Acid-base balance in ducks (*Anas platyrhynchos*) during involuntary submergence

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SHIMIZU, MANABU, AND DAVID R. JONES. *Acid-base balance in ducks (Anas platyrhynchos) during involuntary submergence.* Am. J. Physiol. 252 (Regulatory Integrative Comp. Physiol. 21): R348–R352, 1987.—Measurements of all the major independent variables [arterial CO_2 tension (Pa_{CO_2}); strong-ion difference ([SID]), and total protein content, which approximate total weak acid concentration in plasma] are essential for understanding changes in acid-base balance in plasma. During involuntary submergence of 1, 2, or 4 min, Pa_{CO_2} in ducks increased and arterial pH (pH_a) decreased. During 1-min dives there were no significant changes in any strong ions. In both 2- and 4-min dives, there was a significant increase in [lactate$^-$], but because of an increase in equal magnitude of [Na^+], [SID] did not change. During recovery from all dives the plasma remained acidotic for several minutes, although Pa_{CO_2} fell below predive levels in <1 min. [Lactate$^-$] increased in the recovery period. There were no changes in total protein content during submergence or recovery. Breathing 100% O_2 before 2-min dives caused a reduction in [lactate$^-$] production and release during and after the dive, although due to a marked increased in Pa_{CO_2}, pH_a fell as low as in 4-min dives after breathing air. After 1 min of recovery, pH_a returned to normal along with the restoration of the predive level of Pa_{CO_2}. We conclude that the acidosis during involuntary submergence is due solely to an increase in Pa_{CO_2}, whereas in recovery it is caused by decreased [SID].

diving; arterial carbon dioxide tension; strong ion difference; lactate ion; total protein

DURING INVOLUNTARY SUBMERGENCE both diving birds and mammals abandon homeostatic controls in favor of attempting to preserve the physiological integrity of those tissues, such as the heart and brain that are crucially dependent upon a continuous supply of O_2. This strategy is effected by redistributing the circulation to the O_2-dependent tissues, whereas metabolism of hypoperfused tissues must be supplemented anaerobically once stored O_2 is exhausted (4, 5).

Despite a plethora of observations on blood gas tensions, contents, and pH in diving birds and mammals, only one study has examined blood acid-base equilibrium during submergence. Andersen et al. (2) submerged ducks for 10–13 min and concluded from measurements of blood Pa_{CO_2}, total CO_2, [lactate$^-$], and arterial pH (pH_a) that the diving acidosis was predominantly respiratory early in the dive and was succeeded by a combined respiratory and metabolic acidosis later in the dive. In the recovery period the acidosis was purely nonrespiratory.

Although these conclusions are intuitively obvious, the conventional approach ([HCO_3^-]/pH) to acid-base balance neglects the influence of strong ions other than as expressed in changes in the apparent p*K*. If [lactate$^-$] is the only ion increasing during submergence (2, 5) then the problem is minimized, but Andersen (1) reported an increase in [K^+] of a similar magnitude as the increase in [lactate$^-$] during submergence in ducks. Obviously any hyperkalemia will negate, in whole or in part, the effects of [lactate$^-$] on acid-base equilibrium, which will affect the explanation of the data of Andersen et al. (2) and therefore their conclusions. Furthermore, determination of total CO_2 is central to the conventional analysis of acid-base equilibrium. It is difficult to see how CO_2 content and partial pressure can be dissociated in a closed system such as a submerged animal. Nevertheless, this occurred in the experiments of Andersen et al. (2) in which total CO_2 fell toward the end of long dives despite steadily increasing Pa_{CO_2}. This observation must have affected their interpretation of acid-base equilibrium in submergence, although it seems most likely due to experimental error.

Recently Stewart (6, 7) has suggested a more quantitative approach. Acid-base equilibrium of the blood depends on only three variables: Pa_{CO_2}, strong-ion difference ([SID]), and total weak acid, which is usually protein. The former and the latter are easily and accurately ascertained, although determination of [SID] presents both conceptual and technical difficulties. In practice strong ions are measured as concentrations, although it is their activities and not concentrations that influence acid-base equilibrium. Also the degree of ionization of divalent cations is difficult to estimate and is probably not constant. Fortunately their concentrations, compared with monovalent ions, are low. Nevertheless, we felt that this approach would be worthwhile because it would allow us to explain acid-base equilibrium in diving ducks using an entirely different approach from that used previously (2). Consequently, in the present series of experiments we attempted to identify the relationship between the acidic condition of plasma and the behavior of the three independent variables (Pa_{CO_2}, [SID], and weak acids) affecting acid-base balance during diving and recovery in ducks.

MATERIALS AND METHODS

Experimental preparation. Fifteen adult female White Pekin ducks (*Anas platyrhynchos*) were used. The average body mass of the ducks was 2.7 ± 0.2 kg (±SE). The ducks were kept in an open pen with free access to food and water before being brought into the laboratory 1 or 2 days before the experiments. In the laboratory the animals were kept in a wire cage under an artificial photoperiod (12 h day and 12 h night). Before surgery food and water were offered ad libitum. After the preparatory procedures, only water was offered to the animal.

Blood vessel cannulations were done under local anesthesia (lidocaine 2%; Astra, Missisauga, Ontario, Canada). Polyethylene tubing (PE-90; Clay-Adams, Parsippany, NJ) was inserted into both brachial arteries. In both cases the tip was advanced until it lay near the aortic root. All catheters were filled with a heparinized saline solution (200 USP U heparin/ml). The heparinized saline was removed from catheters before experiments began because of the necessity of obtaining serum. Surgery was not done under aseptic conditions, but all catheters, surgical instruments, and solutions were sterilized before the operation. The animals were allowed 12–36 h to recover from surgery. After experiments the ducks were killed by lethal intra-arterial injection of pentobarbital sodium.

Measurements. Heart rate (HR) was obtained from the electrocardiogram (ECG) recorded by placing electrodes subcutaneously in the left shoulder and in the right thigh. Mean arterial blood pressure (MAP) was recorded through one of the arterial catheters, which was connected to a pressure transducer (Narco Bio-System, Houston, TX). Expired tidal volume (V_T) and breathing frequency (f) were monitored with a pneumotachograph (no. A547; Hewlett-Packard, Waltham, MA) attached to a body plethysmograph (Fig. 1). Minute ventilation ($\dot{V}_E$) was calculated as the product of V_T and f.

Blood samples (2.5 ml) were collected anaerobically into a 3-ml polyethylene syringe through the other arterial catheter. The syringe was immediately disconnected from the catheter, sealed tightly, and placed in an ice bath. After an experiment the blood sample was processed. A well-mixed 0.2-ml portion of the blood sample was used to measure Pa_{CO_2}, Pa_{O_2}, and pH_a using a blood gas analyzer (model IL813; Instrumentation Laboratories, Lexington, MA). Plasma lactic acid concentration was determined with an enzymatic assay kit (no. 826-UV; Sigma, St. Louis, MO). Concentrations of strong inorganic ions ($[Na^+]$, $[K^+]$, and $[Cl^-]$); total calcium; and total protein in serum were determined with a sequential multiple-analyzer computer system (SMAC system; Technicon Instruments, Tarryton, NY). An aliquot of 0.8 ml of the separated serum was frozen at −20°C until analyzed. The samples were analyzed within 3 days of the experiments. The content of Mg was determined by an atomic absorption spectrophotometer (model 2380; Perkin-Elmer, Norwalk, CN). A flow spoiler with mixtures of acetylene and air was used in the spectrophotometer. Absorption measurements were taken at a wavelength of 285.2 nm.

[SID] was calculated from $[Na^+]$, $[K^+]$, $[Ca^{2+}]$, $[Mg^{2+}]$, $[Cl^-]$, and $[lactate^-]$. The $[Ca^{2+}]$ was calculated from the total serum calcium content. The $[Ca^{2+}]$ was determined on the basis of the assumption that ionic calcium comprised 47.5% of total plasma calcium (3). Mg in serum was assumed to be totally ionized.

Protocol. The animal was placed in a temperature-controlled body plethysmograph (Fig. 1). Wings and legs were lightly restrained with filament tape. The head of the animal extended out of the plethysmograph through a collar of dental dam. The catheters and ECG leads were fed out though an airtight hole. The pneumotachograph was attached to a port in the plethysmograph to measure the airflow created by breathing movements of the animal. The head of the animal was placed in a restrainer so that the head could be lowered into the water. Once the animal was placed in the plethysmograph and all of the connections (i.e., catheters and ECG leads) were made, the animal was left undisturbed for 30–60 min before the experiment began so that it could reach a steady state. Hematocrits were measured before and after each dive.

Nine ducks were forcibly submerged once or twice for periods of 1, 2, or 4 min at random. All measurements were made between 1 min before submersion and 10 min after emersion. Six blood samples were collected: 1 min before submersion (predive sample), 15 s before emersion

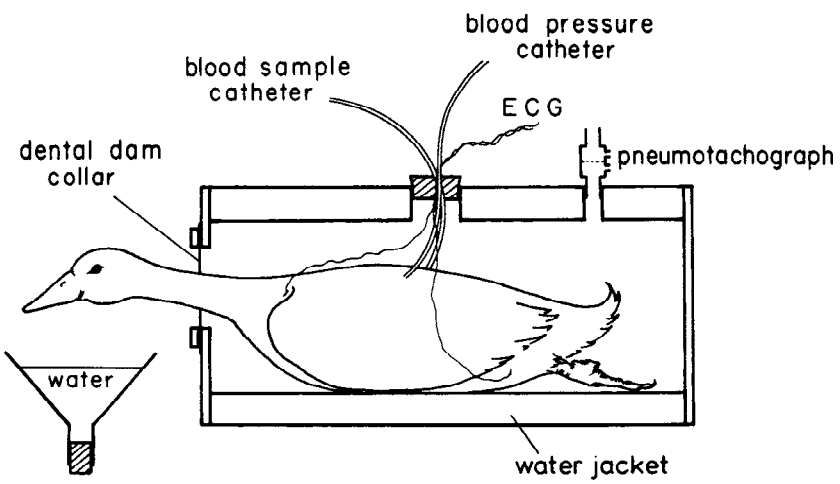

FIG. 1. Diagram of the experimental apparatus. Body plethysmograph is 150 mm (diam) by 500 mm (length) in chamber dimension, and volume of chamber is ~8.8 l. ECG, electrocardiogram.

(end-dive sample), and 1, 3, 5, and 10 min after emersion. If the hematocrit measurement at the end of the first dive showed more than a 5% drop, then the second dive with that animal was canceled. When two dives were done, the duck was allowed at least 2 h between dives to recover.

Six ducks were forcibly submerged twice, each time for a period of 2 min, after breathing 100% O_2 for 5 min. A plastic bag with an O_2 inlet was placed over the head. The head was lowered into the empty funnel (Fig. 1). O_2 was administered at a rate of 5 l/min. After 5 min the plastic bag was removed and water was poured into the funnel to begin a dive. The animal was surfaced into room air. The rest of the procedure was the same as described above except that only 0.6 ml blood was collected for each blood sample because the strong inorganic ions were not measured.

Statistical methods. Statistical significances were based on results of analysis of variance or Student's t test. The critical limit for significance was set at a probability of 5% ($P < 0.05$). Values are means ± SE, N is the number of animals, and n is the number of observations.

RESULTS

HR started to fall noticeably between 20 and 40 s after submersion in most of the dives and reached its lowest level 80 s from the beginning of the 2- and 4-min dives (Table 1). Arterial O_2 tension declined from 93.5 ± 5.8, 102 ± 3, and 100 ± 3 Torr to 55.8 ± 3.2, 45.2 ± 2.8, and 32.5 ± 2 Torr at the end of 1-, 2- and 4-min dives, respectively ($N = 5$ and $n = 5$ for each dive). Once the animals surfaced, HR increased above predive values (Table 1). MAP did not show any great changes during the experiments, except in 2- and 4-min dives when a slight hypotension occurred at the end of the dive and in all dives when a short period of hypertension occurred immediately after emersion. Breathing increased significantly compared with predive levels after all dives, and hyperventilation was more prolonged after the longer dives (Table 1).

Predive values of Pa_{CO_2}, concentrations of strong ions, SID, and pH_a are presented in Table 2. pH_a was converted to $[H^+]$ so that all changes referred to predive levels could be expressed quantitatively. Both Pa_{CO_2} and $[H^+]$ in arterial blood increased significantly and more or less linearly as dive duration increased (Fig. 2, *A* and *B*). Plasma [lactate$^-$] increased significantly during dives except in the 1-min dive. At the end of the 4-min dive [lactate$^-$] was significantly higher than at the end of the 2-min dive (Fig. 2*C*). Serum $[Na^+]$ also increased (Fig. 2*D*). The concentrations of the other ions and total serum protein showed no significant change. [SID] increased slightly, and the value at the end of the 4-min dive was significantly higher than predive (Fig. 2*E*).

When the first postdive measurements were made, Pa_{CO_2} was lower than predive values regardless of the length of the preceding dive. Pa_{CO_2} continued to decrease until 3 or 5 min after emersion (Fig. 3*A*). $[H^+]$ remained significantly increased from predive values 1 min after emersion and was similar to the end-dive levels (Fig. 3*B*). $[H^+]$ gradually decreased toward predive values in recovery. Large increases in [lactate$^-$] were observed after all dives, although after the 1-min dive the increase was less than after the two longer dives (Fig. 3*C*). [Lactate$^-$] slowly returned to predive values but remained significantly above predive levels, even after 10 min of recovery, except after 1-min dives. Serum $[Na^+]$ was lower than during diving and returned to the predive level in a few minutes (Fig. 3*D*). Serum $[K^+]$ increased 2 meq/l 1 min after the 4-min dive but returned to the predive value by the next measurement (Fig. 3*E*). The other strong ions and the total protein did not change significantly during the recovery period. The [SID] was significantly lower than the predive value in all cases, but after the 1-min dives the decrease in [SID] was significantly less than after the two longer dives (Fig. 3*F*).

After 4 min of breathing 100% O_2, predive Pa_{O_2} was 355 ± 7 Torr. Pa_{O_2} decreased to 327 ± 9 Torr by the end of the 2-min dive. It was reduced to 120 ± 2 Torr 1 min after the animal surfaced into air. $\dot{V}E$ returned to the predive level 3 min after surfacing. In dives after breath-

TABLE 1. *Heart rate, mean arterial blood pressure, and minute ventilation before, during, and after 1-, 2-, and 4-min dives*

	Predive	End of Dive	Time After Emersion, min			
			1	3	5	10
HR, beats/min						
1-min dive	170±12	52.4±1.7*	158±7	185±8	189±8	183±8
2-min dive	239±24	21.4±1.6*	315±51*	275±11*	289±21*	386±70*
4-min dive	178±17	20.6±1.3*	247±50*	239±7*	245±15*	238±16*
MAP, mmHg						
1-min dive	166±6	174±15	168±13	156±7	157±8	162±8
2-min dive	180±11	142±9*	185±9	169±11	166±14	171±12
4-min dive	194±16	149±10*	201±11	167±14	175±10	175±12
$\dot{V}E$, l/min						
1-min dive	0.805±0.034		1.37±0.14*	0.924±0.094	0.950±0.163	0.745±0.078
2-min dive	1.23±0.21		2.31±0.49*	1.64±0.24*	1.56±0.22	1.40±0.15
4-min dive	0.921±0.081		3.17±0.47*	2.66±0.34*	2.07±0.33*	1.50±0.19*

Values are means ± SE; $n = 5$. HR, heart rate; MAP, mean arterial blood pressure; and $\dot{V}E$, minute ventilation. * Significantly different from predive value.

TABLE 2. *Values measured before 1-, 2-, and 4-min dives*

	Before 1-min Dive	Before 2-min Dive	Before 4-min Dive
Pa_{CO_2}	34.5±1.9	30.2±1.6	31.8±1.9
[SID]	38.9±0.4	39.3±1.5	39.2±0.8
[Lactate$^-$]	1.88±0.32	1.90±0.24	1.81±0.38
[Na^+]	144±1	142±3	140±1
[K^+]	3.2±0.1	3.9±0.5	3.3±0.2
[Ca^{2+}]	3.8±0.4	3.9±0.5	4.7±0.3
[Mg^{2+}]	1.6±0.1	1.9±0.1	1.7±0.1
[Cl^-]	112±0	110±1	108±1
pH_a	7.441±0.014	7.435±0.031	7.432±0.015

Values are means ± SE; $N = 5$, and $n = 5$. Pa_{CO_2}, arterial CO_2 tension (Torr); SID, strong-ion difference (meq/l); pH_a, arterial pH. Ion concentrations in meq/l.

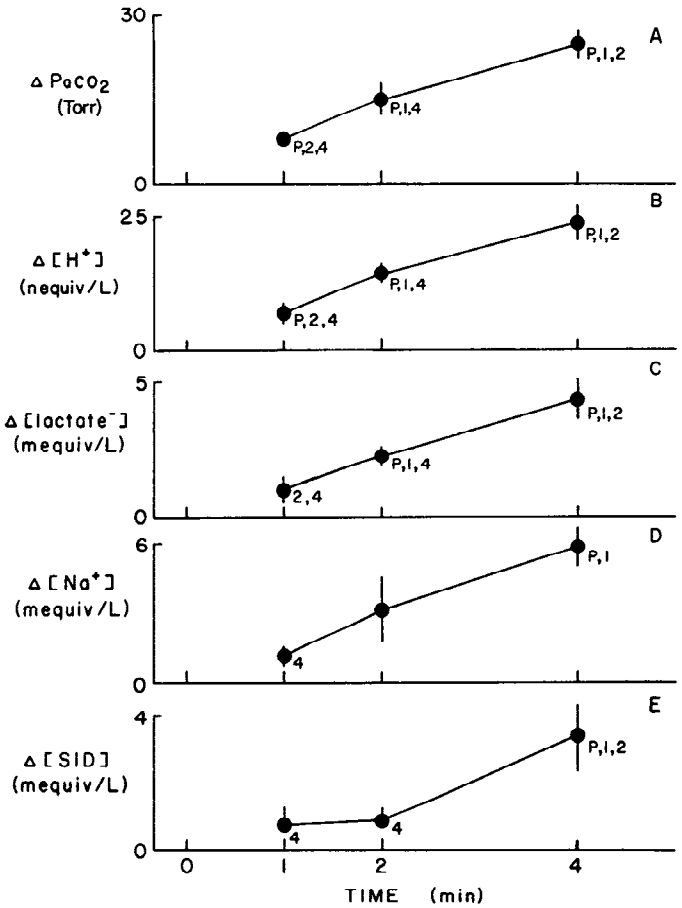

FIG. 2. Changes in arterial CO_2 tension (Pa_{CO_2}), [H^+], [lactate$^-$], [Na^+], and strong-ion difference [SID] from predive values plotted against length of submergence. Each *point* was obtained from measurements made at end of dives whose duration was as indicated in figure. *Labels* on *points* indicate that given pairs of *points* are significantly different from each other. P, predive; 1, end of 1-min dive; 2, end of 2-min dive; and 4, end of 4-min dive. $N = 5$; $n = 5$. See text for further explanation.

ing 100% O_2, HR and MAP did not change significantly during the dive and recovery. Pa_{CO_2} increased significantly during diving but returned to the predive level 1 min after emersion. There was a significant drop in pH_a during the dive, but pH_a also returned to the predive level 1 min after surfacing (Fig. 4*B*). The increase in Pa_{CO_2} and the reduction in pH_a were comparable to those seen in a 4-min dive in the previous series of experiments

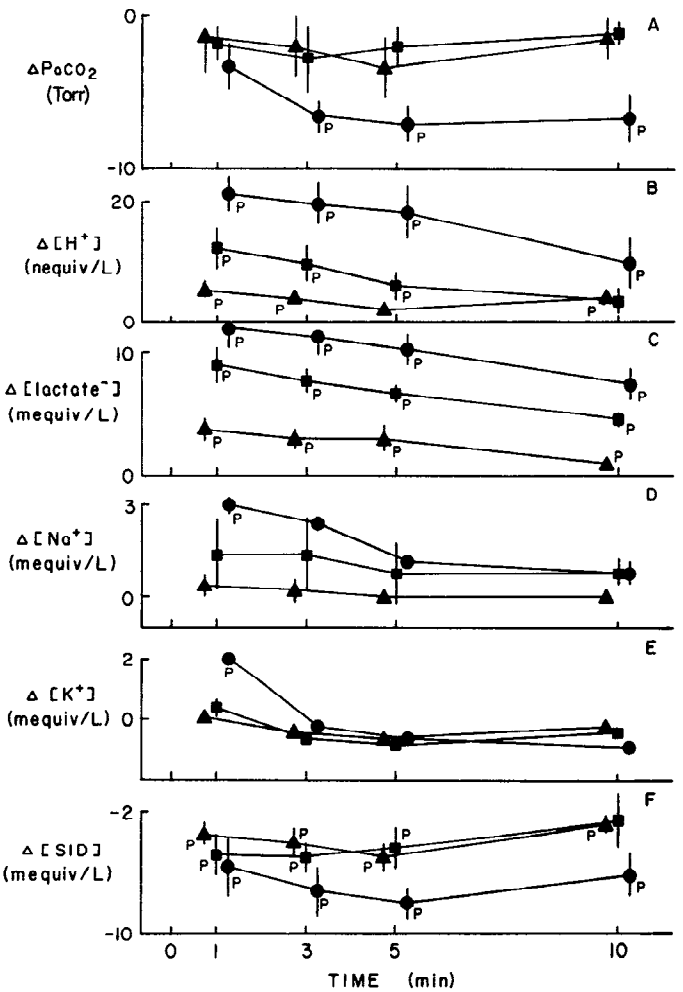

FIG. 3. Changes in arterial CO_2 tension (Pa_{CO_2}), [H^+], [lactate$^-$], [Na^+], [K^+], and [SID] from predive values plotted against time after end of 1- (*closed triangles*), 2- (*closed squares*) and 4-min (*closed circles*) submergence. *P* indicates value is significantly different from predive value. $N = 5$; $n = 5$. See text for further definition.

(compare Figs. 2 and 4). Although there was a significant increase in [lactate$^-$] in recovery, the increase was significantly less than any of the increases seen in the previous experiments (compare Figs. 3 and 4).

DISCUSSION

The Pa_{CO_2} in arterial blood rose more or less linearly with increasing dive duration and was largely responsible for the plasma becoming acidotic during diving. Serum protein content did not change during dives, so total weak acid had no effect on acid-base balance. Among strong ions, [lactate$^-$] increased slowly in the arterial circulation during submergence; however, the other strong-acid anion, [Cl^-], did not change. Among strong cations, only [Na^+] increased during submergence. This conflicts with the report by Andersen (1), who found increased [K^+] during more prolonged forced dives than were used in the present experiments. The increase in [Na^+] generally exceeded that in [lactate$^-$], and as a result [SID] increased slightly. Although the effect of this slight change in [SID] is small, it is also in the direction of alkalinity rather than acidity. That the acidotic condition developing during diving solely depended

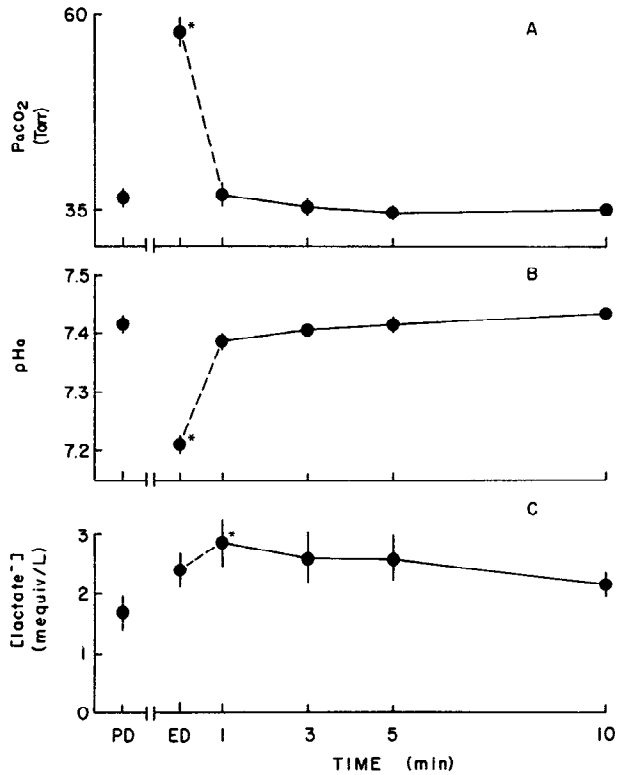

FIG. 4. Absolute values in arterial CO_2 tension (Pa_{CO_2}); arterial pH (pH_a), and [lactate$^-$] before (PD), at end (ED), and in recovery from 2-min dives after breathing 100% O_2 for 5 min before submergence. *Asterisk* indicates value is significantly different from predive value. $N = 6$; $n = 12$.

on the increase in Pa_{CO_2} was confirmed by experiments in which ducks breathed O_2 predive. In 2-min dives after breathing 100% O_2, Pa_{CO_2} increased to more or less the same level as was seen in 4-min dives after breathing air, and the decrease in pH_a was also similar despite the fact that [lactate$^-$] did not increase significantly.

One minute after the end of a dive Pa_{CO_2} was below predive values, particularly after the longer 4-min dives. These Pa_{CO_2} levels would drive plasma acid-base balance in the opposite direction from that during diving, yet pH_a remained just as acidotic as during the dive. In fact, during recovery, the major independent variable controlling the plasma acid-base balance must be [SID]. There were large decreases in [SID] from predive levels except after the 1-min dive. The [Na^+] decreased to the predive value in the 1st min of recovery except after the 4-min dive in which [Na^+] was a few milliequivalents per liter higher than predive for the first 5 min of recovery. There was also an increase in [K^+] 1 min after emersion from the 4-min dive, but then [K^+] fell to predive values for the rest of the recovery period. After the 4-min dives, there was an increase in [Cl^-], although it was not statistically significant when compared with predive values. Obviously the decreased [SID] was due to the large increase in [lactate$^-$] since there were no large changes in the other strong ions. [Lactate$^-$] produced during forced diving is largely retained in the hypoperfused tissues to be released once the circulation is restored, so a larger quantity of [lactate$^-$] was released after the longer dives, and [SID] was therefore lower. However, if the postdive increase in [lactate$^-$] was small, such as in dives after breathing 100% O_2, pH_a returned to predive values rapidly in recovery.

In the present study we were unable to measure concentrations of some strong ions directly. Both of the divalent cations (Ca^{2+} and Mg^{2+}) were measured as content in plasma rather than ionic concentration, and [Ca^{2+}] and [Mg^{2+}] were estimated. There is some doubt about the degree of ionization of calcium and magnesium in blood plasma, and ionization will also vary depending on the status of the acid-base balance. Therefore the [SID] values include some errors; nevertheless changes in [SID] that were observed are valid because they are larger than any possible error, and the contribution of [SID] changes to acid-base balance in involuntary submergence and during recovery is important.

Despite extremely different approaches, the results of this and the only previous study on acid-base balance during diving (2) are in quite good agreement. The major difference concerns whether there is a combined respiratory and metabolic acidosis late in a dive. In our experiments the effect of increasing [lactate$^-$] on acid-base equilibrium was negated by a like increase in [Na^+]. It may be that in extremely long dives (10–13 min) the increase in [lactate$^-$] is far greater than that of the strong cations. Alternatively, the dissociation between Pa_{CO_2} and content observed by Andersen et al. (2) may have influenced their conclusions. Obviously, both approaches to analyzing this relatively simple system are viable, although we feel that our quantitative approach has more to offer in terms of explaining cause and effect.

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