eliminate CO_2 This fact seemed to leave open the possibility that the receptors eliciting the response to CO_2 elevation in the ambient water might be located in the efferent branchial vessels or on the external surface of the fish. The possibility also remains that the reflex change is instigated by olfactory stimulation. Johansen (19G6), working on air-breathing habits of the teleost *Symbranchus marmoratus,* similarly noted a depression of branchial breathing at increased ambient pCO_2 which was quickly abolished upon transfer to CO_2-free water. This finding was found to be suggestive of the presence of external chemoreception for CO_2.

On the basis of the present findings, the conclusion seems warranted that pCO_2 as a chemoreflex stimulant in *Neoceratodus* exerts its regulatory effect by preventing influx of external CO_2 rather than being concerned with the removal of metabolically produced CO_2.

Viewed in a larger phylogenetic contest, the refinement in the control of internal stability and the high operational ability displayed by the highest vertebrates have evolved gradually. The lower vertebrates do not show a similar degree of internal stability and their success is closely linked with the selection of optimal external conditions and avoidance of unfavourable strata. Such behavior can be regarded as a primary integrated response to buffer the internal environment.

An optimal efficiency in avoidance and relocation requires a prompt responsiveness to a changing environment and external rather than internal sensors for important environmental stimuli seem advantageous. The integrity of higher vertebrates is based on a retention of some external sensors which become even more refined as the organism gains more independence from the environment. The idea seems justified that external sensors primarily guided the behavior pattern and that interdigitation of these with secondary developed internal sensors provided the input to integrating portions of the nervous system responsible for successful homeostasis.

REFERENCES

ATZ J. NV. (1952) Normal breathing in fishes and the evolution of internal mares. Quart. *Rev. Biol. 27,* 366-377.

BARRELL J. (1916) Influence of Silurian-Devonian climates on the rise of air-breathing vertebrates. *Bull. geol. Soc. Am.* 27, 357-436.

BASU S. P. (1959) Active respiration of fish in relation to ambient concentrations of oxygen and carbon dioxide. Y. Fish. Res. Bd *Can. 16,* 175-212.

DEAN B. (1906) Notes on the living specimens of the Australian lungfish, *Ceratodus forsteri,* in the Zoological Society's collection. *Proc. Zool. Soc. Land.* 74, 168-178.

DUBALE M. S. (1951) A comparative study of the extent of gill-surface in some representative Indian fishes, and its bearing on the origin of the air-breathing habit. J. *Univ. Bombay 19,* 90-101.

FRY F. E. J. (1957) The aquatic respiration of fish. In *Physiology of Fishes* (Edited by BROWN M. E.) Vol. 1, pp. 1-63. Academic Press, New York.
GRIGG G. C. (1965a) Studies of the Queensland lungfish, *Neoceratodus forsteri* (Krefft)-I Anatomy, histology and functioning of the lung. *Aust. J. Zool. 13,* 243-253.
GRIGG G. C. (1965b) Studies on the Queensland lungfish, *Neoceratodus forsteri* (Krefft)-I 11. Aerial respiration in relation to habits. *Aust. J. Zool. 13,* 413-421.
HABERICH F. J. (1965) The functional separation of venous and arterial blood in the univentricular frog heart. *Ann. N.Y. Acad. Sci. 127,* 459-476.
HALL F. G. (1930) The ability of the common mackerel and certain other marine fishes to remove dissolved oxygen from sea water. *Am. J. Physiol. 93,* 417.
HAZELHOFF E. H. (1938) Uber die Ausnutzung des Sauerstoffs bei verschiedened Wassertieren. Z. *vergl. Physiol. 26,* 306-327.
HUGHES G. M. & SHELTON G. (1962) Respiratory mechanisms and their nervous control in fish. In *Advances in Comparative Physiology and Biochemistry.* (Edited by LOWENSTEIN O.) Vol. 1, pp. 275-364. Academic Press, New York.
JOHANSEN K. (1962) Double circulation in the amphibian, *Amphiuma tridactylum. Nature, Lond. 194,* 991-992.
JOHANSEN K. (1966) Airbreathing in the teleost, *Symbranchus marmoratus. Comp. Biochem. Physiol. 18,* 383-395.
JOHANSEN K. & HAN D. (1967) Respiratory and circulatory responses to hypoxia in the dogfish, *Squalus suckleyi.* (In preparation.)
JOHANSEN K. & LENFANT C. (1966) Gas exchange in the cephalopod, *Octopus dofleini. .Am. J. Physiol. 210,* 910-918.
KERR J. G. (1902) The development of *Lepidosiren paradoxa. Quart. J. microsc. Sci. 46, N.S.,* 417-459.
LENFANT C. & AUCUTT C. (1966) Measurement of blood gases by gas chromatography. *Respir. Physiol. 1,* 39S-407.
LENFANT C. & JOHANSEN K. (1966) Respiratory function in the elasmobranch, *Squalus* suckleyi G. *Respir. Physiol. 1,* 13-29.
LENFANT C., JOHANSEN Ii. & GRIGG G. C. (1966) Respiratory properties of blood and pattern of gas exchange in the lungfish *Neoceratodus forsteri* (Krefft). *Respir. Physiol.* (In press.)
OGDEN E. (1945) Respiratory flow in *Mustelus. Am. J. Physiol. 146,* 134-139.

OSTLUND E. & FANGE R. (1962) Vasodilation by adrenaline and noradrenaline and the effects of some other substances on perfused fish gills. *Comp. Biochem. Physiol. 5,* 307-309.
PARKER W. N. (1892) Anatomy and physiology of *Protopterus annectens. Trails. R. Ir. Acad. 30,* 109-230.
RAHN H. (1966) Aquatic gas exchange: Theory. *Respir. Physiol. 1,* 1-13.

RAMSAY E. P. (1876) On the habits of living ceratodi in captivity. *Proc. zool. Soc. Lond. 44,* 69S-699.
SAUNDERS R. L. (1962) The irrigation of the gills in fishes-11. Efficiency of oxygen uptake in relation to respiratory flow, activity and concentrations of oxygen and carbon dioxide. *Can. J. Zool. 40,* 817-862.
SCHMIDT C. F. & COMROE JR., J. H. (1940) Functions of the carotid and aortic bodies. *Physiol. Rev. 20, 115-157.*
SCHUMAN D. & PIIPER J. *(1966)* Der Sauerstoff-Bedarf der Atmung bei Fischen nach Messungen an der markotisierten Schleie (Tinca *tinca). Pflugers Arch. ges. Physiol. 288, 15-26.*
SEMON R. *(1899) In the Australian Bush.* Macmillan, London.

SIMONS J. R. *(1959)* The distribution of the blood from the heart in some Amphibia. *Proc. zool. Soc. Lond. 132, 51-64.*
STEEN J. B. & KRUYSSE A. *(1964)* The respiratory function of teleostean gills. Comp. *Biochem. Physiol. 12, 127-142.*
STEGGERDA F. R. & ESSEX *H. E. (1957)* Circulation and blood pressure in the great vessels and heart of the turtle, *Chelydra serpentina. Am.* J. *Physiol. 190, 320-326.*
VAN DAM L. *(1938)* On the utilization of oxygen and regulation of breathing in some aquatic animals. Doctoral dissertation, University of Groningen.
WHITE F. N. *(1956)* Circulation in the reptilian heart, *Caimon sclerops. Anat. Rec. 125, 417-432.*

WINTERSTEIN H. *(1908)* Beitrage zur Kenntniss der Fischatmung. *Pflugers Arch. ges. Physiol. 125, 73.*