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AESTIVATION OF THE AFRICAN LUNGFISH *PROTOPTERUS AETHIOPICUS*: CARDIOVASCULAR AND RESPIRATORY FUNCTIONS

BY R. G. DELANEY, S. LAHIRI AND A. P. FISHMAN

Cardiovascular-Pulmonary Division, Department of Medicine, University of Pennsylvania, Philadelphia, Pa. 19104, *U.S.A.*

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SUMMARY

The present study was undertaken to elicit the temporal sequence of changes in cardiovascular and respiratory function during aestivation. Twelve lungfish (2–6 kg) equipped with ECG electrodes, arterial and buccal cannulae, were studied while aestivating in mud or in artificial cloth-bag nests. The periods of observation ranged from 0·5 to 9·5 months. The mean arterial blood pressure gradually decreased from control values of 20–28 mm Hg to a range of 14–18 mmHg during the first 30 days of aestivation, whereas the heart rate dropped more gradually (22–30 beats/min to 11–16 beats/min in 60 days). Ventilatory frequency increased 2- to 5-fold during the first 30 days of encystment and then returned to the control range (2–10 h) within 45 days. The arterial P_{CO_2} increased from control values of 25–30 mm Hg to 45–70 mmHg; arterial pH decreased concomitantly from 7·55–7·60 to 7·40–7·26 after the cocoon was formed. The arterial P_{O_2} increased from the control range of 25–40 to 50–58 mmHg during the first 10 days and then returned to the control range. Therefore, the sequential cardiopulmonary changes during the onset of aestivation are gradual and do not parallel the decline in oxygen consumption. Aestivating lungfish also respond promptly to sensory disturbances and thus do not appear to be in a deep torpor. Aestivation is pictured as a state of dormancy, gradual in onset, and the consequence of a complicated physiological interplay.

INTRODUCTION

The African lungfish, *Protopterus aethiopicus*, is an obligatory air breather which inhabits the shallow waters of lakes and rivers in central Africa (Greenwood, 1966). During the torrid season, as ambient waters evaporate, the lungfish escapes desiccation by burrowing into the mud, forming a chamber in which it remains for months until the waters return. As the mud of the burrow hardens, the fish becomes covered in its entirety with a presumably waterproof cocoon that is open only at the mouth for breathing (Smith, 1930; Johnels & Svensson, 1954). While encased in this subterranean nest, the lungfish undergoes a series of physiological adjustments in order to survive starvation and partial dehydration. During its encasement in the burrow, the lungfish is said to be in a state of aestivation.

Only fragmentary information exists concerning the physiological features of

aestivation: (1) the paucity of body movements has been interpreted as a manifestation of a state of torpor (DuBois, 1892; Smith, 1930); (2) the decrease in oxygen consumption to less than 50% of its resting rate in water reflects a striking decrease in metabolic activity (Smith, 1930; Swan, Jenkins & Knox, 1968; Lahiri, Szidon & Fishman, 1970); (3) an increase in muscle glycogen and the cessation of ammonia production indicate a shift in metabolic pathways (Janssens, 1964); (4) the accumulation of urea and other nitrogenous metabolic end-products is, in part, attributable to marked oliguria (Smith, 1930; Forster & Goldstein, 1966; Janssens & Cohen, 1968). In addition remarkable changes occur in the circulation and respiration, as typified by slowing of the heart rate (from 25 beats/min while in water to 3 beats/min in the cocoon) and a slowing of the respiratory frequency (from 6/h to 1–2/h) (Smith, 1930).

It should be noted that the above observations on oxygen consumption, heart rate and respiratory frequency are exceedingly sparse, generally representing occasional determinations over long intervals (e.g. after one year in the mud), rather than the sequential changes of aestivation. Consequently, they provide little insight into the respiratory and circulatory adaptations that occur during the switch from life in water to life in the cocoon. In the present study we undertook to determine the features of the cardiovascular and respiratory adjustments of *Protopterus aethiopicus* during transition from life in water to artificial aestivation.

MATERIAL AND METHODS

Preparation of animals

Protopterus aethiopicus (2–6 kg) from Lake Victoria near Kampala, Uganda, were transported to Philadelphia by air freight in plastic bags partially filled with water and O_2-enriched air. The duration of transport varied from 2 to 3 days. After arrival, the lungfish were maintained in individual tanks filled with dechlorinated tap water to a depth of 12–16 cm at 21–25°C. Aquatic fish were fed Purina Trout Developer (Ralston Purina Co., St Louis, Missouri) *ad libitum*, every other day.

Eighteen fish were used in the aestivation studies. All had eaten well, seemed to be thriving, and had been in our laboratory for at least 1 month. Implantations of electrodes and cannulae were done under general anaesthesia induced by submersion in 0·5% ethyl *m*-aminobenzoate methane sulphonate (Eastman Organic Chemicals, Rochester, New York) in dechlorinated tap water.

For arterial blood sampling and for monitoring blood pressure, polyethylene tubing (90 cm lengths, PE 160, 1·1 mm I.D. or PE 190, 1·15 mm I.D., Clay Adams) was placed in the artery of the third branchial arch. The cannula was filled with heparinized saline solution (20 i.u./ml) and capped. Oxytetracycline HCl (Liquamycine, Pfizer), 50 mg/kg, was administered once daily for three days postoperatively via the arterial catheter.

Blood pressure was monitored, using a strain gauge transducer (P23Db, Statham Instruments) connected to a bridge amplifier channel of an oscilloscopic recording apparatus (Electronics for Medicine). The transducer was positioned at the level of the heart; and the pressure equivalent of the depth of water between the level of the heart and the surface of the water was subtracted from gauge pressure in order to correct the blood pressure for ambient hydrostatic pressure.

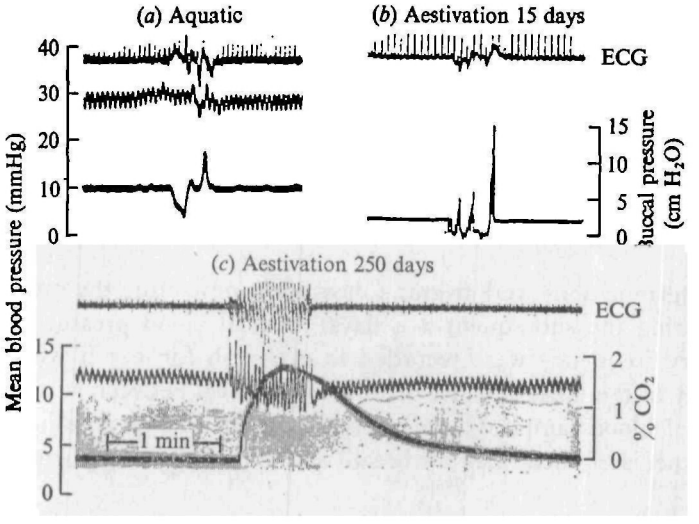

Fig. 1. Lung breath recordings demonstrating the deflexions in the ECG pattern coincidental with air breathing. (*a*) ECG, arterial blood pressure, and buccal pressure in an aquatic lungfish during an air breath. (*b*) ECG and buccal pressure in a lungfish encysted in a mud nest during a breath. (*c*) ECG, blood pressure, and % CO_2 in the region of the mouth during a breath of a lungfish encysted in a cloth sack for 250 days.

The electrocardiogram (ECG) was recorded in all 18 fish. Bipolar ECG electrodes were made from 10 cm lengths of teflon-insulated Flexon-Steel electrode wire (David & Geck) and connected to a 24-gauge hookup wire. The indifferent electrode was sutured to the cartilaginous portion of the right subopercular bone. The other electrode was attached to a curved needle which was passed through the skin, at the level of the heart, and was manipulated until a good ECG record was obtained. Invariably this manipulation placed the electrode in the vicinity of the pericardium. After anchoring the uninsulated portion of the electrode subcutaneously, the insulated ends of the wires were led to the dorsum and sutured in place behind the head for connexion with the recording apparatus. Depending on the amplitude of the signal, either a conventional electrocardiographic or electroencephalographic amplifier was used for recordings. This recording not only gave heart rate, but because of the characteristic electrical artifact coincidental with an air breath, also allowed measurement of respiratory frequency (Fig. 1 *a*, *b*, *c*). The lung breath also produced characteristic distortions in the blood pressure record (Fig. 1 *a*, *c*). The reliability of these artifacts as a measure of respiratory frequency was confirmed in two ways: (1) by relating them to phasic changes in the concentration of CO_2 in air sampled continuously from the vicinity of the mouth (Fig. 1 *c*) using an infra-red analyser (Godart capnograph), and (2) by comparison with changes in buccal pressure. Buccal pressure was recorded using a water-filled polyethylene catheter, 75 cm long (PE 160), the distal end of which was lodged in the buccal cavity via the opercular slit (Fig. 1 *b*). During aestivation each breath counted represented a series of respiratory movements which occurred within 10–60 sec. In several instances these movements appeared to be associated with repeated expiration of CO_2 noted by the capnograph.

Blood gases

The pH, P_{CO_2} and P_{O_2} in arterial blood were determined at 25°C using micro-electrodes (Radiometer BMS-3) which were calibrated in the conventional way (Siggaard-Andersen, 1964).

Experimental observations

Control

Control determinations were begun 2 days after implanting the catheters and were continued during the subsequent 2–4 days. Arterial blood pressure, the ECG and the respiratory frequency were recorded in each fish for 1–5 h while the fish was resting quietly in the tank. Because of the long interval between surfacings for an air breath, arterial blood samples (1 ml) were drawn at a fixed time in the respiratory cycle (i.e. immediately before an air breath as the fish began to surface).

Aestivation

Two types of aestivation experiments were done: in mud and in cloth bags.

Mud. In the initial studies, mud was used as the aestivation medium. The fish was placed on the surface of semi-liquid mud contained in a large styrofoam box. The fish remained quietly on the surface breathing rapidly (30–60 breaths/h, compared with 2–10 breaths/h while in water) for a variable period (from 5 min–2 h). It then began to burrow and, within a few minutes, disappeared under the mud. Under the surface, it formed an aestivation nest and reappeared via a fresh opening. Immediately after surfacing, it gulped several breaths of air and then withdrew into its burrow. Thereafter, it surfaced periodically for its breath of air, gradually withdrawing down into the burrow during the ensuing days to weeks as the mud began to dry. Finally, within 1 month, when the surface had crusted, the lungfish was encased in its cocoon, connected to the surface by the thin breathing channel (about 15 cm long and 1–2 cm in diameter).

It was extremely difficult to maintain arterial cannulae patent in the fish that aestivated in mud. Thus, in only four of the eight fish was it possible to record blood pressure and to sample blood for 24 h or more; in two of these, sampling and recording of blood pressure could be continued for 5 and 15 days respectively, when the catheters clotted. With two other fish, the catheters became obstructed within two days. The other four dislodged the arterial catheters while burrowing and exsanguinated.

Cloth bags. Because of the technical difficulties and high mortality associated with burrowing in mud, aestivation was induced in cloth bags, using a modification of the technique devised by Godet (1961) and developed by Janssens & Cohen (1968). After control measurements in water (described above), the cannulated fish was lightly anaesthetized and placed in a muslin sack (15–25 cm diameter and 30 cm long) so that its body formed a U with the head toward the top of the bag; this is the normal posture for a fish aestivating in mud (Johnels & Svensson, 1954). Recording leads and cannulae were brought out through a small hole in the side of the bag. The sack was then suspended inside a styrofoam container filled with water. The tank was then allowed to drain slowly so that it took 5–10 days for the water level to fall be[illegible]

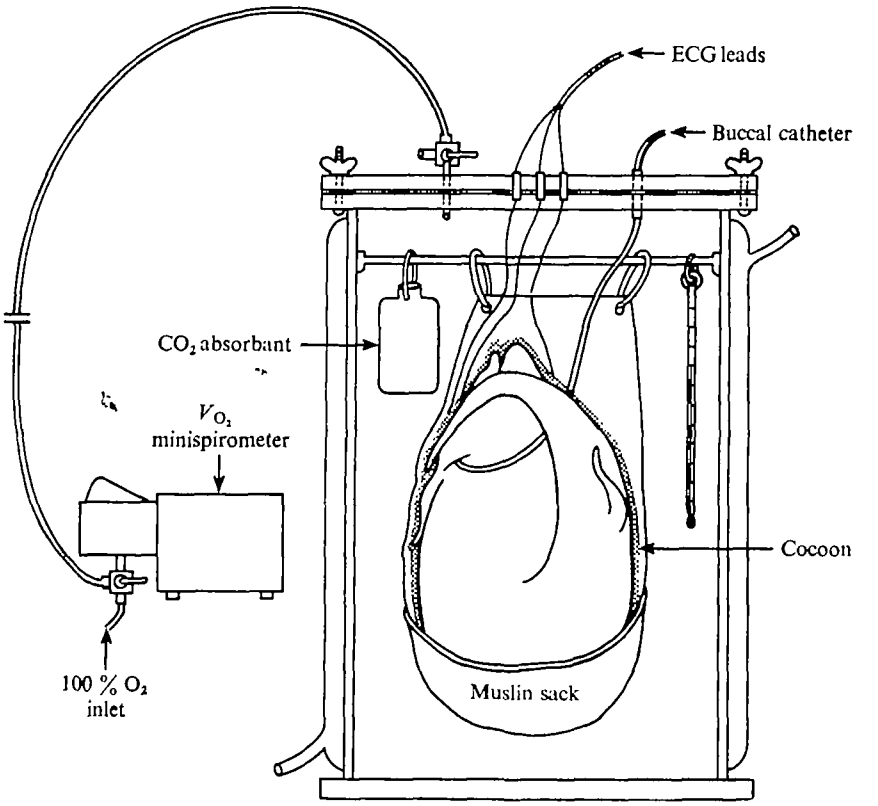

Fig. 2. Schematic representation of the system used for determination of oxygen consumption.

the body of the fish, and for the sack to become completely dry. Formation of the cocoon was observed through the small opening at the top of the sack.

The cannula and ECG leads remained attached to each of the ten lungfish in this group. In 6 of these, serial determinations of arterial blood pressure, heart rate, and air breath frequency were made for prolonged periods, up to 9·5 months. In the other 4, clotting of the arterial cannulae restricted the periods of observation to 2–18 days. In one lungfish, in which the arterial catheter had become occluded after 3 weeks, the cannula was replaced after 80 days of aestivation, using methoxyflurane (Metofane, Pitman-Moore, Fort Washington, Pennsylvania) administered through an ether cone as the inhalation anaesthetic. Blood sampling and recordings were then continued as described above.

Oxygen consumption

In several experiments the oxygen consumption of lungfish was monitored during the onset of aestivation in cloth sacks. The decline in volume of 100% oxygen in a spirometer, which was connected to a cylindrical plexiglass chamber containing the fish, was used to determine the oxygen uptake (Fig. 2). This system maintained the inspired air at a constant oxygen concentration approximating room air at the ambient atmospheric pressure. The procedure was a modification of a technique originally developed by S. Lahiri & B. B. Lloyd (unpublished). The metabolic chamber was a

23 l, thermojacketed (25°C), sealed cylinder containing barium hydroxide granules as a CO_2 absorber. A 40 cc minispirometer filled with 100% oxygen was connected to the cylinder by a 90 cm length of polyethylene tubing (PE 90) to reduce the diffusion of oxygen into the chamber. Tests were run prior to each experiment to ensure that there were no leaks or diffusion of gases into, or out of, the system. The lungfish was placed in the cylinder filled with 3 l of water for aquatic measurements. The preparation for sack aestivation was as previously described. The encased fish was then suspended in the chamber (Fig. 2) filled with water. The water level was lowered daily until the chamber was dry in 7–10 days. Each day, following equilibration with room air for ½ h, the exit ports were closed and the minispirometer was connected to the chamber. The decline in volume of the spirometer reservoir was continuously monitored by a photo-coupled DC signal which was recorded along with heart rate and buccal pressure. The spirometer reservoir was periodically filled, and calibrated at the end of each 7–10 h recording period. The oxygen consumption, corrected to STPD, was averaged for each day's recording period. The gas composition of the chamber was periodically measured to ensure that the concentration of CO_2 was $< 1\%$ and the oxygen remained between 20 and 21%.

Air breathing in non-aestivating fish out of water

To determine the consequences of removing fish from water without access to either mud or a sack for aestivation, two fish were allowed to remain at the bottom of their tanks after the water had been drained. Although air in the tank was moist, there was no layer of water as is provided in the cartons in which they travel for 48–72 h from Uganda. Arterial blood was sampled and blood pressure, heart rate and respiratory frequency were determined as described above for the 2-day control period and the subsequent test periods.

Both lungfish curled on the floor of the tank, head to tail. Their white undersurfaces appeared flushed and congested as though the vessels to the skin had undergone vasodilation. Large quantities of glossy, whitish, tenacious mucus covered the entire body. After 20 h, one of the two fish was transferred to the surface of moist mud as in the mud-aestivation experiments. This fish entered the mud, proceeded to form a cocoon as usual, and survived for over 6 weeks. The other fish, kept moist and undisturbed in its tank, continued to elaborate mucus but did not form a cocoon. It died in 3 days.

Statistical methods

Statistical significance of changes from control was determined using the Student *t*-distribution method for small samples (Alder & Roessler, 1964). In extended studies, when technical difficulties precluded accumulation of sufficient data for their statistical treatment, data were calculated as mean values and their range.

RESULTS

I. *Aestivation in mud*

Cardiovascular. In the four lungfish that survived burrowing, the heart rate during the control period before aestivation varied between 22 and 30 beats/min. W[illegible]

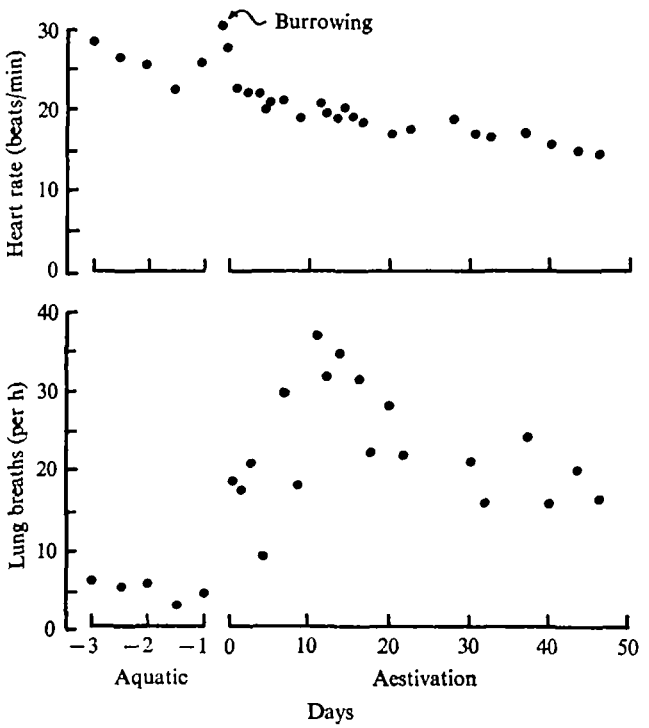

Fig. 3. Consecutive changes in mean heart rate and lung breath frequency of a 2·5 kg lungfish during the initial stage of aestivation in a mud burrow.

burrowing to form the aestivation nest, the heart rate accelerated, reaching 30–32 beats/min. Once settled in the burrow, the heart rate gradually slowed to reach values of 12–17 beats/min by the end of 1–1·5 months in the mud. Fig. 3 shows the sequential changes in one fish. The serial changes in blood pressure (not shown in this figure) paralleled the changes in heart rate, increasing by 2–9 mmHg during burrowing but returning to the aquatic value of 23–26 mmHg within 36 h. During the next 2 weeks, the blood pressure continued to fall, reaching a new level of 20–22 mmHg by day 15. Subsequent determinations of blood pressure could not be made.

Respiratory. In the four fish, respiratory frequency ranged from 2 to 9/h during the control aquatic period. During the first 2 weeks in mud (Fig. 3), it increased 2- to 5-fold, thereafter decreasing toward, but not quite reaching, control values.

To correlate changes in ventilation with blood gas composition, repeated blood samples were obtained from two of the four lungfish (see Methods) during the first 5 days of their encasement in mud. As the respiratory frequency increased, the mean arterial P_{O_2} increased from 35–44 mmHg to 80–90 mmHg by the 5th day. However, the arterial P_{CO_2} also increased to 46–48 mmHg from the control aquatic value of 27–30 mmHg. Concomitantly, the blood pH decreased from 7·59–7·55 to 7·45–7·47.

II. *Aestivation in cloth sacks*

Cardiovascular. In these fish, more prolonged observations were made. As may be seen in Fig. 4, the early changes in heart rate in muslin sacks were similar to those

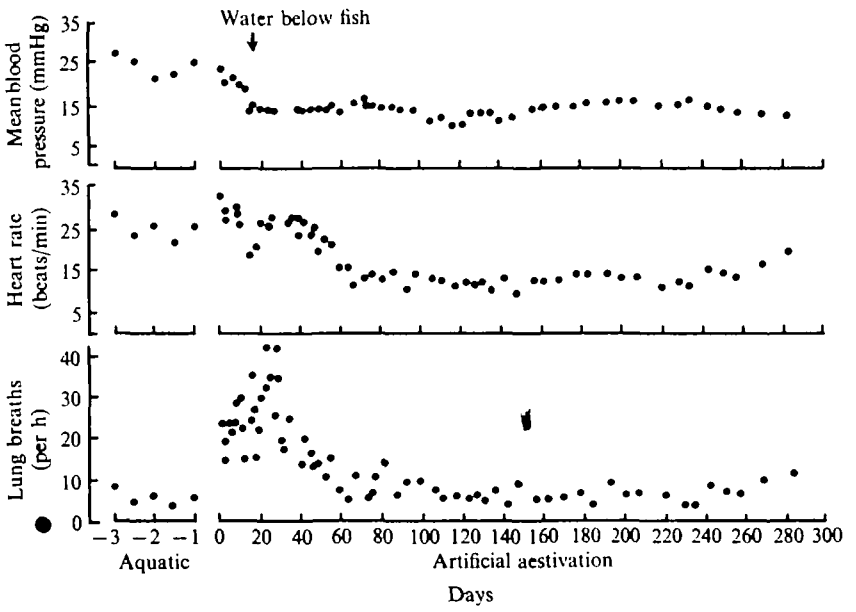

Fig. 4. Consecutive changes in mean blood pressure, heart rate and lung breath frequency of a 3·4 kg lungfish during 283 days of artificial aestivation in a cloth sack.

in mud. Thus, from a mean heart rate of 25 beats/min (range 22–29 beats/min) during the control period, the heart rate increased to 35 beats/min after placement in the sack, followed by a decrease to 28/min within 2 days. During the next 12 days, as the water level dropped (see Methods), the heart rate remained within the control (aquatic) range. After the 12th day, when the water level had fallen below the fish, the heart rate gradually slowed to reach 11–16 beats/min in 6 weeks. This bradycardia persisted throughout the remainder of the experimental period of 283 days.

The pattern of change in blood pressure is also illustrated in Fig. 4. For the first 2 weeks, while the water level was being lowered, the blood pressure slowly decreased from the control mean of 24 mmHg to 15 mmHg. Subsequently, it varied between 12 and 17 mmHg.

Respiratory. Each fish increased its ventilatory frequency after placement in the cloth sack. The typical pattern is illustrated in Fig. 4 in which a maximum frequency of 42 breaths/h was recorded by the 22nd day. During the next 40 days, the breath frequency returned to the control (aquatic) range averaging 6–8 breaths/h (range 5–11 breaths/h). It remained at this level until the advent of the preterminal infection (283 days).

Serial blood samples were drawn from one lungfish during the first 15 days in the aestivation sack. The changes in arterial P_{O_2} and P_{CO_2} are shown in Fig. 5. The control arterial P_{CO_2} was 28–30 mmHg. As the water level dropped during the first week in the sack, arterial P_{CO_2} increased markedly, reaching 45 mmHg by the 15th day. The arterial pH changed synchronously, decreasing to 7·36–7·38 by day 15 from the control range in water of 7·54–7·58. The corresponding arterial P_{O_2} increased from control values of 22–40 mmHg to 49–59 mmHg by the 11th day, returning thereafter to the control range. Thus, despite an increased respiratory frequency and raised

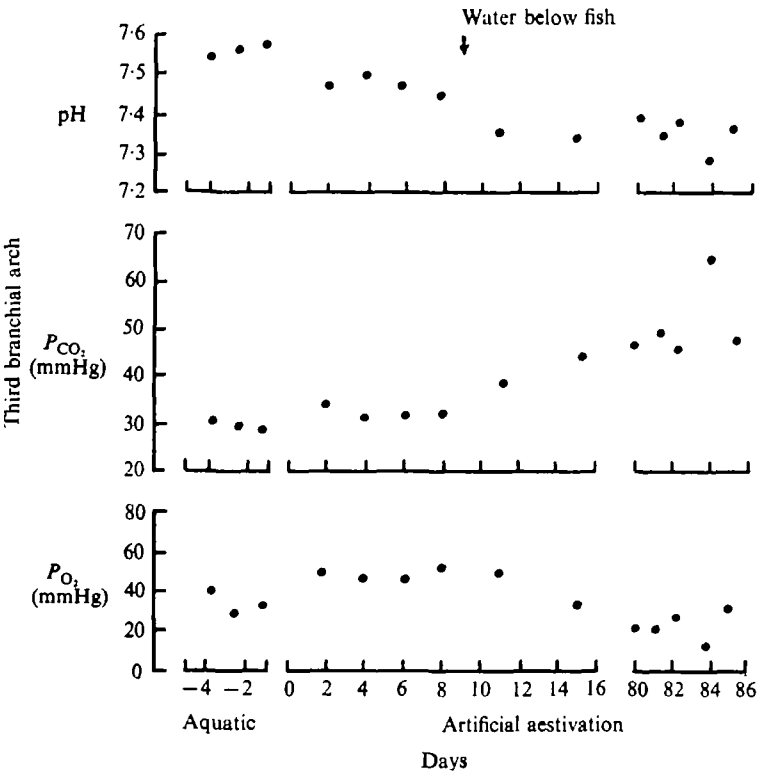

Fig. 5. Consecutive changes in pH, P_{CO_2} and P_{O_2} of arterial blood (third branchial artery) during the onset of cocoon formation and after 80–85 days of artificial aestivation in a muslin sack. Samples were taken just before a breath.

unchanged arterial P_{O_2} the arterial P_{CO_2} increased and the arterial pH decreased during the 15 days of observation.

In this lungfish, which had its arterial cannula replaced after 80 days in the sack, the blood gases were quite different from those observed after 15 days: arterial P_{CO_2} was higher, ranging from 45–70 mmHg, and arterial P_{O_2} was only slightly lower, ranging from 13–42 mmHg. Arterial pH ranged from 7·26 to 7·37, following the arterial P_{CO_2}.

III. *Oxygen consumption during the onset of aestivation*

The oxygen consumption of 5 aquatic lungfish ranged from 12 to 31 ml/kg.h. The lower values were obtained from fish that were not eating well prior to the measurement of oxygen consumption, and the highest values were recorded in lungfish which were actively moving about in the chamber. Fig. 6 shows the change in oxygen consumption, breath frequency, and heart rate of a 4·1 kg lungfish during the onset of aestivation. The oxygen consumption varied from 23 to 26 ml/kg.h while the fish was in water immediately before aestivation. The oxygen consumption declined in a logarithmic fashion. Within 1 day after placement in the aestivation sack the oxygen consumption had fallen to 14 ml/kg.h. After 5 days the oxygen uptake was 12 ml/kg.h and by day 15 was 7 ml/kg.h. From then on the decline in oxygen [redacted]ke was very gradual and after 2 months was 5·05 ml/kg.h. The body weight of the

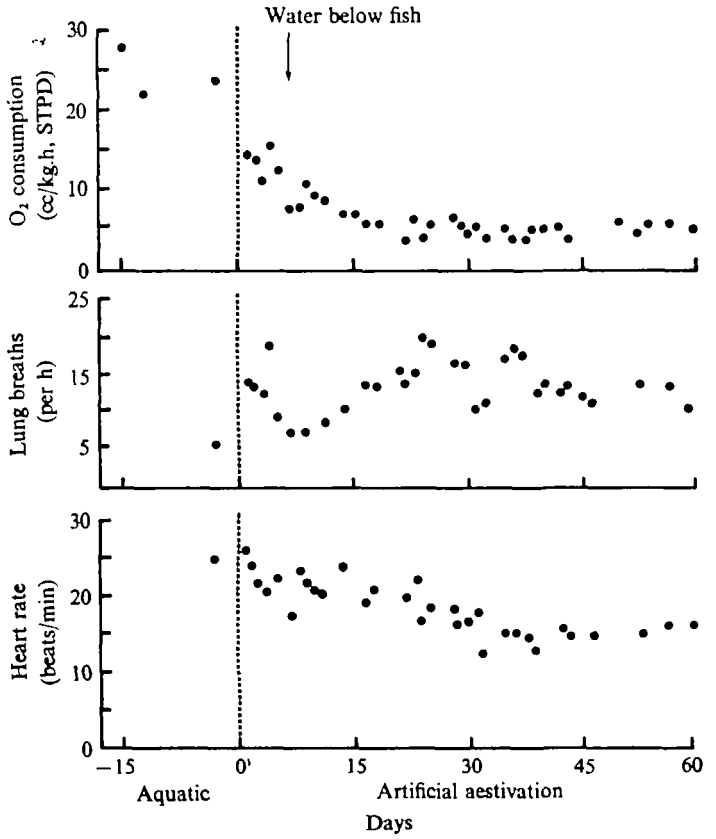

Fig. 6. Sequential changes in oxygen consumption, breath frequency, and heart rate during the onset of aestivation of a 4·1 kg lungfish.

lungfish declined rapidly during the 1st month from 4·1 to 3·4 kg and then continued to fall at a slower rate, reaching 3·1 kg after 60 days in the sack.

The breath frequency and heart rate showed the same pattern of change as previously described.

IV. *Combined observations on aestivation (mud and sack)*

Since the data on respiratory frequency, heart rate and blood pressure in the two groups of experiments (mud vs. sack aestivation) were similar, they are summarized together in Fig. 7. Because of the technical difficulties, sufficient data for statistical appraisal were only available for the first few weeks. However, in one fish, repeated determinations of all three parameters were made for 9 months and in three fish for more than 4 months.

Respiratory frequency increased gradually during the first 2 weeks, from a mean control value of 6/h to 21/h. This change was statistically significant ($P < 0.001$). Thereafter it began to return toward control values reaching a mean value of 8–10 breaths/h within 2 months. Heart rate decreased from a mean control of 25 beats/min to 17 beats/min in about a month and stabilized thereafter at 14–16 beats/min. The slowing of the heart rate by the second month was highly significant statisti

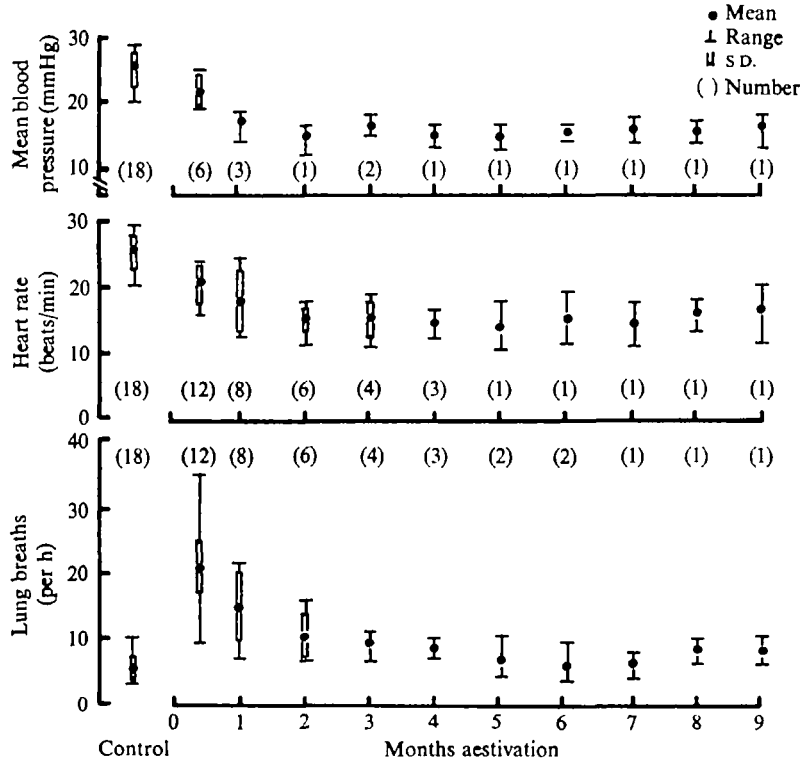

Fig. 7. Composite of changes in mean blood pressure, heart rate and lung breath frequency during aestivation of *Protopterus* in mud and cloth sacks. The mean (circle), range (thin bar) and standard deviation (thick bar) are shown for surviving lungfish (n) during consecutive months of observation.

($P < 0{\cdot}001$). Mean blood pressure decreased gradually but consistently, falling within 1 month from a mean control value of 25 mmHg to a plateau of 14–16 mmHg.

V. *Air exposure without aestivation*

The effects of prolonged air breathing without aestivation are illustrated in Fig. 8. During the first 1·5 h of exposure to air, the ventilatory frequency (lung breaths) increased from 8/h to 40/h and arterial P_{O_2} increased from approximately 40 mmHg to 85–111 mmHg; both the respiratory frequency and arterial P_{O_2} remained high during the subsequent 19–20 h. In contrast, the arterial P_{CO_2} first increased on air exposure (from 28 to 32 mmHg), then gradually decreased during the next 20 h (to 12–13 mmHg). The arterial blood pH changed concurrent with P_{CO_2}, first decreasing slightly (from 7·60 to 7·50) and then increasing to a peak of 7·87. Although the heart rate and blood pressure increased slightly during air exposure, they did not differ significantly from the control values of 25 beats/min and 25 mmHg, respectively.

After 20 h this lungfish was placed on mud. It soon burrowed and went through the same sequence as that described above for aestivation in mud. The respiratory frequency and the P_{O_2} began to decrease once the fish had settled, falling in 5 days to 18/h and 60 mmHg, respectively. The decrease in respiratory frequency was associated with a gradual increase in arterial P_{CO_2} which reached 47–53 mmHg in

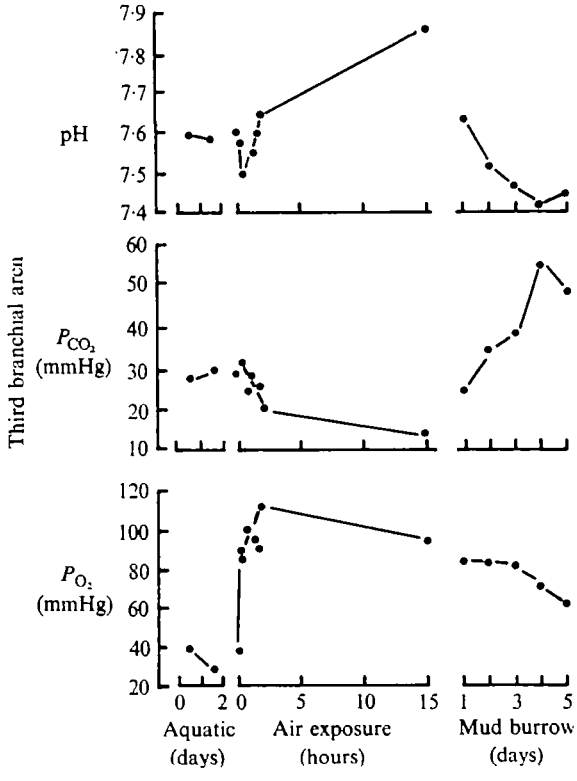

Fig. 8. Consecutive changes in arterial blood pH, P_{CO_2} and P_{O_2} in a non-aestivating lungfish first exposed for 20 h to moist air and then allowed entry into mud for aestivation.

4 days, and a corresponding decrease in arterial pH to 7·45–7·42. Although further sampling of blood became impossible after the 5th day when the catheter clogged, the fish survived in its cocoon in the mud for 6 weeks.

DISCUSSION

This study showed that the removal of ambient water and the formation of a cocoon are prerequisites for aestivation in the African lungfish, *Protopterus aethiopicus*. In addition, it provided some insight into the serial adjustments in the respiration and circulation that are associated with aestivation.

Starvation and desiccation

Our experience is consistent with that of Smith (1935) and Janssens (1964) who found that *Protopterus* in water can survive for many months without food. This ability to survive prolonged starvation is even more dramatically displayed during aestivation when it lives out of water for months or years without food or water (Smith, 1930). However, we have also demonstrated that *Protopterus* cannot withstand prolonged absence of ambient water unless it is sheltered by the cocoon that it generates during aestivation. Consequently, it is pertinent to examine the relationships between desiccation, starvation and aestivation.

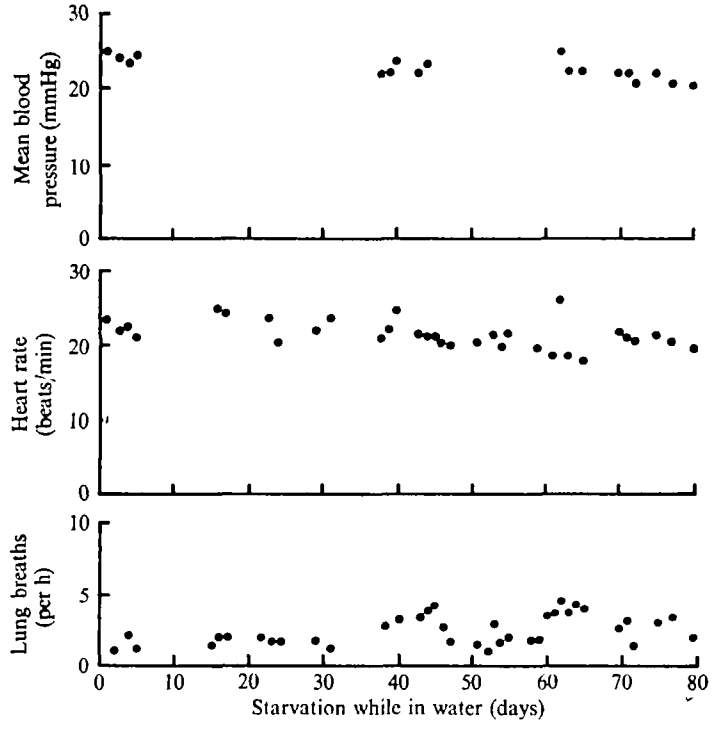

Fig. 9. Consecutive changes in mean blood pressure, heart rate, and lung breath frequency of a lungfish (3·60 kg decreased to 3·38 kg) during 80 days of starvation. Throughout this period the lungfish remained in its tank as in its control observations but was not fed.

Desiccation is a continuing threat during aestivation since no water is ingested and small, but appreciable, quantities of water are eliminated by the lungs during breathing. The cocoon affords considerable protection against excess water loss, this function being facilitated by severe oliguria (Smith, 1930). Indeed, if the normal urinary output of the lungfish, about 2–5 ml/kg.h (Sawyer, 1966, 1970; DeLaney, S. Lahiri & A. P. Fishman, unpublished observations), were to continue during aestivation, 1 kg of water would be lost in 8–20 days. Therefore, the aestivating lungfish must diminish urinary output to survive for weeks to months. The time of onset of the oliguria, and its underlying mechanisms, is not known. But an inevitable consequence of this oliguria, despite diminished metabolic activity, is an accumulation of metabolic wastes (Smith, 1930; Janssens, 1964). What these products contribute to the onset and perpetuation of the state of aestivation is unclear.

Starvation, while the fish is in water, does not appreciably decrease its resting heart rate or systemic arterial blood pressure; nor does it change its ventilatory frequency or pattern. An example is shown in Fig. 9 for a fish starving for 80 days. In contrast, starvation has been reported to decrease O_2 uptake (Smith, 1935) as seen during aestivation in this study and by Smith (1930), and Lahiri, Szidon & Fishman (1970). In fact, the decline in oxygen consumption during the onset of aestivation (Fig. 5) from the last day of feeding (1 day prior to placement in the sack), appears to follow logarithmic regression similar to that reported by Smith (1935) during aquatic

starvation. Thus, although a decrease in O_2 uptake is common to both starvation and aestivation, there is a difference in cardiovascular and respiratory behaviour (i.e. no change during starvation and disproportionate changes during aestivation). This dissociation of O_2 uptake and cardiorespiratory function during starvation emphasizes that the effect of aestivation on heart rate, blood pressure and respiration is not simply a consequence of lack of food intake.

There are also important metabolic differences between aestivating and starving lungfish. For example, Janssens (1964) reported a 50% *increase* in muscle glycogen and 10-fold decrease in glutamic-pyruvate transaminase (GPT) after 6 months in aestivation. In contrast, lungfish starved for 6 months had a 75% *decrease* in muscle glycogen and a 22% increase in liver GPT. Consequently, although starvation is an essential component of aestivation, it cannot account for the entire syndrome.

Circulatory and respiratory changes

The decrease in heart rate, systemic arterial blood pressure and breathing frequency is gradual. However, the patterns of change in these haemodynamic parameters were somewhat dissimilar: the blood pressure reaches its new level within a few days after the ambient water is withdrawn (Fig. 4); in contrast, the respiratory frequency and the heart rate may take 2 months to settle down to their new levels. But asynchrony exists in all three during the first 2 months of encasement: not only does the blood pressure reset sooner than do heart rate and respiratory frequency, but the increase in respiratory frequency during the first few weeks is also disproportionate to the circulatory changes and oxygen consumption. Nonetheless, despite the initial perturbations, the new steady state seems to be set in approximately 2 months. This prolonged and gradual entry contrasts with the onset of hibernation which, in the ground squirrel, requires only 5–10 h to achieve its characteristically low levels of cardiovascular, respiratory and metabolic performance (Landau & Dawe, 1958; Lyman & O'Brien, 1960).

Heart rate. Despite the consistent slowing of the heart rate observed in the present study, the lowest mean values were of the order of 10/min rather than the 3/min reported by Smith (1930). He reported only a single value for heart rate during aestivation which was indirectly obtained from a lungfish (250 g) after 389 days of aestivation at 19°C. Our lungfish were not only much larger (2–6 kg) but our ambient temperature was higher (22–26°C) and our longest period of observation was shorter (283 days).

The mechanisms responsible for the persistent bradycardia of aestivation are not known. Among the potential mechanisms are the high concentrations of K^+ in the plasma during aestivation (6 m-equiv./l after 2⅓ months compared with 2–4 m-equiv./l control aquatic values), and the high levels of metabolic products that accumulate in blood during aestivation. However, we observed that an infusion of 8 m-equiv. of K^+ (as KCl) over a period of 15 min into an aquatic lungfish (3·4 kg) did not decrease the heart rate.

The spontaneous bradycardia during aestivation is exaggerated by sudden noises in the environment. For example, vibrations produced by the start of a noisy exhaust fan immediately slowed the heart rate from 15 to 4–8 beats/min accompanied by a premature lung breath. But as the disturbance continued, the heart rate gradual

turned to 15 beats/min without any further change in respiratory (lung) frequency. This 'fright bradycardia' indicated that the aestivating lungfish was not in a deep torpor.

Blood pressure. The decline in blood pressure in part appears to be related to a partial desiccation of the lungfish during the onset of aestivation. However, the mechanisms responsible for its dissociation from the heart rate are not clear since we lack information about the cardiac output, circulating blood volume, distribution of blood flow or autonomic regulation of the heart and circulation. The lungfish has an autonomic nervous system, but its regulatory role on the heart and lung is not known (Nicol, 1952). However, the likelihood of venous pooling as a consequence of the fixed position in the cocoon and haemoconcentration due to dehydration suggest that a decrease in effective blood volume and cardiac output may contribute to the genesis of the arterial hypotension. Indeed, the aestivating lungfish may be manifesting orthostatic hypotension. The accumulation of metabolic waste products might also add to the decline in cardiac output by decreasing myocardial contractility.

Respiratory frequency

Several different influences may be contributing to the increase in respiratory (lung breath) frequency observed after entry into mud. (1) Cutaneous stimulation, as seen in the increased respiratory frequency that follows removal of a lungfish from water (Lenfant & Johansen, 1968; McMahon, 1970; Johansen, 1970) may be important initially, but there is no evidence that this stimulus persists. (2) The partial collapse of the lungs as buoyancy is lost may reduce lung volume and also stimulate a deflation reflex; we have found that gentle deflation of the lungs via a catheter, in an aestivating or non-aestivating lungfish increases respiratory frequency. (3) The increase in arterial P_{CO_2}, resulting from diminished CO_2 elimination by skin and gills and the increase in respiratory dead space produced by the channel between the mouth and the surface, also may act as a stimulus. We have confirmed Smith's observation (Smith, 1930) that aestivating lungfish increase respiratory frequency when CO_2 is added to inspired air. Since arterial oxygenation is well maintained during aestivation, hypoxia was apparently not involved in the respiratory stimulation.

Despite persistent hypercapnia during aestivation, respiratory frequency gradually slowed. In part, this decrease may be related to the progressive decrease in O_2 consumption. On the other hand, it may represent a gradual decline of the ventilatory response. Further studies involving acid–base of arterial blood and cerebrospinal fluid will be necessary to determine the nature of the compensation involved.

Theories of aestivation

DuBois (1892) pictured the aestivation of *Protopterus* as a state of sleep mediated through a cutaneous sensory reflex. Smith (1930) believed aestivation to be a state of deep torpor initiated by a postural reflex elicited by immobilization. Smith's criteria for torpor were not explicitly defined, but appeared to be based on the ability of the fish to remain dormant (i.e. did not break out of the cocoon) in the face of a variety of stimuli including the administration of thyroxine, a change in ambient temperature, or mild sensory stimulation.

In the present study we have found that mild ambient noise produced prompt

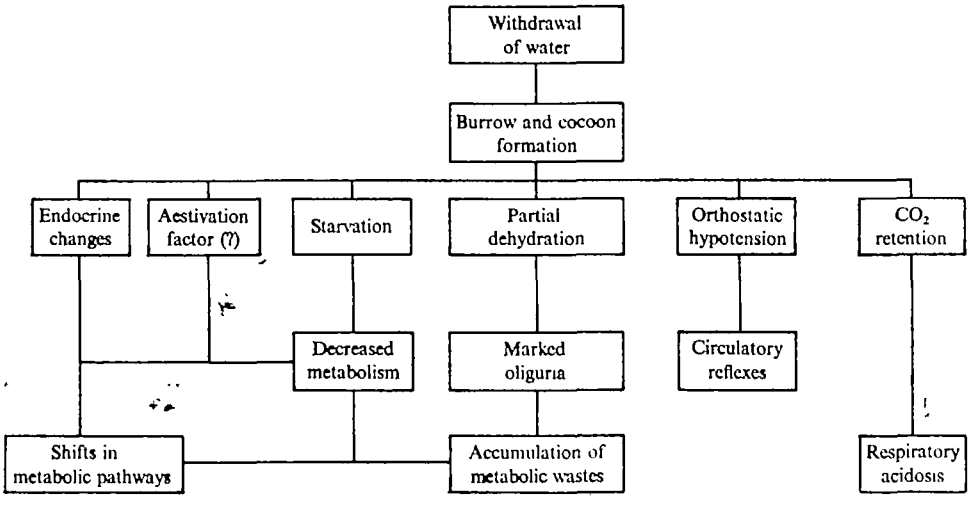

Fig. 10. Schematic representation of factors that may be involved in the genesis (and maintenance) of aestivation in *Protopterus*.

changes in breathing and heart rate. A similar 'startle' response to noise can be elicited in the hibernating ground squirrel only during the arousal phase (Landau & Dawe, 1958), but not during the deep torpor of prolonged hibernation (Kayser, 1961). In this respect, therefore, our observations support the view of Hudson & Bartholomew (1964), whose considerations of aestivation in mammals led them to conclude that aestivation is a form of 'light' or shallow torpor.

The possibility of a humoral control of aestivation was first tested experimentally by Godet & Pieri (1961). They found that an extract of brown fat from an aestivating lungfish caused a depression in body temperature in rat. Swan, Jenkins & Knox (1968) were able to extract an 'antimetabolite' from the brain of aestivating *Protopterus aethiopicus* which elicited a 35% drop in metabolic rate and a fall of 5 °C in body temperature within 1 h after intravenous injection in rats. These observations were interpreted as indicating the existence of a biochemical factor(s) in tissues which is elaborated during aestivation. Whether this factor is specific for aestivation, how it exerts its effects, and what its relationship is to the 'sleep factor' described, for example, by Fencl, Koski & Pappenheimer (1971), is as yet unknown.

From the observations that have been summarized above, it is possible to list the factors that are associated with aestivation (Fig. 10). The process begins with removal of ambient water in a setting (mud or sack) which allows the formation of a cocoon. Starvation and dehydration are inevitable sequelae. In time, the lack of water and food results in haemoconcentration, a reduction in blood volume, and, presumably, a decrease in cardiac output. But, before these changes become appreciable, urine formation decreases to the point of marked oliguria. This oliguria is presumably reflex, being possibly a consequence of venous pooling in the periphery. The ensuing accumulation of metabolic waste products may contribute to a torpid state.

The stimulus for cocoon formation is unclear. But the cocoon does form gradually, thus preventing water loss but also promoting CO_2 retention. The sustained hypercapnia and the persistent postural hypotension produced by fish in the head-u

position in the burrow may contribute to the mild torpor by adding the factor of diminished cerebral blood flow to the biochemical factors in the internal environment.

Starvation in the lungfish decreases oxygen consumption. But starvation, *per se*, cannot account for the entire syndrome of aestivation. The extent to which the decrease in metabolic rate and shifts in endocrine function contribute to the light torpor of aestivation remains to be investigated.

At present, it seems that aestivation in *Protopterus* is a state of dormancy, which is gradual in onset, and involves an interplay of different influences. The pattern is consistent, suggesting a reproducible series of adaptive changes that culminate in a new steady state. Whether a specific neurohumour is involved in the genesis of this state through regulation of metabolism, or by some other means, is as yet unknown.

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