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ACID-BASE RELATIONSHIPS IN THE BLOOD OF THE TOAD, *BUFO MARINUS*

I. THE EFFECTS OF ENVIRONMENTAL CO_2

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SUMMARY

An abrupt increase in ambient CO_2 resulted in a marked respiratory acidosis which took place within 30 min. During this time there was a considerable reduction in the P_{CO_2} difference between arterial blood and inspired gas caused by an increase in ventilations. Prolonged CO_2 exposure (24 h) showed that there was some compensation for the acidosis in that plasma bicarbonate concentrations increased substantially. At the same time, however, the P_{CO_2} of arterial blood always rose so that the net result was usually only a small increase in pH. Upon return to air, the blood was backtitrated along a buffer line elevated above and parallel to that seen during the initial response to hypercapnia. The fall in arterial blood P_{CO_2} during the early stages of recovery often led to pH values higher than those seen in the untreated animal. After 48 h in air, recovery had gone further with P_{CO_2}, pH and $[HCO_3^-]$ levels approaching but rarely reaching the pre-exposure values.

INTRODUCTION

In vertebrates, the evolutionary transition from water to land and development of aerial gas exchange has resulted in a substantial change in acid-base regulatory mechanisms. The properties of water as a medium for gas exchange and the design of fish gills together ensure that the levels of P_{CO_2} are low with small CO_2 gradients between arterial blood and water. Mammals, on the other hand, maintain large CO_2 gradients in the lung and can substantially alter P_{a,CO_2} by changes in ventilation so as to adjust the HCO_3^-/CO_2 ratio and therefore pH_a.

Amphibians occupy an intermediate position between aquatic and terrestrial vertebrates. As evidenced by many studies, pulmonary gas exchange in the Amphibia is supplemented by skin-gas transfer by directing a portion of the circulation to a highly vascularized and very permeable skin (Krogh, 1904; Hutchison, Whitford & Kohl, 1968; Toews, Shelton & Randall, 1971). Thus, bimodal gas exchangers such as the Anura may alter P_{a,CO_2} and therefore pH_a by either changing their pattern of lung

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ventiliation or adjusting CO_2 removal across the skin. In addition, renal hydrogen ion excretion (Frazier & Vanatta, 1971, 1973; Ludens & Fanestil, 1972), as in most terrestrial tetrapods, may also be involved in pH regulation. Finally, as in the gill epithelium of fish, Cl^- uptake across the frog skin is coupled to HCO_3^- efflux (Motais & Garcia-Romeu, 1972) so CO_2 may be excreted as molecular CO_2 or HCO_3^- and either process may be modulated to regulate pH_a.

The marine toad *Bufo marinus* lives in diverse habitats ranging from tropical rainforests to deserts (McCutcheon & Hall, 1937; Stuart, 1951) which suggests that it can adapt physiologically to a wide variety of environmental conditions. For example, under natural conditions in the Amazon, *Bufo marinus* may encounter very hypercapnic waters (Toews & Macintyre, 1978) where CO_2 gradients could be reversed across the skin. In addition, Macintyre (1975) has studied the defensive inflation in these animals and has shown that during long periods of apnoea (up to 20 min), P_{CO_2} in lung gas progressively increases to levels reaching 35 mmHg.

Hypercapnic conditions are therefore not uncommon in the natural habitat of *Bufo marinus*. Exposure to similar conditions in the laboratory enabled us to observe the response to an increased acid load and describe some of the processes associated with CO_2 and hydrogen ion regulation in these animals.

MATERIALS AND METHODS

The animals used in this study (*Bufo marinus*) were collected in Mexico and supplied commercially by the Mogul-Ed. Corp., Oshkosh, Wisconsin, U.S.A. Healthy animals of both sexes, ranging in weight from 300 to 500 g were maintained at room temperature (25 ± 2 °C) in large aquaria with water at one end.

In vivo *experiments*

After the animals were anaesthetized in a 1·5 g/l solution of tricaine methane sulphonate (Sandoz MS–222), the femoral artery of one leg was occlusively cannulated in the region of the thigh with an indwelling catheter prepared from Clay–Adams polyethylene tubing (P.E. 60; i.d. 0·76 mm, o.d. 1·22 mm). Prior to insertion, catheters were filled with heparinized amphibian Ringer (250 i.u./ml) to prevent clotting, and 0·1 ml/kg of the solution was injected immediately following placement of the cannula. The catheter was then secured to the surrounding tissues with nylon suture and the wound was closed with Michel skin clips. All blood samples were taken from unanaesthetized free-moving animals following a minimum recovery period of 24 h.

For experimentation, an animal was placed in a 4 l wide-mouthed jar which provided room for limited movements. The jar was placed on its side and 3–4 cm of tap water covered the floor of the chamber. Three holes (0·5 cm diameter) were bored in the bung closing the jar to allow passage of gases and cannulae. Toads were allowed a 3–5 h period of adjustment to the chamber before the experiments began. Following a period of time while the animals were breathing water-saturated air, a 5 % CO_2–95 % air mixture was introduced into the chamber. The size of the jar allowed for abrupt changes between normal and elevated levels of ambient CO_2. Several samples of blo for measurement of arterial blood pH (pH_a), arterial CO_2 tension (P_{a,CO_2}) and total

CO_2 content (C_{a,CO_2}) were taken during the normal, hypercapnic and post-hypercapnic periods.

For P_{a,CO_2} measurements, the blood was allowed to flow, using the animal's blood pressure, through the catheter into a Radiometer thermostatted cell and CO_2 electrode (Type E5036, with silicone membrane). The output was displayed on a Radiometer PHM 72 acid-base analyzer. The electrode system reached a constant reading some 3 min after the blood sample was admitted to the cell, after which blood was again allowed to flow through the cell so that a completely fresh sample was admitted. This procedure normally resulted in a further increase in the P_{CO_2} reading. After P_{CO_2} measurement, blood was returned to the animal by applying pressure to the system with a tuberculin syringe. We have shown that certain response characteristics of Radiometer CO_2 electrodes (Type E5036) necessitate the use of such sample replacement methods for accurate determinations of P_{CO_2} (Boutilier *et al.* 1978). The CO_2 electrode was calibrated frequently using water-saturated gases of known composition, corrected each day for atmospheric pressure and temperature effects.

Measurements of pH_a were made at 25 °C using a Radiometer microelectrode unit (Type E5021) coupled with a Radiometer PHM–71 acid-base analyzer. A 40 μl blood sample was drawn from the arterial catheter and following determination, the sample was discarded. The pH unit was frequently calibrated at measurement temperature against Radiometer precision buffer solutions S1500 and S1510. Both CO_2 and pH electrodes were held and calibrated at the same temperature as the animal. Calculations of means were carried out directly on the pH data without converting to H^+ concentration. The differences resulting from the two calculations were small and had negligible effect on data plotted in the Figures.

Total CO_2 determinations were made on 100 μl blood samples using the electrode and cuvette method described by Cameron (1971). Calibration of the instrument was carried out using standard bicarbonate solutions that were prepared fresh daily. The system was maintained at 36 °C so that the response time of the CO_2 electrode was reduced at this higher temperature.

The amount of blood lost from the animal following each determination of pH, P_{CO_2} and total CO_2 content was 140–160 μl.

In vitro *experiments*

CO_2 combining curves and blood buffer lines were determined on oxygenated whole blood which was drawn from either the aortic arch or femoral artery of pithed animals. Each series of determinations required 8 ml of blood which was very often pooled from several animals. Samples of blood (0·6 ml) were immediately transferred by pipette to heparinized round-bottomed tonometers.

For each set of determinations, the tonometric vessels were placed in a temperature-controlled horizontal shaker bath maintained at 25 °C. The blood was equilibrated with gas mixtures containing 0·6 %, 1 %, 2 %, 3 %, 5 %, and 7 % CO_2, the balance of which was air. All of these gas mixtures were water-saturated and were continuously supplied from Wösthoff gas mixing pumps. After 1 h of equilibration, each blood sample was taken up with a gas-tight syringe and immediately (within 15 s) introduced into the electrode chambers for determination of P_{CO_2}, total CO_2 content and pH. The equilibrating gases continued to flow as the blood samples were removed from the tonometers.

CO_2 combining curves and buffer lines were also constructed for true plasma at 25 °C using the same methods. True plasma curves were obtained by equilibrating whole blood samples (2 ml) with the various CO_2 gas mixtures and then anaerobically centrifuging off the plasma for measurement of C_{CO_2} and pH at each P_{CO_2}. To avoid CO_2 losses through diffusion to the air during centrifugation, the equilibrated blood samples were taken up in gas-tight Hamilton syringes and introduced into the centrifuge tubes under a thin layer of mineral oil. The tubes were then immediately sealed and the plasma was separated from the red cells in less than 90 s. Samples of the true plasma were obtained at once by puncturing the seal with the needle of a gas-tight syringe.

Because the CO_2 solubility coefficient (α_{CO_2}) and apparent first dissociation constant of carbonic acid (pk'_1) were not known, an additional series of experiments were undertaken to determine these constants for *Bufo marinus* plasma. The solubility of CO_2 in toad plasma was determined after acidification of the plasma by the addition of a small amount of pure lactic acid (Van Slyke *et al.* 1928; Bartels & Wrbitzky, 1960). Aliquots (2 ml) of the acidified plasma were placed in thermostatically controlled tonometers and equilibrated with 100% CO_2 at 25 °C following the procedure of Van Slyke *et al.* (1928). Carbon dioxide content was measured using 50 and 100 μl samples by the method of Cameron (1971). The solubility coefficient of CO_2 in toad plasma (α_{CO_2}) was found to be 0·033 m-mol.l^{-1}.$mmHg^{-1}$ (Table 1). This value is considerably lower than the value of 0·0404 m-mol.l^{-1}.mmHg for mammalian blood plasma at 25 °C (Severinghaus, 1965) and the reason for the discrepancy is not known.

The pk'_1 of separated plasma was determined gasometrically at different pH values (Truchot, 1976; DeLaney *et al.* 1977) by equilibrating 2 ml aliquots in temperature controlled tonometers (25 °C) with gas mixtures containing 2, 5 and 10% CO_2 in air, obtained from Wösthoff gas mixing pumps. After 30 min of equilibration, CO_2 contents and pH were measured as described before. The bicarbonate concentration was estimated using the formula:

$$[HCO_3^-] = C_{CO_2} - \alpha_{CO_2} . P_{CO_2}$$

and the pk'_1 was then calculated using the Henderson–Hasselbalch equation (pk'_1 = 6·05, Table 1). These experimentally derived constants (α_{CO_2} and pk'_1) were then used in the *in vivo* experiments to calculate plasma bicarbonate concentrations of the experimental animals from measurements of pH and P_{CO_2}.

Haematocrit measurements were made using a Clay–Adams microhaematocrit centrifuge.

RESULTS

In vivo *determinations on arterial blood*

The relationships between the measured values of arterial blood P_{CO_2} and pH seen during the course of a typical experiment on an individual *Bufo marinus* are plotted in Fig. 1. Plasma bicarbonate concentrations are also shown in the diagram as diagonal lines, the positions of which were calculated using the values for pk'_1, (6·05) and α_{CO_2} (0·033) determined for *Bufo* plasma at 25 °C (Table 1). The experimental protocol adopted in all these experiments was to take samples of blood (in replicate if possibl

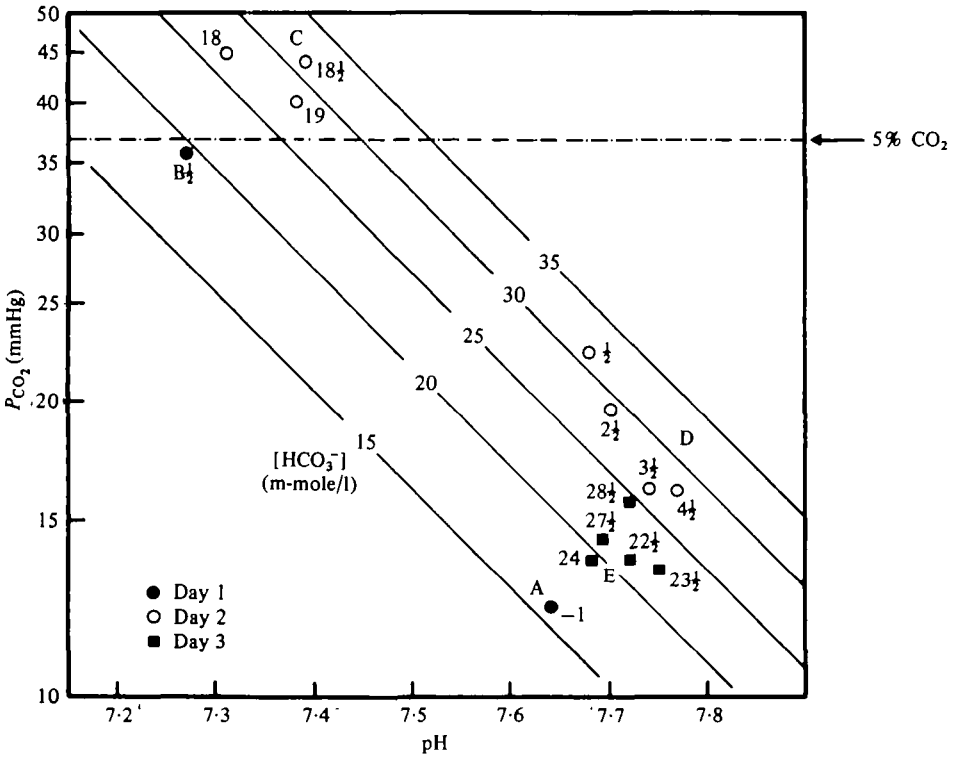

Fig. 1. A pH-log P_{CO_2} diagram showing the relationships between measured values of arterial blood pH and P_{CO_2} during a typical hypercapnia experiment on an individual *Bufo marinus*. The positions of the diagonal lines indicate the plasma bicarbonate concentrations which were calculated using the Henderson–Hasselbalch equation. Numbers adjacent to individual data points from A to C represent the CO_2 exposure time in hours. Similarly, those from C to E represent the time course of movements during recovery.

Table 1. In vivo *and* in vitro *respiratory characteristics of* Bufo marinus *whole blood and plasma at* 25 °C. *Values are means* ± *standard error of the mean*

pHa	7·79 ±0·02
$P_{a.CO_2}$ (mmHg)	12·10 ±0·39
Plasma [HCO_3^-], m-mol/l	22·30 ±1·32
Buffering capacity (m-mol $HCO_3^- \cdot l^{-1} \cdot pH^{-1}$)	
True plasma (*in vitro*)	−20·40
True plasma (*in vivo*)	− 6·20
Whole blood (*in vitro*)	−15·90
Plasma pk_1'	6·05 ±0·03
α_{CO_2} in plasma (m-mol $\cdot l^{-1} \cdot mmHg^{-1}$)	0·033 ±0·002
Haematocrit (%)	25·0 ±1·48

from the animal after it had been allowed to settle in the chamber and was breathing air (point A, Fig. 1). A 5% CO_2–95% air mixture was then admitted and further samples taken after 30 min and, in many cases, at times during the first 3–4 h of CO_2 exposure (point B). The 5% CO_2 mixture was left running for a period of approximately 24 h and further samples were taken during the final 6 h of CO_2 exposure (point C). Finally, air was readmitted to the chamber and, as the animal recovered, samples of blood were taken over the first 4 h (point D) and the following 24–48 h

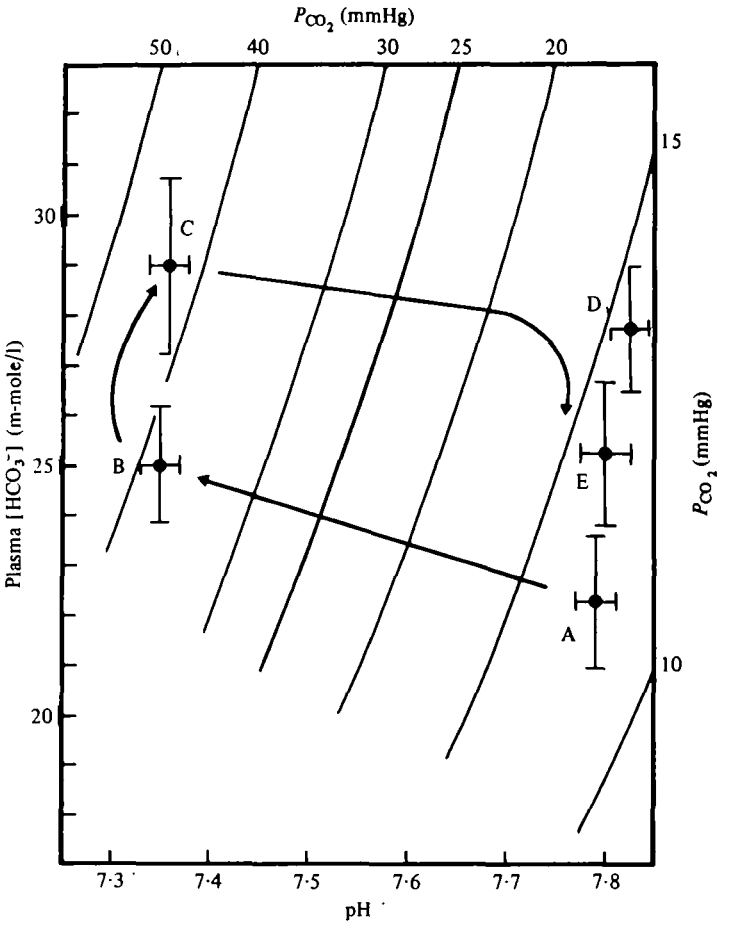

Fig. 2. Davenport diagram showing arterial blood acid-base changes that occurred in six animals during the hypercapnia experiments. Mean (±S.E.) plasma bicarbonate concentrations are plotted as a function of plasma pH for the experimental stages A to E. The curved lines are calculated P_{CO_2} isopleths. Arrows indicate the major movements.

(point E) if the catheter remained patent. In Fig. 2, data from six animals have been used to determine plasma bicarbonate concentrations from meaurements of P_{CO_2} and pH. The mean (± S.E.) of these bicarbonate concentrations are plotted against pH in a Davenport diagram for each of the five experimental stages described above and labelled A–E on both Figs. 1 and 2.

The initial effects of hypercapnia on arterial blood (A–B in Figs. 1 and 2) were rapid and usually complete within 30 min. No consistent changes were then seen in the next 4 h. The fall in pH and elevation of plasma bicarbonate represents a shift along a whole body buffer line substantially different from that determined for whole blood samples equilibrated *in vitro* (Fig. 5). During this initial respiratory acidosis it seems likely that an important response by the animal was a considerable, CO_2 stimulated, hyperventilation since the P_{CO_2} difference between arterial blood and inspired gas was considerably reduced (12 mmHg when the animal was breathing in air, as against 2 mmHg during the initial exposure to 5% CO_2). More prolonge

exposure to 5% CO_2 led to a considerable movement of arterial blood away from the *in vivo* buffer line. Samples taken after 18 h of exposure to high CO_2 showed that there was some compensation for the respiratory acidosis in that bicarbonate concentrations in the plasma increased substantially (point C, Figs. 1 and 2). At the same time, however, the P_{CO_2} of arterial blood always increased, suggesting that changes were also taking place in respiratory regulation causing a decrease in the CO_2 induced hyperventilation. The net result of these two changes was usually an increase in pH which could be substantial in some individuals (Fig. 1, B–C). Because of the rise in P_{CO_2}, the pH compensation was often much less marked (Fig. 2, B–C). Since the arterial pH and P_{CO_2} were measured towards the end of the 24 h period of CO_2 exposure, it is difficult to be certain that complete equilibrium had been reached, especially as the changes were occurring quite slowly. No clear-cut trends were obvious in the final samples taken before air was readmitted to the chamber, however, and it is probable that the major adjustments had occurred by the end of the 24 h period.

When the animal was allowed to breath air, the plasma pH increased and bicarbonate decreased along an *in vivo* buffer line (C–D, Figs. 1 and 2), above and probably parallel to that seen during the initial response to hypercapnia. It was noticeable that the early stages of the recovery process were more gradual than those observed at the onset of hypercapnia, and during the first 3 or 4 h of air breathing, P_{CO_2} continued to fall and pH to increase. The sudden removal of a high level CO_2 stimulus must cause the respiratory control system to produce a substantial hypoventilation at first, thus slowing the recovery process. The eventual fall in P_{CO_2} levels during the early stages of recovery often led to pH values higher than those seen in the untreated animal (Fig. 1) but during this time, plasma bicarbonate concentration was also beginning to decrease and pH stablilzed around the 7·7 level. After 24 h in air, recovery had gone further with P_{CO_2}, pH, and bicarbonate levels approaching (Figs. 1 and 2, point E), but rarely reaching during the period monitored, those seen prior to CO_2 exposure.

The changes in total CO_2 of whole blood are plotted in Fig. 3, the widths of the histogram blocks indicating the time spans over which blood samples were taken that contribute to the mean values shown. The effect of breathing a 5% CO_2 mixture on the total blood CO_2 concentration was to cause changes similar in direction but different in quantitative detail from those already described for plasma. There was an initial increase of some 3–4 m-mol/l total CO_2, and as hypercapnia was prolonged so that bicarbonate-pH compensation and respiratory changes occurred, the levels increased by a further 2–3 m-mol/l CO_2. When air breathing was resumed there was an initial rapid fall in total CO_2, but even in the longer term the animals were unable to bring their blood CO_2 back to the original air breathing levels. Plasma bicarbonate concentrations were calculated from P_{CO_2} and pH as before so that the distribution of CO_2 between plasma and red blood cells could be determined. It was assumed that the plasma carbonate was negligible and that the haematocrit was 25%, this being the mean value as measured in arterial blood (Table 1). The difference between total CO_2 in 1 l of blood and the sum of bicarbonate and dissolved CO_2 in 750 ml plasma represented the CO_2 content of 250 ml of cells. The partitioning thus calculated is shown in Fig. 3 for both air breathing and hypercapnic periods. The assumption that haematocrit remains constant during hypercapnia was not justified experimentally. It

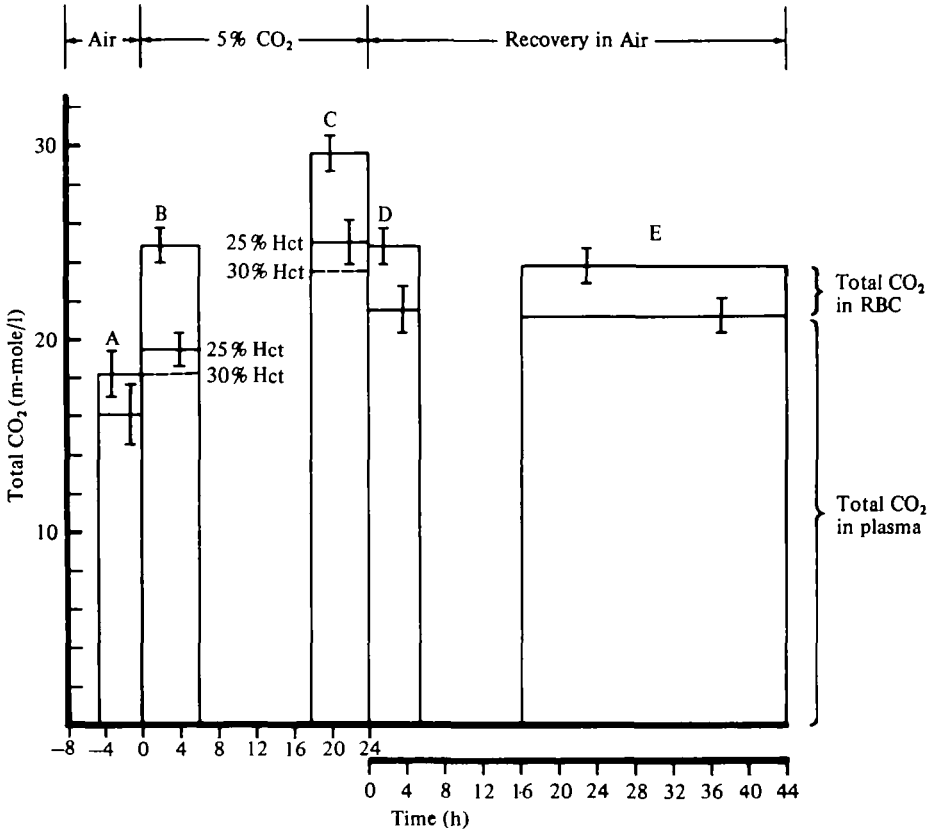

Fig. 3. Histogram showing the mean (±S.E.) changes in total CO_2 content (C_{CO_2}) as partitioned for red cells (upper part of histogram block) and plasma (lower part), during *in vivo* hypercapnia experiments on six *Bufo marinus*. Distributions were calculated on the basis of 25% haematocrit throughout. The effect on distribution of an increase to 30% haematocrit is indicated on histogram blocks within the 5% CO_2 period. Widths of the histogram blocks indicate the time span over which blood samples were taken that contribute to the mean values shown. The blocks are labelled A to E as described in text.

is clear that the calculations will be in error if the red cells swell when carbon dioxide concentrations rise. Under these conditions, the plasma contribution to CO_2 carriage will obviously be reduced. The partitioning that would be found if the haematocrit went up to 30% during the hypercapnia has also been indicated in Fig. 3.

As Fig. 3 illustrates, much of the increase in CO_2 content of the blood during the early stages of hypercapnia was due to the greatly elevated levels in the red cells. As hypercapnia continued and total CO_2 increased even further, there was a substantial redistribution so that most of this later increase was reflected in the rise in plasma concentration. Following the return to air breathing, the CO_2 contained in the cells fell fairly rapidly, though, as has been noted above, the recovery processes were on the whole slow and incomplete.

In vitro *determinations on pooled blood samples*

Carbon dioxide combining curves for *Bufo marinus* whole blood and true plasma are shown in Fig. 4. The curves were obtained from three independent sets of determinations on pooled blood samples from nine animals. True plasma is that which is in

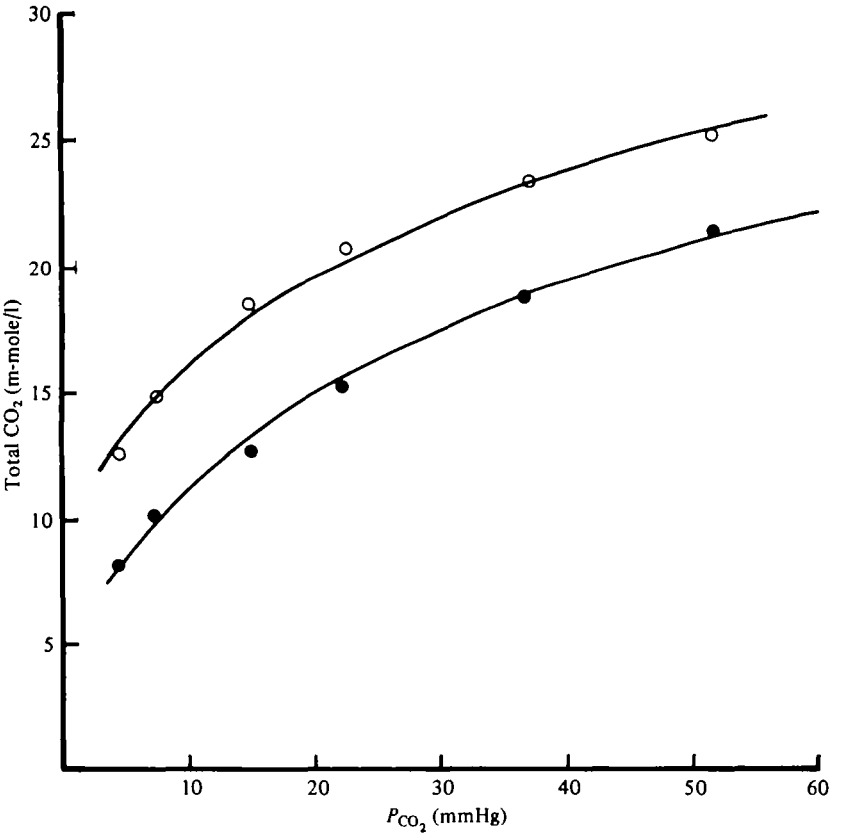

Fig. 4. CO_2 combining curves for *Bufo marinus* whole blood (●) and true plasma (○) determined *in vitro* at 25 °C. Data points are means from at least six measurements. Curves fitted by eye.

equilibrium with each different gas concentration and also in equilibrium with the red blood cells and differs markedly from separated plasma which would be in equilibrium with the erythrocytes only at the time of centrifugation (Roughton, 1964). The true plasma CO_2 curve is similar in shape but elevated above that of the whole blood indicating that *in vitro*, as was seen *in vivo* (Fig. 3), greater concentrations of CO_2 are carried as bicarbonate in the plasma than in any form within the RBC.

The buffering capacity of the non-bicarbonate blood buffers is defined as the negative slope of the $[HCO_3^-]$ *v.* pH line ($\Delta HCO_3^-/\Delta pH$). Over the physiological range of arterial blood P_{CO_2} in *Bufo*, the whole blood and true plasma bicarbonate concentrations (calculated from the C_{CO_2} measurements in Fig. 4 and the α_{CO_2} in Table 1) were found to vary linearly with pH (Fig. 5). As determined by least squares regression analysis, the slope of the true plasma buffer curve, and thus its buffering capacity, is greater than that of whole blood (Table 1, Fig. 5).

The slope of the true plasma buffer line obtained *in vivo*, during whole body CO_2 titration (i.e. Point A to B, Fig. 2) is markedly lower than that found *in vitro* (Table 1, Fig. 5). Furthermore, at a given plasma pH (over the range studied), the bicarbonate concentration *in vivo* is always considerably elevated above that seen *in vitro* (Fig. 5).

Although the slope of the *in vitro* buffer line is higher, the levels of bicarbonate

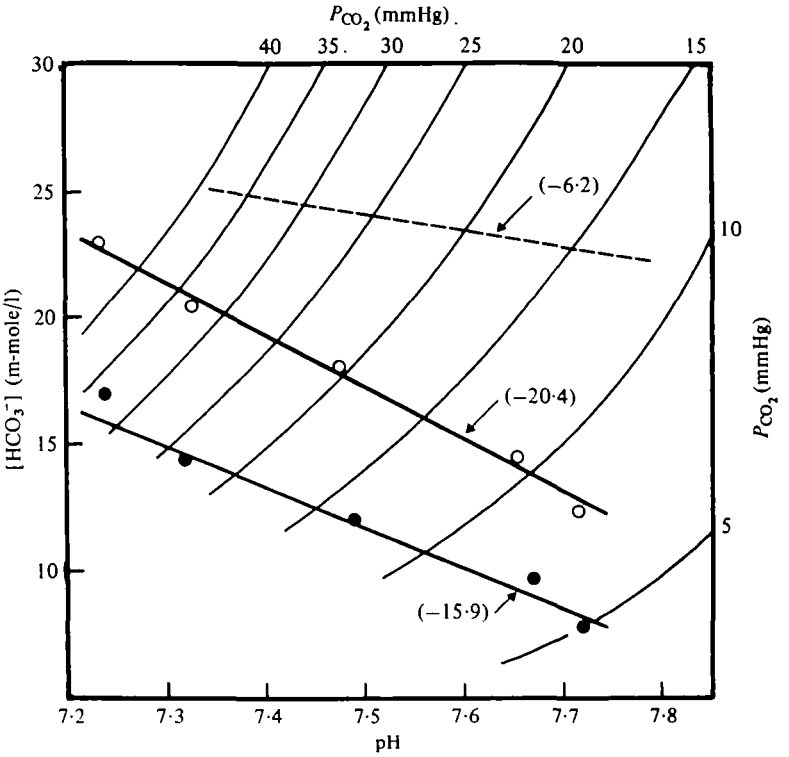

Fig. 5. Davenport diagram showing *Bufo marinus* whole blood (●) and true plasma (○) buffer lines determined *in vitro* at 25 °C as in Fig. 4. Data points are means from at least six measurements. Lines fitted by least squares regression analysis, whole blood (r^2 = 0·99), true plasma (r^2 = 0·92). Dashed line is *in vivo* buffer line taken from data in Fig. 2. Numbers in brackets indicate the buffering capacity ($\Delta HCO_3^-/\Delta pH$). Curved lines are calculated P_{CO_2} isopleths.

measured *in vitro* are considerably lower than those seen *in vivo* at the same pH. This depression in plasma [HCO_3^-] is likely caused by the presence of non-respiratory (i.e. metabolic) acids which may have been added to the circulation during blood collections. Seymour (1973), for example, collected blood from toads by cardiac puncture and found that only 30 s of struggling led to a fivefold increase in blood lactate levels. In our case, the struggling activity during pithing (20–30 s) is compounded by the additional time required (2–3 min) to expose the heart and draw a sufficiently large blood sample. It should be pointed out, however, that the appearance of metabolic acids in the blood will not affect the slope of the buffer line since they do not influence the concentations of non-bicarbonate blood buffers (principally haemoglobin).

DISCUSSION

When *Bufo marinus* are exposed to elevated ambient CO_2 tensions, there is an initial rapid increase in arterial blood P_{CO_2} to a level above that in the environment. During this time, the number of lung ventilations increase and breathing patterns are altered so that lung inflations predominate (Macintyre & Toews, 1976). This hyperventilatory response must therefore lead to the considerable reduction in the differen between P_{a,CO_2} to P_{I,CO_2} (points A and B, Fig. 1). The slow rise in blood P_{CO_2} at

constant but elevated P_{I,CO_2} (B–C, Fig. 1) probably occurs when changes in the carbon dioxide receptor systems diminish breathing. There can be no doubt that CO_2 sensitivity exists in the respiratory control system of these animals but it seems likely that prolonged exposure to hypercapnic conditions causes some adaptive changes in the overall receptor system. Such changes are well established in the chronic hypercapnic state in the mammal where pH adjustments of the cerebrospinal fluid, by increases in $[HCO_3^-]_{CSF}$, causes some decrease in ventilatory drive. It is clear that *Bufo* is responding in the same overall fashion.

With the onset of hypercapnia, several extracellular processes are set in motion with each of the mechanisms proceeding simultaneously over progressively lengthening time courses. Figs. 1 and 2 illustrate that the blood acid-base disturbance associated with a step increase in P_{I,CO_2} occurs in two distinct stages: (1) an uncompensated respiratory acidosis, and (2) a subsequent compensatory phase. The initial response (A–B) is rapid and reaches steady-state values of plasma pH, P_{CO_2} and $[HCO_3^-]$ by 30–60 min. This process is analogous to that seen in man during whole body CO_2 titration where the time necessary for interstitial fluid to reach steady-state levels (10–60 min) is a measure of how long it takes for blood and interstitial fluids to mix (Woodbury, 1974). Unlike the *in vitro* buffer line, the titration curve observed *in vivo* has a much reduced slope (Fig. 5, Table 1) which illustrates the effect that a largely unbuffered interstitial fluid has on the distribution of plasma $[HCO_3^-]$.

The involvement of compensatory processes such as transmembrane ion exchange, release of fixed carbonates and renal compensation are probably slow acting, as in mammals (Woodbury, 1974), so that during the initial stage of chemical buffering and distribution (i.e. A–B, Figs. 1 and 2) they probably exert only a negligible effect on extracellular fluid bicarbonate. This appears to be the case in *Bufo* where the initial respiratory acidosis persists at a steady state for approximately 4 h (point B, Figs. 1 and 2) before any of the known processes giving rise to transients in pH and $[HCO_3^-]$ of plasma are effectively set in motion. Assuming that the blood buffering capacity stays the same, the Davenport analysis clearly illustrates that the compensatory gain of plasma bicarbonate (B–C, Fig. 2) must be caused by processes other than an increased solubility of CO_2 in the blood. The origin of this plasma $[HCO_3^-]$ was not investigated in these experiments but additions from the tissues and hydrogen ion excretion from the plasma space represent the most likely candidates.

For example, fixed carbonates can be mobilized during hypercapnic stress in frogs (Sulze, 1942) and lead to rapid increases of base excess in the blood (Simkiss, 1968). Furthermore, HCO_3^-/Cl^- exchanges across the skin (Garcia-Romeu, Salibian & Pezzani-Hernandez, 1969) or bicarbonate retention by the kidney are other possible tissue sources. On the other hand, hydrogen ion excretion into the bladder and the subsequent acidification of the urine has been well documented for *Bufo marinus* (Frazier & Vanatta, 1972, 1973; Ludens & Fanestil, 1972; Ziegler, Ludens & Fanestil, 1974). Moreover, in similar experiments to ours, Vaughan (1976) has shown that urine NH_4^+ production substantially increases when toads are exposed to 5% CO_2 for 24 h. Since the fluid volumes released when *Bufo marinus* urinate average 21% of their total body weight (Boutilier *et al.* 1979) it is clear that an increased frequency of bladder emptying during hypercapnia could be one strategy for hydrogen ion elimination. The excretion of plasma H^+ in this way would be functionally equivalent to

additions of bicarbonate from the tissues. Regardless of its origin, the rise in plasma $[HCO_3^-]$ had only a minor influence on pH_a restoration over the 24 h period examined. Any substantial effect it may have had on pH_a was probably offset by the rise in blood P_{CO_2} that occurred over the same period of time. Even without the aggravation of P_{CO_2}, the Davenport analysis shows that regulation along a constant P_{CO_2} isopleth and perfect pH compensation is highly unlikely at the levels of hypercapnia that were induced in these experiments. Since, after 24 h of CO_2 exposure, it was not obvious that any further changes were about to occur it is possible that equilibrium had been reached and that all available buffering resources had been exhausted. If so, the compensatory mechanisms had little effect on restoring the blood acid-base disturbance although they did serve to minimize any further depression in arterial blood pH.

Figs. 3 and 4 show that a much greater proportion of the overall blood CO_2 content (principally in the form of bicarbonate) is carried in the plasma than in the red blood cells, reflecting the generally lower erythrocytic pH. The proportionally greater increase in erythrocytic C_{CO_2} as compared to plasma C_{CO_2} during the early stages of hypercapnia (Fig. 3) probably reflects the enhanced capacity for bicarbonate formation in the RBC. As CO_2 penetrates the erythrocyte it dissociates to form H^+ and HCO_3^-. By virtue of the buffering power of the negatively charged haemoglobin molecule, the H^+ are mopped up and thus more HCO_3^- can be formed intracellularly; by comparison, H^+ are only weakly buffered in the plasma. In mammalian erythrocytes, the majority of bicarbonate ions formed when H^+ combine with haemoglobin are exchanged for plasma Cl^- (i.e. chloride shift). A similar outward migration of HCO_3^- in the blood of *Bufo* would serve to explain why the whole blood buffer value is lower than that of true plasma (Table 1). Because the increased RBC $[HCO_3^-]$ would increase the total number of osmotically active particles within the RBC it seems likely that H_2O entry and swelling would also occur when blood P_{CO_2} is elevated. Although the swelling phenomenon was not investigated during these experiments, subsequent data has shown that the maximum erythrocytic volume is reached asymptotically as blood P_{CO_2} tensions approach the 5 % level (unpublished data).

During prolonged hypercapnia (B–C), the carriage of CO_2 by the RBC remained unchanged even though both P_{a,CO_2} and plasma $[HCO_3^-]$ increased considerably (Figs. 1–3). If we assume that CO_2 can still readily penetrate the erythrocytic membrane, then upon a further increase in P_{a,CO_2} (B–C), it is surprising that the RBC CO_2 pool does not increase as a direct result of the intracellular hydration of CO_2, as was the case during the initial stages of hypercapnia (i.e. A–B). In fact, it appears that either HCO_3^- ions are excreted or H^+ ions are taken up by the erythrocytes during this period since P_{CO_2} levels rise. What is clear is that only a small portion of the large gain in plasma $[HCO_3^-]$ seen during the B–C shift could be contributed by the red cells.

The blood acid-base changes accompanying the recovery period involve two processes whose time courses are different. Firstly, the plasma $[HCO_3^-]$ decreases such that an *in vivo* buffer line (C–D) develops parallel to and elevated above that of the initial response to hypercapnia (Fig. 2). In effect the animal is backtitrated along the original *in vivo* buffer slope to the pre-exposure pH_a with the time course most likely being governed by ventilation. The redistribution of C_{CO_2} between red cells and plasma during the early stage of recovery (Fig. 3) further suggests that this initial recovery process (C–D) reflects the reverse of those interactions between blood and interstitia

fluid during whole body CO_2 titration. Because the differences in plasma [HCO_3^-] between points D–A and B–C are similar, it seems likely that any further changes were slow to act and were the result of a redistribution of the base excess contributed by the tissues. Only three of the six animals studied approximated a return to original blood acid-base levels (one of which is shown in Fig. 1) and this occurred only after several days of recovery.

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