Respiration and acid-base status of turtles following experimental dives

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When an animal's normal gas exchange is interrupted, changes in gas tensions and acid-base status occur in its body fluids. In diving animals these changes are slowed and restricted by characteristic responses which adapt these animals to prolonged apnea. These responses include a reduction in overall metabolism (1, 10, 15, 19) and a selective distribution of arterial blood which favors the heart and brain (7, 19). Even in diving animals, however, internal homeostasis is disturbed so that the adaptation to diving must include a tolerance to these internal changes and the ability to rapidly restore the normal condition following the dive. Since most diving vertebrates depend on a continuous supply of oxygen to the vital central organs, the duration of submergence is interrupted, changes in gas tensions and acid-base status occur in its body fluids.

Freshwater turtles, in contrast, can survive without oxygen for long periods because all their vital functions can be supported for a time by anaerobic metabolism (5, 14). While this metabolic capacity greatly extends the length of time turtles can remain apneic, it also tends to exaggerate the disturbances to the internal environment. For example, Robin et al. (18) kept turtles of the genus Pseudemys sub-

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Metabolism and respiratory acidosis. Recent studies on respiratory control in the turtle Pseudemys scripta elegans demonstrate that this animal is quite sensitive to increased CO2 (unpublished data) or decreased O2 (13) in the inspired air, and since hypercapnia and hypoxia are prominent features at the conclusion of a dive, considerable hyperventilation could be predicted on this basis when breathing resumed.

The object of the present study was to describe various aspects of the recovery pattern from a standardized experimental dive in the turtle Pseudemys scripta elegans. As will be seen, the ventilatory response was marked and quite effective, restoring blood pH to normal, whereas the correction in the disturbances to blood lactic acid and bicarbonate concentration was a much slower process.

METHODS

Animals. Male turtles Pseudemys scripta elegans weighing 150-750 g were studied. All experiments were conducted at 24°C, and the turtles were kept at that temperature for at least 1 day prior to study.

Ventilation and metabolic rate. Pulmonary ventilation was measured using the buoyancy principle (12). In brief, the turtle was suspended into a temperature controlled water bath from a calibrated strain gauge. Although submerged and restrained from swimming movements, the turtle could freely elevate its head above the water surface into a ventilated chamber and breathe. When it did so, its underwater weight or buoyancy changed in proportion to the volume of air inspired, and this change in weight was sensed by the strain gauge and recorded on a polygraph as a tidal breath. Respiratory minute volume was averaged over intervals of 5-20 min (expressed as ml (min)/kg ⋅ min).

Oxygen consumption was measured by a conventional open-circuit method. The breathing chamber was ventilated at a monitored rate from a compressed air cylinder. After
passing through the chamber, the gas was dried and directed through a paramagnetic oxygen analyzer (Beckman model F-3), which provided a continuous recording of the oxygen content of the gas. Flow rate was adjusted according to the animals' oxygen uptake. Oxygen consumption was averaged for time intervals from 5 to 70 min (expressed as ml (STPD)/kg·min). No correction was made for possible differences in the volumes of inspired and expired air.

**Blood sampling and analysis.** In most experiments blood was sampled from a chronically placed catheter (PE-90) in the subclavian artery. The catheterization was carried out on turtles lying supine in a tray of ice following equilibration overnight at 5°C. The artery was exposed through a hole trephined in the plastron, and the catheter, after insertion in the artery, was led out through the skin at the base of the neck. The original disc of plastron was cemented back in place and the area was covered with dental acrylic. During the time the shell was open, a rubber finger cot tightly enclosed the head of the turtle to prevent lung collapse. This, of course, prevented the turtle from breathing, but the procedure lasted less than 1 h, which, at the low temperature of the animal, was not a severe apneic stress.

In some experiments (which will be specified) turtles were killed by decapitation and blood was sampled by heart puncture within 5 min. Samples were also taken of pericardial fluid, peritoneal fluid, and urinary bladder urine. The volume of each of these latter three fluids was also estimated.

The pH of blood and the other fluids was measured promptly with a glass microelectrode (Radiometer) to within 0.01 pH units. The blood was centrifuged and plasma CO₂ concentration was determined in duplicate using a Van Slyke micromanometric apparatus. In certain cases, an additional sample of plasma was deproteinized and analyzed enzymatically for lactic acid concentration using a test kit (Boehringer Mannheim Corp.). Lactic acid was also analyzed in the other body fluids when they were collected.

**Experimental procedure.** All experiments were conducted on turtles submerged in water at 24°C and consisted of a period of breathing, a forced dive for 2–4 h, followed by a recovery period lasting up to 24 h. This protocol is similar to that employed in our previous investigation in which we did direct calorimetry of this same species of turtle during dives at 24°C lasting 4 h (10). In most of the experiments to be reported, the turtles were catheterized and arterial blood samples were taken prior to the dive, at the end of the dive before the resumption of breathing, and at various intervals following the dive. Blood samples were 0.5–1.0 ml in volume, and at most 10 samples were taken on a single animal. In one turtle which weighed 618 g, the hematocrit of the first sample was 26% and the 10th sample, taken 24 h later, had a value of 24%. The estimated blood volume of this animal, based on these measurements, was about 50 ml or 8.4% of the body weight. In other animals the calculated volume was about 10% of the body weight, which agrees with values determined by Hutton (9).

In the experiments in which pericardial fluid, peritoneal fluid, and urine were collected, a single blood sample was taken from each animal following decapitation. Four groups of turtles were tested. The first group (6 animals) were control animals and did not dive; the second group (6 animals) were killed at the end of 4-h dives before breathing; the third group (6 animals), 1 h after a 4-h dive; and the fourth group (5 animals), 24 h after a 4-h dive.

**RESULTS**

**Effects of apneic diving.** Experimental dives lasting 2–4 h drastically altered blood gas and acid-base parameters (Table 1). The turtles were anoxic and had severe respiratory and metabolic (lactic) acidosis. A surprising feature was the significant fall in total plasma CO₂ content which occurred. Within 24 h, however, normal acid-base balance was restored, although lactate concentrations continued to be slightly elevated.

**Ventilation and O₂ consumption.** The turtles exhibited a dramatic increase in ventilation following the dive (Fig. 1). Respiratory minute volume increased steadily to a peak which averaged about 9 times predive levels, but this peak response was not reached until about 30 min into the re-

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**TABLE 1. Blood gas and acid-base values of turtles before, at the conclusion of, and 18–20 h following diving**

<table>
<thead>
<tr>
<th></th>
<th>Predive</th>
<th>End of Dive</th>
<th>Next Day</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pH</strong></td>
<td>7.604 ± 0.047</td>
<td>6.809 ± 0.087</td>
<td>7.604 ± 0.054</td>
</tr>
<tr>
<td><strong>Paco₂, torr</strong></td>
<td>31.8 ± 0.7</td>
<td>131 ± 33.5</td>
<td>30.8 ± 5.7</td>
</tr>
<tr>
<td><strong>PaO₂, torr</strong></td>
<td>81 ± 14.1</td>
<td>3.8 ± 1.7</td>
<td>72 ± 3.5</td>
</tr>
<tr>
<td><strong>CO₂ content, mM</strong></td>
<td>38.6 ± 5.4</td>
<td>28.6 ± 6.5</td>
<td>38.9 ± 5.8</td>
</tr>
<tr>
<td><strong>Lactate, meq/liter</strong></td>
<td>2.3 ± 0.9</td>
<td>28.0 ± 7.5</td>
<td>5.8 ± 2.0</td>
</tr>
</tbody>
</table>

All data are from the same 10 experiments. Values are mean ± SD. Numbers of observations are in parentheses.
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The ventilation decreased exponentially, usually reaching the predive values some 3 h after breathing resumed.

The increase in ventilation was due both to increased tidal volume and increased breathing frequency. The pattern of change of these parameters differed, however. In the first few minutes of recovery the breaths were very deep and irregular, whereas in the following 30-40 min the breaths were less deep but at high frequency and more regular. Thus, the peak response in tidal volume was reached almost immediately, whereas the peak response in frequency was delayed and approximately coincided in time with the peak response in minute volume.

Oxygen uptake reached very high values when breathing was restored and then declined back to the predive level in about 2 h (Fig. 2).

**Blood gas and acid-base parameters.** The course of recovery from the dive is revealed by the observed changes in blood pH, \( P_{\text{aO}_2} \), \( P_{\text{aCO}_2} \), and plasma \( [\text{HCO}_3^-] \) (Figs. 3-6). The blood gases \( P_{\text{aO}_2} \) (Fig. 3) and \( P_{\text{aCO}_2} \) (Fig. 4) generally changed in accordance with the ventilation changes. \( P_{\text{aO}_2} \) increased rapidly following the end of the dive and reached the peak value (average of 6 experiments = 111 torr) within 30 min; that is, at the time of peak ventilation. \( P_{\text{aCO}_2} \) decreased more slowly and reached the minimum value about 60 min after breathing resumed. Even after 4-5 h \( P_{\text{aO}_2} \) was higher than normal and \( P_{\text{aCO}_2} \) was lower than normal, which indicates continued hyperventilation.

Plasma \( [\text{HCO}_3^-] \), which fell during the dive, was reduced further by hyperventilation to an average minimal concentration of 15.9 mM (\( n = 10 \)), which was also reached at about the time of maximal ventilation (Fig. 5). The severity of the acidosis was apparently related to the lowest value of plasma \( [\text{HCO}_3^-] \). The one animal which never hyperventilated following the dive and which died several hours later had a \( [\text{HCO}_3^-] \) of slightly less than 7 mM. No data from this animal have been included. Recovery was slow and even after 4-5 h \( P_{\text{aO}_2} \) was higher than normal and \( P_{\text{aCO}_2} \) was lower than normal, which indicates continued hyperventilation.

**FIG. 2.** Oxygen consumption of 6 turtles before and after diving. Each predive point is value for a different animal averaged over at least 15 min. Postdive points are mean values for intervals lasting from 5 to 60 min. These points are situated on time axis at midpoint of recorded interval.

**FIG. 3.** Arterial \( P_{\text{aO}_2} \) values of turtles before and after diving. Values measured at end of dive average 131 mmHg (see Table 1) and are thus off scale in this figure.

**FIG. 4.** Arterial \( P_{\text{aCO}_2} \) values of turtles before and after diving. Values measured at end of dive average 7.58. These values were not significantly below the predive pH values (7.60) as judged by the paired \( t \) test (\( P > 0.5 \)). Normal pH was then maintained during the balance of the recorded recovery period. The restoration of normal blood acidity and its maintenance can be primarily attributed to compensatory hyperventilation and consequent hypocapnia. The degree of hyperventilation required was gradually reduced as \( [\text{HCO}_3^-] \) concentration was increased.

**TABLE 2. Peak ventilatory response following diving**

<table>
<thead>
<tr>
<th>Ventilation, ml/kg min</th>
<th>Predive</th>
<th>Peak postdive</th>
<th>Time-to-Peak Response, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>32.3 ± 14.7</td>
<td>287.2 ± 89.7</td>
<td>28 ± 17</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. Summary of eight experiments.

Further recovery period (Table 2).
Lactic acid. Plasma lactate concentrations were measured in 14 turtles following 3- to 4-h dives at 24°C. Peak values averaged 29.6 meq/liter. In four of these animals, blood samples were taken periodically, and both lactate and \( \text{HCO}_3^- \) were determined (Fig. 7). Peak concentrations of circulating lactate were reached by the end of the apneic period, and no consistent additional increase was noted when breathing resumed. The increase in lactate was closely matched by the decrease in bicarbonate in these animals, and the course of recovery back toward normal was also similar. As the insert in Fig. 7 shows, the changes in these substances were similar, although the increase in lactate tended to exceed the decrease in bicarbonate. This disparity probably reflects the buffering of lactic acid by buffers in the blood other than bicarbonate, presumably by red cell hemoglobin. The largest difference between lactate increase and bicarbonate decrease was observed in a turtle whose hematocrit was 30%. The hematocrits of the other turtles were in the range of 15-20%.

The fate of the lactate ion was not fully assessed by this study, although much of it was presumably dealt with metabolically by conversion back to glycogen or oxidation via the Krebs cycle. A significant amount, however, was excreted by the kidney, as evidenced by the presence of lactate in the urine stored in the urinary bladder of turtles following dives. No lactate was present in the bladder urine of turtles prior to diving. There was also no lactate present in the bladder urine following 4-h dives before the resumption of breathing, although plasma lactate was very high at this time. This suggests a shutdown of renal circulation and urine production in this species during diving. In six animals examined 1 h following a dive, bladder urine lactate was quite variable, but averaged 5.6 meq/liter (range 0.8-17.4 meq/liter), whereas in four animals, studied 24 h following a dive, mean [lactate] was 8.0 meq/liter (range 3.6-9.9 meq/liter). Since bladder volumes were quite different in these animals, the absolute amount of lactate also differed widely, but in two of the 24-h group, the amount of lactate in the bladder urine was about half as great as the total estimated lactate in the plasma of turtles shortly after a dive.

Small amounts of lactate were buffered by the highly alkaline pericardial and peritoneal fluids following 4-h dives (Table 3). The effect was most notable in the pericardial fluid where a small decrease in \( \text{HCO}_3^- \) was also detected. No change occurred in peritoneal \( \text{HCO}_3^- \), although total fluid volume was decreased at the end of diving. This raises the possibility that bicarbonate rich fluid was transferred to the plasma and interstitial fluid compartments to enhance buffering capacity there. The absence of large changes in
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TABLE 3. Effect of diving on pericardial and peritoneal fluids of turtles

<table>
<thead>
<tr>
<th></th>
<th>Pericardial Fluid</th>
<th>Peritoneal Fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vol, ml/kg</td>
<td>[HCO₃⁻], meq/liter</td>
</tr>
<tr>
<td>Predive</td>
<td>1.9 ± 0.3</td>
<td>109 ± 31.6</td>
</tr>
<tr>
<td>End of dive</td>
<td>1.4 ± 0.8</td>
<td>103 ± 47.7</td>
</tr>
<tr>
<td>1-h recovery</td>
<td>1.7 ± 0.6</td>
<td>99 ± 29.1</td>
</tr>
<tr>
<td>24-h recovery</td>
<td>2.4 ± 1.1</td>
<td>92 ± 24.4</td>
</tr>
</tbody>
</table>

Values are means ± SD. Numbers of measurements are in parentheses.

[lactate] and [HCO₃⁻] of these fluids casts serious doubt on their importance as major buffer sites following apneic diving.

DISCUSSION

Our data agree in most respects with previous information on diving animals, but there are several important differences which will be considered. These differences concern the changes in blood pH, [lactate], and CO₂ content during and immediately after the dive. In previous studies, typified by the classical investigation of the seal by Scholander (19), central arterial acidity and [lactate] rose during diving, but only slightly; the major increases occurred after the resumption of breathing. The interpretation for this sequence is that the skeletal muscles, the principle site of anaerobiosis, were virtually without blood flow during the dive. When breathing resumed, the circulation to the muscles was reestablished, and lactic acid was flushed into the general circulation, lowering the blood pH in the process. With respect to blood CO₂ content, Scholander found that an increase occurred during the dive due to the accumulation of metabolic CO₂. After the dive, CO₂ content fell sharply due to lactate buffering and hyperventilation. This general pattern of change in the blood acid-base status of diving animals has been observed in the penguin (19), the duck (4, 19), the beaver (8, 19), and the alligator (2). In several of these studies, blood CO₂ content rose initially during diving, but then decreased during the latter part of the dive (2, 4, 8).

The pattern of change in the turtle was different. Plasma [lactate] reached high concentrations during the dive and rose little, if at all, when breathing was restored (Fig. 7). The change in arterial pH reflected this change in plasma [lactate] (Fig. 6). The plasma CO₂ content of the turtles, in contrast to other diving animals tested, fell significantly during the dive (Table 1). After the dive, CO₂ content fell further, but this fall was due to hyperventilation and buffering of lactate just as in the previous studies.

Why did the turtles exhibit a pattern of acid-base change different from the other animals? The answer, we believe, is that the turtles were totally anoxic during much of the dive. Consequently, lactic acid was produced by all tissues, not just by the peripheral structures. In our previous study, turtles, experimentally submerged under similar conditions, exhausted lung and blood oxygen by the end of 1 h (10). A number of other studies, on related species, have conclusively documented the capacity of freshwater turtles to survive total anoxia for extended periods (5, 14, 18). Furthermore, vital central organs of the turtle, such as the heart (17) and central nervous system (6), continue to function in the absence of oxygen. In the present study, arterial PO₂ was near zero at the end of diving (Table 1).

Unlike the other diving animals, therefore, the turtle had to deal with sharply elevated lactate in the circulating blood prior to the resumption of breathing. In the extracellular fluid this acid load had to be buffered principally by bicarbonate; however, this is a poor buffer in a closed system, since the carbonic acid (and dissolved CO₂) cannot be eliminated. We suggest that this titration of the extracellular fluid with lactic acid elevated PCO₂ values to such an extent that significant loss of CO₂ to the environment ensued. A beneficial consequence was that the effectiveness of bicarbonate as a buffer was enhanced and acidosis was less severe.

We performed one simple experiment to test CO₂ loss from the turtle during apnea. A turtle weighing 611 g was placed in a sealed, air-filled chamber with a rubber finger cot enclosing its head to prevent breathing. Over the course of 4 h, the PCO₂ of the enclosed air space increased by 10 torr. Based on the volume of the space, this represented an extrapolmonary CO₂ loss of over 18 ml, which is of the right order of magnitude to account for the observed fall in plasma CO₂.

Like other diving animals, the turtle hyperventilated following the dive. The effectiveness of the hyperventilation was shown by the fact that arterial PO₂ was above normal within 30 min and arterial pH was indistinguishable from normal by the end of 2 h. Regulation of pH at its normal value throughout the remainder of the recorded recovery period, despite continued metabolic acidosis, indicates that respiratory compensation was essentially complete.

Early in the recovery period, all the known chemical factors in the blood concerned with respiratory control were displaced far from their normal values. On the basis of our previous studies, both low PO₂ (18) and high PCO₂ (unpublished data) produce hyperventilation in the turtle. Also, experimental metabolic acidosis apparently induced respiratory compensation in this species (11). Within the first 0.5 h of recovery, however, arterial PO₂ was already above normal and Paco₂ was at or below normal. Thus, at the time when the peak ventilatory activity was in progress, neither of the respiratory blood gases could have been acting as effective respiratory stimuli. During this phase of the recovery, the hyperventilation must be attributed solely (in terms of blood parameters) to the acid pH. Once pH re-
turned to normal (in 2 h), none of the measured parameters could account for the continued respiratory stimulation. The true nature of the effective stimulus to the central chemoreceptors is unknown, of course, and may not be revealed accurately by these blood changes.

Late in the recorded recovery period (3–5 h) the data are difficult to interpret. Arterial PO$_2$ was higher than normal (predive value) and Pa$_{CO_2}$ was less than normal. This blood gas picture indicates continued hyperventilation. In most experiments, however, respiratory minute volume had reached or gone below the predive ventilation by this time and O$_2$ consumption was generally at or above the predive level. This relationship between ventilation and metabolic rate indicates possible hypoeventilation. We believe that the blood gas data are more valid, since accurate assessment of ventilatory state by the second method requires knowledge of alveolar ventilation and CO$_2$ exchange; minute volume and O$_2$ uptake are only approximations. For example, CO$_2$ storage was occurring during the recovery which could help to account for the apparent discrepancy.

At the beginning of the recovery period, oxygen uptake was very high, but it rapidly subsided and returned to the predive level by approximately the end of the 2nd h (Fig. 2). The excess V0$_2$ during this portion of the recovery period was calculated for the four experiments in which plasma [lactate] was also measured. The average excess VO$_2$ in these experiments was about 43% of the total oxygen debt which would have accrued had the predive metabolic rates of these animals prevailed throughout the diving period. However, after the VO$_2$ had returned to the predive level, plasma [lactate] still exceeded the normal concentration by an average of 21.4 mM. Thus, a substantial fraction of the lactacid oxygen debt was not yet repaid. The magnitude of the debt remaining was uncertain because we do not know the distribution of lactate in the other major body fluid compartments.

An estimate of the actual oxygen debt can be made from our previous study in which total metabolic rate of turtles was measured under similar circumstances by the method of calorimetry study. The true nature of the effective stimulus to the central chemoreceptors is unknown, of course, and may not be revealed accurately by these blood changes. Consequently, the metabolism of the present animals during diving, based on the calorimetry study, would be almost 60% of their predive metabolic rate. Based on these rather tentative calculations, approximately one-third of the total oxygen debt still remained to be repaid at the end of 2 h, when VO$_2$ had apparently returned to the control level. If this were true, the excess lactate which we observed at this time must have been confined solely to the plasma compartment. Alternatively, the estimated oxygen debt is too low and the total metabolic rate was not reduced at all during diving in these experiments. Evidence for a reduction in total metabolism during experimental diving has been obtained in a variety of animals (1, 13, 19) including the turtle (10), but we cannot verify its occurrence in the present study.

Many species of freshwater turtle, including Pseudemys scripta elegans, possess specialized pericardial and peritoneal fluids. Rather than being ultrafiltrates of plasma as in most vertebrates, these fluids are highly alkaline with large concentrations of bicarbonate ion. The pericardial fluid of some turtles, in fact, is virtually pure isotonic NaHCO$_3$ (21). The volumes of these fluids are also comparatively large. When Smith (21) described these characteristics in 1929, he speculated that the fluids may be buffer reserves available to help deal with the acid load produced by diving. This suggestion was supported by the observation that exogenous lactate penetrated the peritoneal fluid of Pseudemys scripta elegans and was buffered there (16). In the same species, the bicarbonate of both these fluids was nearly depleted (and replaced by chloride) when metabolic acidosis was experimentally induced by daily intragastric infusion of dilute HCl (11). Despite the attractiveness of Smith’s hypothesis and the suggestive evidence supporting it, our data have failed to confirm that these fluids play a significant part in lactate buffering following diving. Lactate does enter the fluids, but the buffering accomplished there accounts for an insignificant fraction of the total acid load handled by the turtle.

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