Acid-base balance and plasma composition in the aestivating lungfish (*Protopterus*)

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The African lungfish, *Protopterus* sp., is generally held to have survived for over 300 million years without marked change in the anatomical and physiological features that characterized the first terrestrial animals (37). For this reason, the lungfish has attracted the attention of those concerned with the adaptive mechanisms involved in the evolutionary transition from aquatic to terrestrial life. In addition, this genus has retained the distinctive ability to aestivate, a type of dormancy that enables it to survive the seasonal periods of drought (3-9 mo) that characterize life in the tropics.

To initiate the process of aestivation, the lungfish burrows into the mud as the ambient waters recede and forms an aestivation burrow. As the surrounding mud dries, the mucous secretions of the skin harden to form a waterproof cocoon that surrounds the body completely except for the small opening at the mouth. In this subterranean nest, which is connected to the surface by a narrow breathing channel, the lungfish is obliged to rely entirely on air breathing for its external gas exchange, and is deprived of access to food or water. Inevitably, the lack of food and water intake and the cessation of gas exchange through skin and gills lead to dramatic changes in metabolic functions (4, 36).

Survival during aestivation also implies accommodation to major derangements in acid-base balance and in the electrolyte composition of the blood. Evidence for this conclusion was adduced in the course of our observations on the circulation and respiration in aestivating *P. aethiopicus* (4). Thus, during the first 2.5 mo of aestivation, we found that the arterial $P_{CO_2}$ increased from the normal aquatic range of 25-30 to 40-70 Torr. Hypercapnia was accompanied by a fall in arterial pH which appeared to be only partially compensated by an increase in plasma bicarbonate. This pattern of adjustment differs from that reported for other animals that undergo transition from aquatic to terrestrial existence. For example, in both the metamorphosis of the bullfrog (8) and the emersion of the intertidal crab, *Carcinus* (38), the transition from water-to-air breathing is accompanied by corresponding increases in bicarbonate concentration which compensate for the rise in arterial $P_{CO_2}$ and maintain arterial pH virtually unchanged from that of the aquatic state. Thus, the air-breathing lungfish fails during aestivation to compensate for respiratory acidosis as fully as other poikilotherms (11).

By analyzing the muscle composition of aestivating lungfish and measuring the rate of excretion of metabolites after the aestivating fish were returned to water, Smith (36) showed that metabolic wastes accumulated within the body during aestivation. Urea and inorganic sulfate were retained in large amounts and renal function appeared to be below measurable limits. Janssens (14) confirmed Smith’s findings and also reported that sodium was excreted in large amounts when aestivation was ended and the lungfish returned to water. These separate observations on acid-base changes and on electrolyte composition prompted us to explore their interplay during aestivation. The only previous determinations of plasma electrolyte composition in the lungfish were made in the nonaestivating state: Smith (36) and Lahiri et al. (20) analyzed plasma samples from *P. aethiopicus*; Urist et al. (39) dealt with plasma composition of three related Dipnoi species. We undertook to determine the sequential changes in acid-base status and plasma composition during 13.5 mo of aestivation in the African lungfish, *P. aethiopicus*.

**METHODS**

African lungfish, *Protopterus aethiopicus* (2-12 kg) obtained from Lake Victoria in East Africa were maintained in the laboratory as previously described (4).
LUNG FISH BLOOD DURING AESTIVATION

Blood Sampling

A polyethylene catheter was placed in the third branchial arch under MS-222 anesthesia (4). Control blood samples were obtained from the undisturbed, free-swimming lungfish 2–3 days after surgery. The in vivo acid-base responses to acute changes in arterial PCO₂ were determined in three aquatic lungfish. The fish were placed in a covered aquarium maintained at 25°C. A mixture of CO₂ (4 or 6.5%) in air was bubbled into the tank to promote equilibrium of the water with the inspired gas mixture. Blood samples were taken from the lungfish after a period of 3-4 h.

The fish used for the studies of the onset of aestivation were anesthetized with thiopental sodium (15 mg/kg) via the arterial catheter after control aquatic samples had been drawn. They were then placed in an artificial aestivation sack which was hung in a tub of water after the fish had recovered from anesthesia, i.e., within 15–30 min after administration of this short-acting barbiturate. The rate of emptying of the tub was adjusted so that the water level gradually fell, in 7–14 days, below that of the body of the fish. During this time, the cocoon began to form around the fish in its characteristic U-shaped posture (4). Arterial samples (3 ml) were collected every 2–3 days during the onset of aestivation until blood could no longer be withdrawn through the catheter. The catheters generally remained patent for 10–20 days.

Blood samples from lungfish aestivating for 1 mo or longer were obtained from individual fish which had not been previously catheterized. The aestivating fish was lightly anesthetized by inhalation of methoxyflurane (Pitman-Moore, Ft. Washington, Pa.) administered by an ether cone placed over the mouth. The cocoon was dissected free from the region of the gill slit and the operculum was opened to expose the branchial arches. The blood sample was collected by an arterial puncture (22-gauge needle) on the third branchial arch or by cannulating this artery as described above.

Urine Collection

In two lungfish, arrangements were made to collect urine before and after the start of aestivation in order to relate changes in urine formation to electrolyte balance. A small polyethylene catheter was sutured to the open operculum was opened to expose the branchial arches. The blood sample was collected by an arterial puncture (22-gauge needle) on the third branchial arch or by cannulating this artery as described above.

Analytical Methods

The arterial pH, Pco₂, and P0₂ of 1.5-ml samples were determined using thermal-jacketed (25°C) microelectrodes (Radiometer PHM 72C system, Copenhagen, Denmark) calibrated with two known buffers (pH 7.410 and 6.865) and two different gas mixtures (2.9% CO₂ in N₂ and 11.2% CO₂, 14.1% O₂, balance N₂, barometric pressure = 758 Torr). The plasma bicarbonate concentration was calculated from the Henderson-Hasselbalch equation

\[ \text{pH} = pK' + \log \frac{[\text{HCO}_3^-]}{\alpha \text{PCO}_2} \]

Since the solubility coefficient of (1/α) (α) and the pK’ of lungfish plasma were not known, a separate series of experiments were undertaken to determine these constants. The solubility of CO₂ in nonaestivating lungfish plasma was determined by manometric analysis of the CO₂ content of 3-ml samples of plasma acidified by the addition of lactic acid (1% solution) and equilibrated with 100% CO₂ at 25°C according to Van Slyke et al. (41). The water content of the plasma was determined by desiccating 2-ml aliquots for 48 h at 100°C. The pK’ of separated plasma at 25°C was determined at different pH’s, by determining the values of pH, PCO₂, and CO₂ content (40) after tonometry with gas mixtures containing 2, 3.5, 7, and 8% CO₂ in the air (2). These values were used in the following formula

\[ pK' - \text{pH} \log \frac{[\text{CO}_2] - \alpha \text{PCO}_2}{\alpha \text{PCO}_2} \]

The hemoglobin concentration of blood was determined as described by DeLaney et al. (5).

Plasma osmolality was determined by the freezing-point depression method using a Precision Systems 2007 osmometer (Waltham, Mass.) calibrated with three known standards (0, 300, and 500 mosmol/l). Concentrations of sodium and potassium in serum were determined by flame photometry using an Instrumentation Laboratories model 143 flame photometer (Boston, Mass.). For the serum K⁺ measurements, the nonheparinized blood was separated immediately after sampling by gentle centrifugation (1,500 rpm, 2 min, 25°C). The decanted serum was then stored at −3°C until analysis.

The serum concentrations of calcium and magnesium were determined using an atomic absorption apparatus (model 404, Perkin-Elmer Corporation, Downer's Grove, Ill.). Concentrations of chloride in serum were determined using an Aminco-Cotlove titrator (American Instrument Company, Silver Spring, Md.). The concentration of inorganic phosphate in serum was determined using a modified Fiske-SubbaRow method (32). The concentration of serum inorganic sulfite was determined as described by Kleeman et al. (19). Human serum of known composition (Lab-Trol, Dade, Miami, Fla.) was analyzed simultaneously for reference.

The total concentration of plasma proteins was determined using a modified Folin-Ciocalteau method of Lowry (32). Plasma protein electrophoresis of the same samples were also run on cellulose acetate. Barbital buffer (pH 8.6) was used in the Beckman electrophoresis cell and samples were run for 45 min at 250 V with the origin being at the center of the membrane (Beckman Instruction Manual 015-983618). The electropho-
retrie pattern was identified by staining the cellulose acetate strips with nigrosin stains. A Beckman densitometer was used to characterize the separation pattern. The protein fraction ratios were determined by quantifying the area under the curve using the densitometer integrator.

The concentration of glucose in whole blood was determined by the hexokinase-UV method; spectrophotometric readings were made at 340 nm (HK method 1S994, Boehringer-Mannheim, New York City). The concentration of urea nitrogen in blood was determined after hydrolysis with urease using Berthelot's method (32). The optical density of the Berthelot reaction was measured on the Gilford spectrophotometer at 580 nm.

The concentration of serum creatinine chromagen was calorimetrically determined using the Jaffe reaction (13). The concentration of lactate in whole blood was determined using the lactate dehydrogenase enzymatic method on 0.5 ml of whole blood (Boehringer-Mannheim, 15972TLAA, UV method).

RESULTS

Acid-Base Status

Control observations. The solubility of CO₂ in plasma of six samples from non-aestivating lungfish at 25°C proved to be 0.6939 ± 0.0023 (SD) ml CO₂/ml plasma (STPD), a value equivalent to 0.0410 mM H₂CO₃/Torr Pco₂. This solubility factor for CO₂ (α) was used in all calculations that follow.

The water content of pooled lungfish plasma used in these determinations was 94.12%. The pK′ of lungfish plasma at 25°C of eight samples from two fish is shown in Fig. 1. The pK′ of aquatic lungfish plasma at pH 7.600 was 6.1420; the linear regression (dpK'/dPH) had a slope of -0.061.

The arterial pH of 10 non-aestivating lungfish resting in water at 25°C averaged 7.602 ± 0.032 (SD); the corresponding arterial Pco₂ averaged 26.4 ± 1.3 (SD) Torr. The plasma bicarbonate level, calculated using the experimentally derived α and pK′ values, averaged 31.4 ± 1.350 (SD) meq/l. The linear regression of the in vivo CO₂ titration curve from 2 of the normal adult lungfish (weighing 3.5 and 5.5 kg) is shown in Fig. 2.

Changes in plasma bicarbonate averaged −1.26 meq/l per 0.1 pH unit.

Aestivation. At the onset of the aestivation process, as the water level fell below the lungfish, the arterial Pco₂ increased considerably, averaging 45.2 Torr at a pH of 7.405 despite a 2- to 5-fold increase in lung breath frequency in the four fish studied (Fig. 2). The plasma bicarbonate rose concomitantly to 33.2 meq/l within this 1-wk period. During the next week, arterial Pco₂ continued to increase but at a slower rate, reaching an average of 49.8 Torr at pH 7.373; the corresponding concentration of bicarbonate was 34.0 meq/l. Thus, as may be seen in Fig. 2, during the first 2 wk of aestivation, the plasma bicarbonate concentration followed the in vivo titration line of the non-aestivating lungfish.

Blood samples were drawn from other lungfish (not previously catheterized) to trace changes in acid-base status during subsequent months of aestivation. A different lungfish was used after 1, 2, 3, and 7 mo of aestivation (total 4 fish). In lungfish aestivating for over 2 mo, the interval between lung breath periods generally ranged from 5 to 15 min similar to that seen in aquatic fish. However, serial blood sampling revealed that between these breathing periods, arterial Pco₂ and pH changed strikingly. Thus, at the start of the apneic interval, arterial Pco₂ began to increase from a level of 40–45 Torr (pH 7.53–7.510) to reach a plateau at 50–55 Torr (pH 7.44–7.40) within 2–3 min; thereafter, there was only a slight and gradual change until the next breath period 3–13 min later. The values for arterial pH, Pco₂, and bicarbonate values in Fig. 2 and Table 1 are from blood samples taken in the apneic period when plateauing was in effect. The bicarbonate concentration in plasma increased slowly to 39 meq/l along the arterial Pco₂ = 50 isopleth during the next 1 2 mo of aestivation. It reached a plateau of 41 meq/l in the lungfish aestivating for 3–7 mo despite a slight further increase.
Plasma Composition

buffering power of its blood was -19.1 meq/l per pH units). The change in slope of the buffer line during decreased concomitantly (slope of -1.91 meq/l per 0.1 arterial Pco2 from 52-57 Torr to a nadir of 32 Torr (pH the samples. Blood samples then showed a decrease in the experimental manipulations involved in obtaining from 6 to 15-18 breath periods/h), apparently related to tion for several hours (an increase in breath frequency during aestivation was complicated by transient hyperventila-

arterial pH in the range of 7.40-7.44. The severe respiratory acidosis tended to maintain the for each g/100 ml of hemoglobin.

The chloride concentration in P. aethiopicus was lower in P. annectens by Urist et al. (39). On the other hand, Smith (36) is much greater than the 5.5 meq/l reported measured ions, urea and protein, was 236 meq/l. This Table 1. The osmolality of plasma, calculated from the measured sodium in serum was only 12 meq/l higher than preaestivation aquatic values. This was considerably less than the increase of 25 meq/l in lung-fish aestivating in a cloth sack, urine flow appeared to stop and remained arrested for the duration of the aestivation.

Aestivation. The changes in plasma composition during aestivation are compared with the preaestivation aquatic values in Table 1. The concentration of most plasma constituents increased throughout aestivation. At the start of aestivation, as the water level fell below the lungfish (see Fig. 3), plasma osmolality increased. For the first 3 days of aestivation, concomitant with only a slight increase in plasma osmolality, urine osmolality increased despite a decline in urine output so that total excreted ions increased (from the normal aquatic range of 22.3 x 10^-3 to 31.2 x 10^-3 mosmol/kg per h to 58.7 x 10^-3 to 67.5 x 10^-3 mosmol/kg per h). However, from day 4 to 7, further decreases in urine volume caused total excreted ions to fall below the preaestiva-

ion rate of excretion and both plasma osmolality and blood urea began to increase rapidly. Within 1 day after the water level fell below the lungfish (after the 7th day in the aestivation sack), urine flow appeared to stop and remained arrested for the duration of the aestivation.

That the cloth aestivation nest technique used in the present study promoted more rapid evaporation of sur-
face water and consequent dehydration than during aestivation in mud was tested in one lungfish that was aestivated in mud and took more than 1.5 mo to dry fully. In this fish, after 2 mo of aestivation, the concentration of sodium in serum was only 12 meq/l higher than preaestivation aquatic values. This was considerably less than the increase of 25 meq/l in lung-fish aestivating in a cloth sack for 1 mo. The varying rates of drying in the sack after ambient water has been withdrawn probably also accounted for the different rates at which concentration of plasma constituents changed from fish to fish during the first 3 mo of aestivation.

After 2-4 wk in the aestivation sack, the rate of

in arterial Pco2 (Fig. 2). This partial compensation of the severe respiratory acidosis tended to maintain the arterial pH in the range of 7.40-7.44.

Sampling in the lungfish that had spent 7 mo in aestivation was complicated by transient hyperventilation for several hours (an increase in breath frequency from 6 to 15-18 breath periods/h), apparently related to the experimental manipulations involved in obtaining the samples. Blood samples then showed a decrease in arterial Pco2 from 52-57 Torr to a nadir of 32 Torr (pH 7.590). As shown in Fig. 2, bicarbonate concentration decreased concomitantly (slope of -1.91 meq/l per 0.1 pH units). The change in slope of the buffer line during aestivation appeared to be directly related to the change in hemoglobin concentration. The in vivo CO2 titration line ( -12.6 meq HCO3^-/l per pH unit), which was measured at a hemoglobin concentration of 6.7 g/100 ml, had a ratio of 1 g/100 ml of hemoglobin to -1.88 meq HCO3^-/l per pH unit in the aquatic lungfish. The lungfish aestivating for 7.5 mo had a hemoglobin concentration of 11 g/100 ml. The linear regression slope describing the concentration of plasma in P. aethiopicus (31.4 meq/l) in aestivation; cocoon formed after 1.5 wk in aestivation sack.

TABLE 1. Changes in plasma composition during aestivation

<table>
<thead>
<tr>
<th>Plasma Component</th>
<th>Preaestivation†</th>
<th>Aestivation†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>2 wk</td>
</tr>
<tr>
<td>Sodium, meq/l</td>
<td>10</td>
<td>101.3 ± 4.4</td>
</tr>
<tr>
<td>Potassium, meq/l</td>
<td>10</td>
<td>3.1 ± 0.6</td>
</tr>
<tr>
<td>Calcium, meq/l</td>
<td>10</td>
<td>4.8 ± 0.7</td>
</tr>
<tr>
<td>Magnesium, meq/l</td>
<td>6</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td>Chloride, meq/l</td>
<td>10</td>
<td>77.3 ± 6.2</td>
</tr>
<tr>
<td>Bicarbonate, meq/l</td>
<td>10</td>
<td>31.4 ± 1.3</td>
</tr>
<tr>
<td>Inorganic phosphate, meq/l</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Inorganic sulfate, meq/l</td>
<td>6</td>
<td>2.6 ± 0.9</td>
</tr>
<tr>
<td>Lactate, meq/l</td>
<td>9</td>
<td>0.58 ± 0.16</td>
</tr>
<tr>
<td>Urea, mmol/l</td>
<td>9</td>
<td>4.2 ± 3.0</td>
</tr>
<tr>
<td>Creatinine, mg/100 ml</td>
<td>9</td>
<td>0.32 ± 0.13</td>
</tr>
<tr>
<td>Total protein, g/100 ml</td>
<td>10</td>
<td>3.1 ± 0.5</td>
</tr>
<tr>
<td>Blood glucose, mg/100 ml</td>
<td>15</td>
<td>54.8 ± 6.6</td>
</tr>
<tr>
<td>Osmolality, mosmol/l</td>
<td>10</td>
<td>234 ± 15</td>
</tr>
</tbody>
</table>

* n = Number of observations on control fish in water. 
† Values obtained for each period are from individual fish in artificial aestivation; cocoon formed after 1.5 wk in aestivation sack.
change in electrolyte concentration slowed but continued during the 13 mo of aestivation. Disproportionately large increments in the concentration of magnesium and inorganic sulfate occurred as compared to the increments in sodium and chloride concentrations. The concentration of inorganic phosphate was the only electrolyte level which appeared to decrease during aestivation (Table 1).

The concentration of urea in blood continued to increase at a rapid rate throughout the 13 mo of aestivation and became an increasingly larger fraction of the osmotically active substances in plasma. Thus, after 13 mo in aestivation, the 203 mmol/l of urea accounted for 31.2% of the plasma osmolality as compared with only 2% in the aquatic lungfish. Despite the marked increase in blood urea, the concentration of creatinine in plasma did not differ significantly from that of the nonaestivating lungfish (Table 1).

The concentration of glucose in blood decreased during the 1st mo of aestivation but returned to within the preaestivating range during the following 13 mo of aestivation (Table 1).

The electrophoretic pattern of the plasma proteins had six identifiable peaks (Fig. 4) in both aestivating and nonaestivating fish. Trace quantities (<1%) of a protein with a mobility similar to that of mammalian albumin were found in only six of the lungfish studied. Fraction A had a mobility resembling that of human β-globulin. Fraction F contained fibrinogen which often masked the presence of the B-fraction protein. In serum samples, the F peak was greatly reduced. Fraction L was the only portion which had a positive staining reaction with the sudan black and oil red O indicating the presence of a lipoprotein. Peak G was in the same region as mammalian γ-globulin.

During aestivation, the plasma protein pattern was essentially the same as the aquatic lungfish even though the total protein content of plasma increased considerably from the control value of $31 \pm 0.5$ (SD) to 5.2–6.4 g/100 ml. Accompanying the increase in total protein was a disproportionate increase in the concentration of fibrinogen (F) and a decrease in the L fraction. The total plasma protein content in aquatic P. aethiopicus was higher than in P. annectens (2.8 g/100 ml, Urist et al. (39); 1.9 g/100 ml Masseyeff et al. (25)) and the increase in plasma protein concentration during aestivation was much higher than the 42% increase in P. annectens after 2 yr aestivation (25).

DISCUSSION

The capacity of the African lungfish to survive periods of drought by entering a state of aestivation has interested naturalists for many years (see review by Johnells and Svensson (18)). However, comparatively little has been known about the sequence of physiological changes that occur during aestivation.

As the lungfish withdraws into its aestivation nest and the cocoon begins to form, the lungs become the principal organ for CO₂ elimination. However, the increase in respiratory dead space produced by the extension of the burrow to the surface, and the decrease in tidal volume (23), reduce the efficiency of CO₂ elimina-
tion by the lungs. Thus, the arterial PCO₂ increases despite an increase in minute ventilation due to increased respiratory rate and a decrease in oxygen consumption. Concomitantly food and water intake cease, and excretory functions are markedly reduced. In addition, metabolic pathways (14–16) and endocrine functions (7) change during aestivation. All of these alterations have profound effect on the acid-base balance and the electrolyte composition of blood.

**Acid-Base Status**

The arterial PCO₂ in *P. aethiopicus* living in water under natural conditions is high (order of 26 Torr) compared with other fish that rely solely on gills for breathing (arterial PCO₂ < 5 Torr) (29). The mature *Protoporus* is an obligatory air-breathing fish which relies on lung ventilation to provide 90% of its oxygen consumption (22, 26). While in water, the gill ventilation of the lungfish is much slower than that of most fish and gill filaments are restricted to the fourth to sixth branchial arches (17). As a result only 60% of the metabolic CO₂ is exchanged across the gills (22, 26).

Despite the high value for arterial PCO₂ in aquatic lungfish, arterial pH is maintained around 7.6 by a high concentration of bicarbonate in plasma, i.e., of the order of 31 meq/l. Thus, the arterial pH at 25°C is within the pH range expected for other water-breathing fish based on the pH vs. temperature plot of Rahn and Baumgardner (30). This suggests that the pattern of acid-base balance in aquatic *P. aethiopicus* corresponds to that of other poikilotherm vertebrates, i.e., a mechanism is present which, by maintaining a constant difference between blood pH and the pH of pure water, results in a constant relative alkalinity of the blood with respect to water at any given temperature (12, 29–31).

The time course of the acid-base changes during aestivation (Fig. 2) indicates two distinct phases: initially, an uncompensated respiratory acidosis develops as the gill and cutaneous exchange of CO₂ diminishes during dehydration, in the subsequent compensatory phase, plasma bicarbonate increases slowly along the course of the isopleth of arterial PCO₂ = 50 Torr to reach a new buffer line that is 6–8 meq/l above the aquatic (preaestivation) in vivo buffer line for the same pH. This suggests that the pattern of acid-base balance in aquatic *P. aethiopicus* corresponds to that of other poikilotherm vertebrates, i.e., a mechanism is present which, by maintaining a constant difference between blood pH and the pH of pure water, results in a constant relative alkalinity of the blood with respect to water at any given temperature (12, 29–31).

The reciprocal exchange of bicarbonate and chloride ions across the gills of teleosts (3, 6), as well as across the skin of the frog (9), appears to be involved in the acid-base balance of these species. Dejours (3) found that fish in which concentrations of chloride in plasma were low tended to have correspondingly high values for plasma bicarbonate. In the aestivating lungfish in which both kidneys and gills ion exchange appear inoperative, there can be no net influx of chloride ion. The increase in plasma bicarbonate concentration is accompanied by only a slight increase in plasma concentration of chloride ion (Table 1). The extent to which tissues and red blood cells may be involved in these changes is discussed below.

**Electrolyte Composition of Blood**

After the 1st mo of aestivation, the rate of change in electrolyte concentrations slowed but the electrolyte balance did not reach a new steady-state level throughout the 13 mo of observation. Since water intake and normal aquatic kidney function had ceased, the shifts in electrolyte content undoubtedly represent exchanges between different tissues and blood.

A reasonable analogy may be drawn between the adaption of the aestivating lungfish and the nephrectomized dog during acute respiratory acidosis (10). An exchange of intracellular Na⁺ and K⁺ for extracellular H⁺ accounted for 51% of the buffering of the carbonic acid and the exchange of extracellular Cl⁻ for bicarbonate across the erythrocyte contributed 29% of the total buffering in the dog. The rise in plasma Na⁺ concentration, and a disproportionately low increase in plasma Cl⁻ concentration appear to follow a similar pattern in the aestivating lungfish as in the nephrectomized dog.

Magnesium secretion is coupled with sodium reabsorption in the kidney of teleosts (27). Also, in hypo-
thermic turtles, the concentration of magnesium in plasma has been found to increase, presumably from inactive skeletal muscle (28). In the aestivating lungfish, the increase in plasma magnesium may also represent an accumulation of magnesium derived from skeletal muscle. This increment balances the large influx of inorganic sulfate that appears to originate from oxidation of protein sulfur in muscle. The low level of inorganic phosphate and creatinine, as well as creatine (36), may also be related to the reduction in muscular activity during aestivation.

Further studies are required to determine the control mechanisms responsible for the marked oliguria during aestivation. The decrease in blood pressure during the onset of aestivation (4) may contribute to the reduction in urine output. Another mechanism might be a reduction in production and/or release of the neurohypophysial hormone arginine vasotocin which has been isolated from the pituitary of *P. aethiopicus* (34). This hormone has been demonstrated to have a diuretic, natriuretic, and vasopressor effect in aquatic lungfish (33, 35).

The accumulation of nitrogenous wastes as urea during aestivation was first described by Smith (36). While in water, *Protopterus* excretes approximately 35% of its nitrogenous wastes as urea, the remainder being excreted as ammonia (15); during aestivation, ammonia production almost completely ceases (16).

Janssens (14) found that muscle of lungfish aestivating for 6 mo had 50% more glycogen than did the preaestivation (aquatic) lungfish, indicating that carbohydrate reserves were increased rather than decreased as postulated by Smith (36). The restoration of the blood glucose level to the normal range after 3 mo aestivation in the present study seems to support Janssens' conclusion that appreciable gluconeogenesis occurs during aestivation.

The increase in the concentration of plasma proteins during aestivation was associated with a disproportionate increase in the fibroprotein fraction (fraction F). The basis for the unusual increment in this fraction is unclear. The concomitant decrease in the lipoprotein fraction (fraction L) may be indicative of a depletion of lipids during aestivation. Fraction G has recently been shown to have immunoglobulin properties (L. D. Plonsky, D. T. Rowlands, R. G. DeLaney, A. P. Fishman, unpublished observations) as in *Neoceratodus forsteri* (24).

**Interrelationship Between Acid-Base Status and Total Plasma Composition**

During aestivation, one of the results of the increase in concentration of plasma components due to dehydration and cessation of normal kidney function which influences acid-base balance and the resulting arterial pH is the increasing ionic strength (μ) of the plasma. An increase in the ionic strength of a solution will decrease the solubility of gases and increase the equilibrium constant of most reactions (see Albers (1)). Therefore, the elevation in μ from 0.13 to more than 0.22 during aestivation must influence acid-base balance. Unfortunately, we could not collect enough blood from aestivating fish to measure the decrease in Pco2 and pK'. Albers and Pleschka (2) reported an α equal to 78% of the solubility of CO2 in pure water, and a plasma pK' of 6.019 in elasmobranchs at pH = 7.40, 25°C. Aquatic (preaestivation) lungfish in the present study under the same conditions had an α equal to 93.4% the solubility of CO2 in pure water, and the pK' was 6.152. During prolonged periods of aestivation, it appears that the plasma α and pK' values may approach those of elasmobranchs as electrolyte and urea concentrations reach comparable values.

In summary, a respiratory acidosis develops during aestivation despite the large decrease in oxygen consumption. This condition is only partially compensated by a slow compensatory increase in the concentration of bicarbonate in plasma. The degree of restoration of arterial pH toward the preaestivation value, while the lungfish is in water, is a complicated function of the change in respiratory dead space, the cessation of normal kidney function, acidosis, the decrease in metabolism and shifts in metabolic pathways, and changes in electrolyte composition and acid-base status of plasma due to dehydration. As a result of this interplay, the concentration of electrolytes and urea in plasma do not reach new steady-state levels even during prolonged aestivation (more than 1 yr) and the plasma osmolality continues to increase progressively, although at a slow rate. In essence, electrolyte and acid base balance continue to change uninterruptedly throughout aestivation.

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**REFERENCES**

9. GARCIA ROMET, P., A. SALERNO, AND PREZIANI-HERAND. The nature of the in vivo sodium and chloride uptake mechanism.


