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

II. THE EFFECTS OF DEHYDRATION

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SUMMARY

Cutaneous CO_2 excretion is reduced as the skin dries during dehydration but an increase in breath frequency acts to regulate the arterial blood P_{CO_2} and thus pH_a. Moreover, the toad does not urinate and water is reabsorbed from the bladder to replace that lost by evaporation at the skin and lung surfaces. The animal does, however, produce a very acid bladder urine to conserve circulating levels of plasma $[HCO_3^-]$ and this together with an increased ventilation effectively maintains the blood acid-base status for up to 48 h of dehydration in air. Water loss and acid production are presumably also reduced by the animal's behaviour; animals remain still, in a crouched position or in a pile if left in groups.

Dehydrated toads are less able than hydrated toads to regulate blood pH during hypercapnia: they hyperventilate and mobilize body bicarbonate stores in much the same fashion as hydrated animals but due to the restrictions on cutaneous CO_2 excretion and renal output, there is comparatively little reduction in the P_{CO_2} difference between arterial blood and inspired gas thereby resulting in a more severe respiratory acidosis. These factors further contribute to the persistent acidosis which continues even when the animals are returned to air.

INTRODUCTION

The dehydration tolerance of those amphibians which inhabit terrestrial environments can often be correlated with the ability to store large volumes of water in the bladder (Shoemaker & Nagy, 1977). Bufonids, being one of the most terrestrial representatives of the Amphibia, have been chosen for several studies dealing with physiological adjustments in body fluid composition during dehydration (Shoemaker, 1965; Claussen, 1969; Fair, 1970). The inability to form a hyperosmotic urine and the fluid volume depletion imposed by evaporative water loss through the skin must pose serious problems with extracellular ion regulation and acid-base balance. In bufonid

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toads, however, the concentration of body fluids remain remarkably constant until bladder reserves are exhausted (Ruibal, 1962; Shoemaker, 1964; Shoemaker & Nagy, 1977). The semi-terrestrial toad *Bufo marinus* is capable, under some conditions, of storing up to 30% of its body weight as dilute bladder urine. It seems likely that this large volume of liquid could be used as a fluid store and may participate in extracellular acid-base balance during at least short periods of dehydration.

Because of its terrestrial habit, pulmonary respiration is of major importance in *Bufo marinus*. Adequate arterial oxygen tensions in the toad can be maintained with a ventilatory rate which is low in comparison with more aquatic forms. The consequence of a decreased minute volume would lead to a considerable CO_2 retention if it were not for the efficiency of the skin as a site for CO_2 elimination (Hutchison, Whitford & Kohl, 1968; Macintyre & Toews, 1976). Despite the limitations to cutaneous gas exchange which must be imposed by the thick cornified epidermis (i.e.: diffusion distance) and large size (i.e.: low surface area per unit body weight) of *Bufo marinus*, they can liberate 76% of their $\dot{V}_{CO_2}$ across the skin at 25 °C (Hutchison *et al.* 1968). During dehydration, limitations on evaporative water loss via the skin and the drying of the epidermis would tend to increase the diffusion barrier to cutaneous CO_2 removal and advance some form of compensation (i.e.: ventilatory and/or bicarbonate adjustments). The problems would be further increased by elevated levels of CO_2 in the environment.

In this paper, the effects of progressive dehydration on extracellular acid-base balance will be examined in normocapnic and hypercapnic *Bufo marinus*.

MATERIALS AND METHODS

Bufo marinus used in this study were collected in Mexico and supplied by a commercial dealer (Mogul-Ed. Corp., Oshkosh, Wisconsin, U.S.A.). Upon arrival, the toads were placed in moistened aquaria at room temperature (25 ± 2 °C) for at least 2 weeks before any experiments.

Dehydration in air

Animals which had been sitting in water for 2 days were weighed and the value was expressed as the gross body weight. The bladder was then emptied by inserting a glass pipette into the cloacal opening while squeezing the abdomen of the toad and allowing the fluid to drain into a thin-necked vial. Each animal was subsequently reweighed and this value recorded as the standard body weight. The difference between gross and standard weight (i.e.: the urine volume) represented the fluid storage capacity of the bladder. All urine depleted toads were able to regain their gross weight after being placed back in water over night. Animals referred to herein as 'hydrated', always had access to water for at least a 24 h period. Gross and standard weights were recorded again after the fully hydrated animals had been dehydrated by exposure to air for either a 24 or 48 h period.

Other animals were anaesthetized and catheters were chronically implanted in the buccal cavity and femoral artery so that breathing records and blood samples could be taken in unison with urine collections as animals were subjected to dehydration. Details of the femoral cannulation are given elsewhere (Boutilier *et al.* 1979*a*). An

samples of urine and blood were analysed for pH, P_{CO_2} and C_{CO_2} by the methods described in Boutilier *et al.* (1979*a*) except that P_{CO_2} measurements in this case were made by injecting a single sample into the electrode chamber and allowing it to remain stationary until the reading was taken (see Results). The buccal cavity was cannulated by passing a piece of polyethylene tubing (P.E. 100) through a small puncture in the upper jaw and anchoring it firmly against the roof of the mouth after heat flaring the implanted end. It was then connected to a Statham pressure transducer (Type P_{23}Db) and the signals displayed on a Beckman Type R511 oscillograph recorder writing on curvilinear co-ordinates. Transducers were calibrated with static columns of water at frequent intervals throughout the experiments. Respiratory rates, patterns and buccal pressures were extracted from the chart records.

Dehydration in 5 % CO_2

The methods for this series of experiments were like those already described for hydrated toads (Boutilier *et al.* 1979*a*), except that the animals were denied access to water. After cannulating the femoral artery, the animals were placed in water until they regained consciousness and subsequently transferred to a dry aquarium for 24 h before the experiments began. For each experiment, a toad was placed in a dry chamber (25 °C) which could be supplied with air or a 5 % CO_2 gas mixture. Repetitive blood samples were taken before, during and after a 24 h period of CO_2 exposure. Measurements of pH, P_{CO_2} and C_{CO_2} were made on each blood sample and plasma bicarbonate concentrations were calculated from the Henderson–Hasselbalch equation using pk'_1 (6·05) and α_{CO_2} (0·033 m-mol.l^{-1}.$mmHg^{-1}$) estimates for *Bufo marinus* plasma detailed elsewhere (Boutilier *et al.* 1979*a*).

RESULTS

I. *Dehydration in air*

Water balance. Bufo marinus has the ability to store large quantities of water as dilute urine. In some toads, fluid volumes in excess of 30 % gross body weight could be collected by draining the bladder. On the average, however, this amounted to 21 % (Fig. 1). More often than not, urine volumes of this magnitude were actively released while handling the animal although undisturbed *Bufo* would often expel similar quantities while sitting in water. In contrast, we observed no active urine release under any circumstances during the time that toads were subjected to dehydration in air.

The initial bladder volume in the hydrated toads progressively declined over 24 and 48 h of dehydration to 10 % and 5 % gross body weight respectively (Fig. 1). The reduction in gross weight over 48 h was 271·7 g.kg^{-1}, 67 % of which was lost over the first 24 h. Similarly, a larger proportion of the overall loss in standard weight (70 % of 120·2 g·kg^{-1}) occurred during the initial 24 h of dehyration. The reduction in standard weight is more reflective of evaporative water losses from the tissues since it removes possible changes in urine volume. The smaller overall reduction in standard as opposed to gross body weight then clearly reflects the role played by the toad bladder as a fluid store during periods of dehydration. Furthermore, all animals, regardless of their state of dehydration, were able to regain their gross body weight within 24 h of being placed back in water.

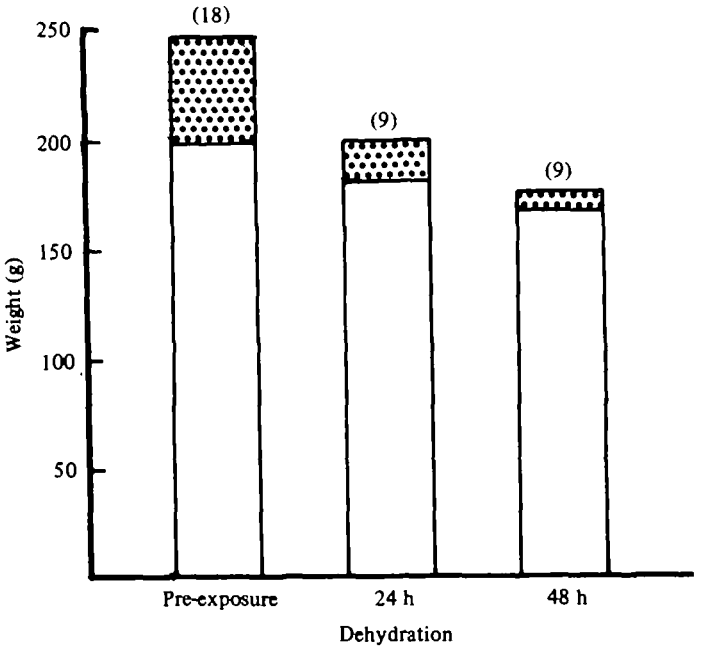

Fig. 1. Histogram showing the gross weight (total bar height), standard weight (open), and urine volume (stippled), in grams for hydrated toads and those subjected to either 24 or 48 h of dehydration in air. Values are means calculated from the number of animals given in parentheses above each bar.

Extracellular acid-base balance. Because of some initial problems encountered with CO_2 electrode performance (Boutilier *et al.* 1978) our urine and arterial blood measurements in Table 1 (our first series of experiments on *Bufo*) are underestimates of the actual P_{CO_2} levels. For all our subsequent experiments (i.e.: Boutilier *et al.* 1979*a*, *b* and this study), these problems were overcome by adopting the fluid replacement protocol described by Boutilier *et al.* (1978). Although the levels of arterial blood P_{CO_2} seen in Table 1 were always lower than those measured later (i.e.: Fig. 3, point A), the error was consistent (4–5 mmHg below the actual P_{a,CO_2}). In further support of a consistent error, the CO_2 tensions of the urine were always in equilibrium with those of arterial blood. Thus, while the absolute levels of P_{CO_2} in Table 1 must be viewed with caution, they can still be used to qualitatively interpret the accompanying changes in pH and total CO_2.

Over 48 h of dehydration exposure there were no marked changes in arterial blood pH, P_{CO_2} or C_{CO_2} (Table 1). Furthermore, the urine P_{CO_2} was always in equilibrium with that of the blood and was not altered by the state of hydration of the animal or the fluid volume in the bladder. Urine pH and C_{CO_2} had considerably declined after 24 h of dehydration and continued to do so over the final 24 h period. Since P_{CO_2} did not change, the progressive acidification of the urine could not have resulted from the addition of respiratory acid (i.e. H_2CO_3).

Respiratory patterns and frequency. Breathing records from five toads showed that the normal patterns of ventilation were modified during dehydration (Fig. 2). *Bufo marinus* exhibit three well defined respiratory movements (oscillations, ventilatio

Table 1. *Acid-base parameters in the arterial blood and urine from hydrated toads* ($N = 10$) *and those subjected to either* 24 ($N = 5$) *or* 48 *h* ($N = 5$) *of dehydration in air. Values are given as means* ($\pm$ S.E.)

	Blood			Urine		
	pH	P_{CO_2} (mm Hg)	C_{CO_2} (m-mol/l)	pH	P_{CO_2} (mm Hg)	C_{CO_2} (m-mol/l)
Pre-exposure	7·79 ± 0·02	8·86 ± 0·41	15·76 ± 0·59	7·02 ± 0·08	8·73 ± 0·50	1·82 ± 0·27
24 h	7·77 ± 0·03	8·08 ± 0·39	16·44 ± 1·34	6·35 ± 0·19	8·48 ± 0·45	1·28 ± 0·42
48 h	7·78 ± 0·03	7·53 ± 0·35	15·80 ± 1·43	6·00 ± 0·23	7·25 ± 0·74	0·55 ± 0·21

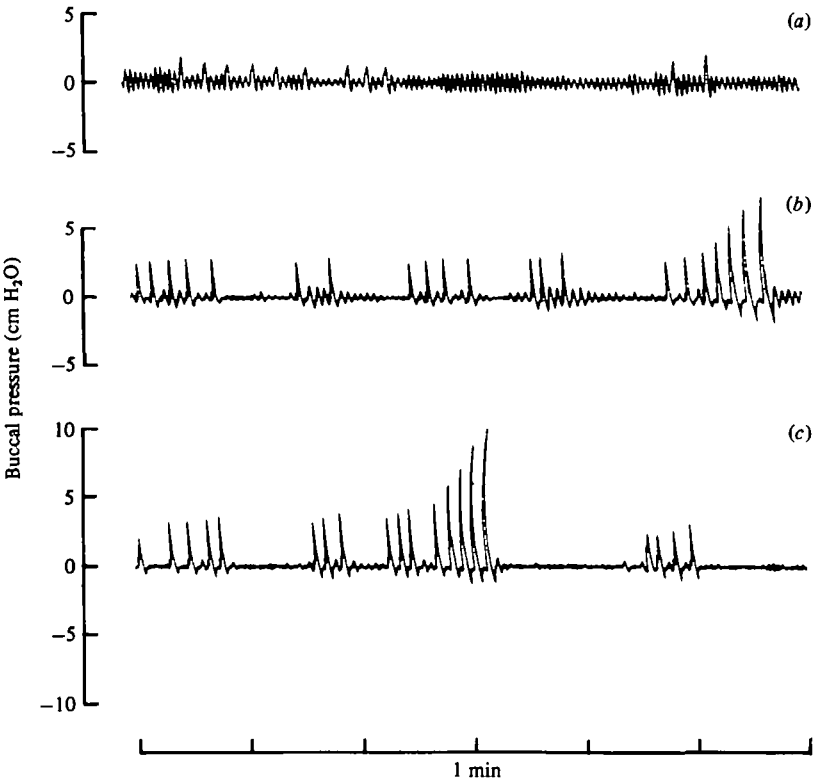

Fig. 2. Typical breathing records from unanaesthetized *Bufo marinus* at 25 °C. (*a*) Fully hydrated; (*b*) after 24 h of dehydration in air; (*c*) after 48 h dehydration in air.

and inflations) which have been reported elsewhere in detail (Macintyre & Toews, 1976). The oscillations serve to flush air into and out of the buccal cavity and appear as the small pressure deflexions in Fig. 2. The larger positive pressure changes are seen during air renewal in the lungs. In hydrated animals, ventilations occurred irregularly and alternated with periods of numerous buccal oscillations (Fig. 2*a*). During dehydration, the ventilations were very often grouped into discrete periods called inflations. Each inflation consisted of a series of uninterrupted ventilations and was recorded in the buccal cavity as 5–8 positive pressure changes that progressively increased in amplitude (Fig. 2*b*, *c*). During an inflation, intrapulmonary pressure

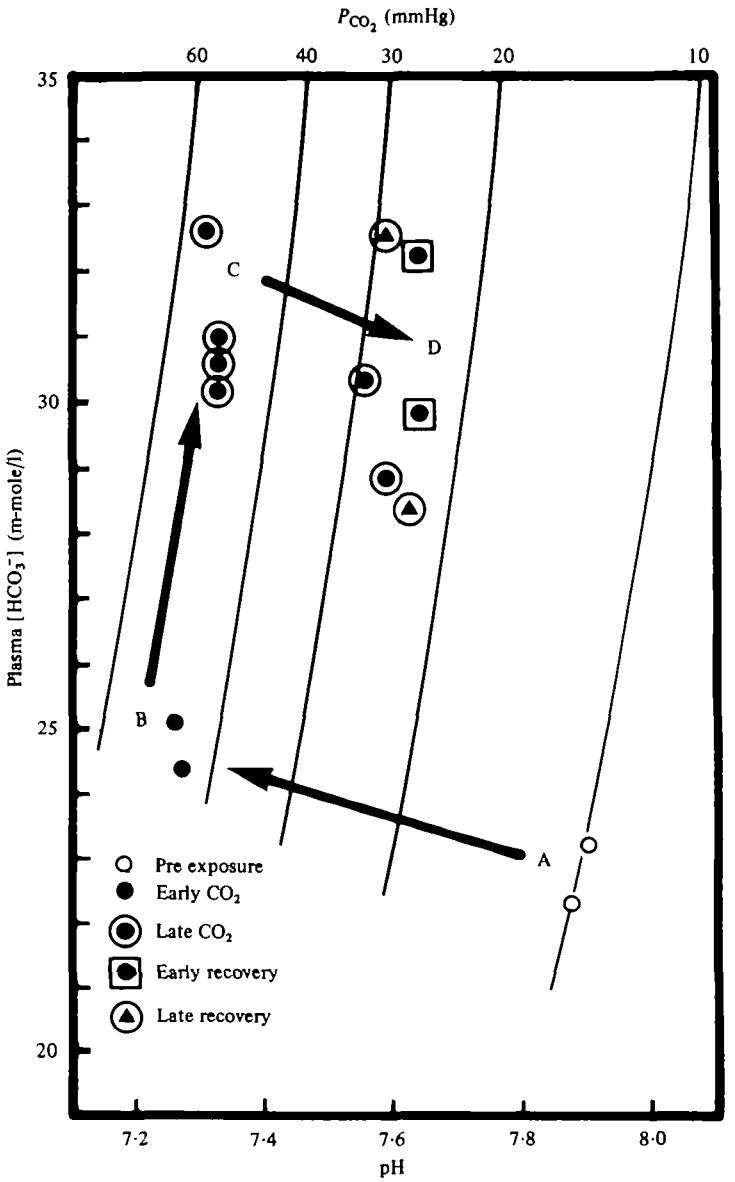

Fig. 3. Davenport diagram showing the arterial blood acid-base relationships seen during the course of a typical hypercapnia experiment on a dehydrated *Bufo marinus*. Arrows indicate the major movements from A to D (see text). Curved lines are calculated P_{CO_2} isopleths.

progressively increases and then is held at a high level for a variable length of time (Macintyre & Toews, 1976). During this time, ventilations were absent and numerous oscillations (Fig. 2*b*) or respiratory pauses (Fig. 2*c*) were recorded until the return to a regular pattern of oscillations and ventilations.

In this study, fully hydrated animals at rest ventilated their lungs at a mean frequency of 13·4 ± 1·6 ($\bar{X} \pm$ S.E.) per minute. Dehydrated animals exhibited increase

ventilatory rates at both the 24 h (mean, 19·9; S.E. 1·9) and 48 h (mean, 21·3; S.E. 2·1) periods of air exposure. In addition, the amplitude of the buccal pressures recorded during a ventilatory movement in dehydrated toads were in some instances three- to fourfold greater (Fig. 2*b*, *c*) than those seen in the hydrated animals (Fig. 2*a*). These high pressure ventilations were usually associated with the inflations.

II. *Dehydration in* 5 % CO_2

The protocol for the series of experiments testing the effects of hypercapnia on dehyrated animals were similar to those reported in the preceding paper (Boutilier *et al.* 1979*a*). Samples of arterial blood (usually two) were taken from the resting animal after it had been subjected to dehydration in air for 24 h (point A). The animal was then made to breath a 5 % CO_2 gas mixture for 24 h and repetitive blood samples were taken at intervals during the first 3–4 h (point B) and over the final 6 h of CO_2 exposure (point C). When returned to a normal atmosphere, further samples were taken within the initial 4 h of recovery (point D) and for as long as the arterial catheter remained patent (up to 4 days).

Measurements of pH and P_{CO_2} made on each blood sample were used to calculate their corresponding plasma bicarbonate concentration. When these bicarbonate concentrations are plotted against pH, the events seen during a typical hypercapnia experiment on *Bufo marinus* are as shown in Fig. 3. The averaged data for six dehydrated animals are similarly plotted in Fig. 4. Such plots can be directly compared to those previously reported (Boutilier *et al.* 1979*a*) for more hydrated toads.

As shown before in Table 1, the blood acid-base status was unchanged by 24 h of dehydration in air (point A, Fig. 4). The initial response to hypercapnia (A–B) resulted in a significantly larger fall in the arterial blood pH of dehydrated animals. Since the *in vivo* buffering capacity in the dehydrated toads ($-6{\cdot}81$ m-mol.HCO_3^-.l^{-1}.ΔpH^{-1}) was similar to that seen in hydrated *Bufo* (Boutilier *et al.* 1979*a* and Fig. 4), the more pronounced acidosis was caused by a considerably greater P_{a,CO_2} increase in the dehydrated animals. Consequently, the P_{CO_2} difference between arterial blood and inspired gas in dehydrated toads was only slightly reduced (11·5 mmHg when the animals were breathing air, as against 10 mmHg during the initial exposure to 5 % CO_2) in comparison with the more hydrated animals (12 mmHg, point A; 2 mmHg, point B). Upon more prolonged CO_2 exposure (B–C), the dehydrated animals showed somewhat larger increases in plasma [HCO_3^-] and pH_a, though the attendant increases in P_{a,CO_2} were not significantly different than those observed in the hydrated animals.

When both groups of animals were allowed to breathe air, the blood was back titrated along an elevated *in vivo* buffer line (C–D). The slopes of these lines were similar to those seen during the initial response to hypercapnia which meant that the buffering capacity of the non-bicarbonate blood buffers (principally haemoglobin) had gone unchanged. The recovery period in hydrated animals (C–E) occurred over two distinct phases: an initial 3–4 h period in which plasma pH and P_{a,CO_2} were restored toward normal and a slow (over days) readjustment of body buffer stores (Boutilier *et al.* 1979*a* and Fig. 4).

Of the six dehydrated animals studied, Fig. 3 illustrates a typical recovery period

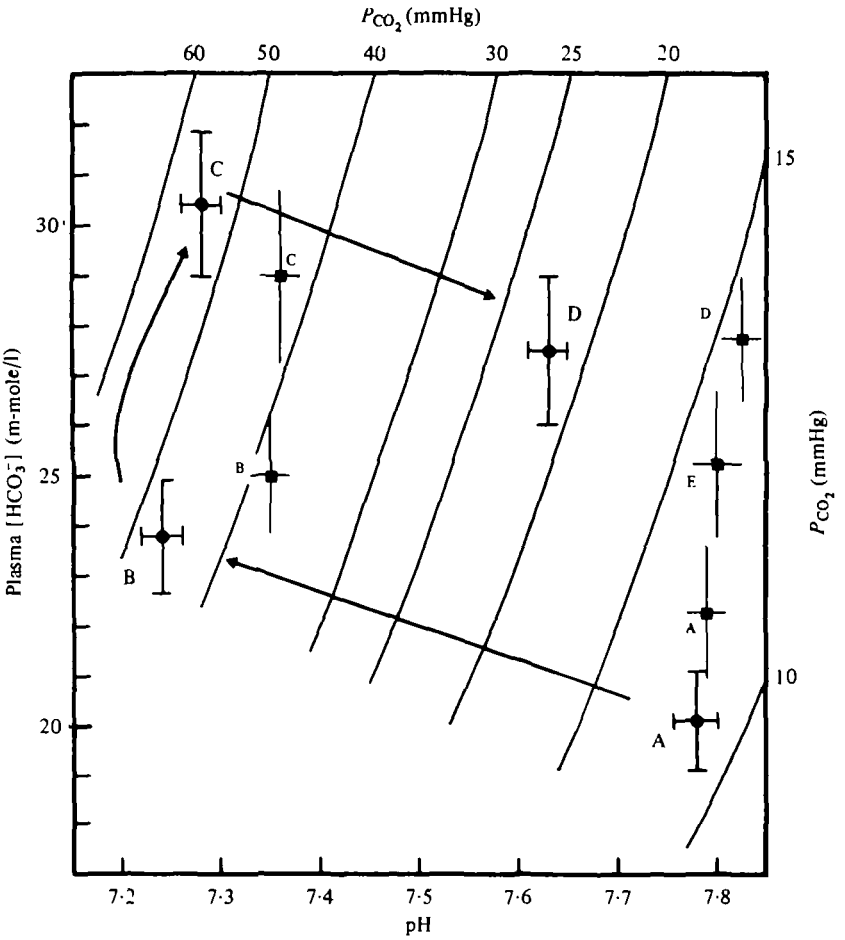

Fig. 4. Davenport diagram showing arterial blood acid-base relationships that occurred in six hydrated (■) and six dehydrated (●) *Bufo* during the hypercapnia experiments. Mean (±S.E.) plasma bicarbonate concentrations are plotted as a function of plasma pH (±S.E.) for each of the experimental stages described in the text. Data for hydrated animals were taken from Boutilier *et al.* (1979*a*). Arrows indicate the major movements (A to D) for the dehydrated toads. Curved lines are calculated P_{CO_2} isopleths.

which, in contrast to the hydrated toads (Fig. 4) does not show any trend (over days) towards an eventual restoration of pre-exposure levels of plasma pH, P_{CO_2} or [HCO_3^-]. The back titration along the elevated buffer line (C–D, Fig. 3) was in itself incomplete and represented the only adjustment in the recovery period of the dehydrated toad. Most noticeable were the persistent high levels of P_{a,CO_2} at point D (Figs. 3 and 4) even though the animals had been returned to air for long periods of time. Thus the recovery period in dehydrated animals was always characterized by a chronic respiratory acidosis.

DISCUSSION

In *Bufo*, almost all of the water lost during dehydration can be accounted for by evaporative water losses from the skin and lungs since urine was not voided. Furthermore, the comparatively lower reduction in standard as opposed to gross body weight (Fig. 1) suggests that as dehydration proceeds water is gradually moved out of the bladder to replace that which is lost by the various body fluid compartments. Whereas the osmoregulatory benefits of a large storage capacity for dilute urine in the bladder of bufonid toads have received much attention (see review by Shoemaker & Nagy, 1977), there is little known about the consequent effects that dehydration has on the role played by the kidney as an organ of acid-base balance.

When acids from metabolism are released into the extracellular fluid of the resting animal, they are buffered by both the non-bicarbonate and bicarbonate blood buffers and the pH of the blood is maintained within a narrow range by ventilatory regulation of P_{a,CO_2} and comparatively slow acting adjustments in extracellular [HCO_3^-] via the kidneys. In bimodal breathers like the toad, the major proportion of V_{CO_2} is removed passively across the skin (Hutchison *et al.* 1968) but the P_{CO_2} levels within the lung and therefore in the blood are controlled by the pattern and frequency of buccal to lung filling and emptying sequences. During dehydration, it seems likely that CO_2 excretion would be impaired by the need to limit evaporative water losses across the epidermis and thereby necessitate some form of compensation. In *Bufo*, the maintenance of a constant P_{a,CO_2} over 48 h of dehydration (Table 1) was correlated with an elevated breathing rate (Fig. 2) which indicated that the lungs had taken on an increased importance in CO_2 removal. Furthermore, the breathing pattern was altered so that high pressure ventilations and inflations became prominent. Inflations are usually seen during some form of respiratory stress in *Bufo* (Macintyre & Toews, 1976; Boutilier & Toews, 1977) and are thought to increase gas transfer per unit time. If so, the reduction of buccal oscillations and of ordinary lung ventilations during dehydration, with an increased reliance on lung inflation cycles, may permit increased alveolar ventilation and at the same time reduce the amount of water lost by evaporation at the alveolar surface.

However, the respiratory system can participate only in the stabilization of plasma carbonic acid levels and any adjustments in the circulating stores of bicarbonate depends on the flow of ureteral urine, the bladder volume and the frequency of urination, all of which are affected by the degree of dehydration. The progressive acidification of the urine at constant P_{CO_2} during dehydration (Table 1) indicates that the depression in pH was caused by non-respiratory acid additions or their functional equivalent (i.e.: bicarbonate reabsorption). Thus it seems likely that both the lungs and the kidneys contributed to the blood acid-base maintenance over 48 h of dehydration since it is the concentrations of both carbonic acid and HCO_3^- (i.e.: the HCO_3^-/CO_2 ratio) which ultimately determine the pH of plasma. Thus, the bladder may well serve as both an osmoregulatory and an acid-base organ during periods of dehydration.

The effects of hypercapnia on arterial blood in dehydrated toads caused changes which were similar in both direction and duration but different in quantitative detail from those of the more hydrated animals (Fig. 4). The fundamental difference must

pivot upon the limitations for cutaneous CO_2 removal in dehydrated animals and thu[illegible] the increased participation of the lung in overall gas exchange. We have already suggested (Boutilier *et al.* 1979*a*) that an important response by the hydrated animal, during the initial stages of CO_2 exposure, was a CO_2 stimulated hyperventilation since the arterial blood to ambient P_{CO_2} gradient was considerably reduced. As shown in Fig. 2, the pattern of ventilation was already modified and working at a high rate after only 24 h of dehydration in air (i.e.: point A, Fig. 4). Thus, the higher levels of CO_2 in dehydrated animals ought to stimulate even greater pulmonary ventilation but it is clear from the results that there are limitations in the animal's capacity for hyperventilation so that the overall result is a more pronounced respiratory acidosis (point B, Fig. 4).

Upon prolonged CO_2 exposure, both hydrated and dehydrated toads exhibited some degree of compensation for their respiratory acidosis in that plasma bicarbonate concentrations increased substantially. This resulted, however, in only a small pH_a adjustment since at the same time the P_{a,CO_2} also increased. The gradual rise in blood P_{CO_2} was most likely caused by a decrease in ventilatory drive due to adaptive changes in the CO_2 receptor systems which stimulate breathing. Consequently, for the same degree of pH_a adjustment (B–C, Fig. 4), the attendant increase of bicarbonate in the plasma of the more acidotic dehydrated toad was 50–60 % greater than that seen in the hydrated toad. If the larger gain of bicarbonate was solely caused by the ongoing desiccation (i.e.: haemoconcentration) we should have observed an increase in the buffering power of the blood when it was back titrated along the elevated buffer line during recovery (C–D, Fig. 4). Since we did not and considering that the time course of the compensatory stage was similar in both groups of animals, it seems more likely that the underlying mechanisms which govern this phase are the same and simply proceed at a comparatively higher rate in the more acidotic condition of the dehydrated animal. Of the known processes giving rise to transients in pH and $[HCO_3^-]$ of plasma, a more accelerated renal compensation (i.e.: H^+ excretion or HCO_3^- reabsorption) seems unlikely in view of the obvious restrictions imposed by dehydration. It does seem possible, however, that the more acid conditions in dehydrated toads could act to liberate greater quantities of fixed carbonates such as the HCO_3^- pool in bone (Simkiss, 1968).

In hydrated toads, the blood acid-base events accompanying the return to normal atmosphere (C–E) are the reverse of those seen during CO_2 exposure (A–C) except that they occur much more slowly (Boutilier *et al.* 1979*a* and Fig. 4). The initial stage of recovery (C–D) represents a back titration along an elevated *in vivo* buffer line which has a similar slope to that seen during early CO_2 exposure (A–B). During this initial phase of recovery, ventilation must govern the time over which pH_a is restored to normal since over the same period of time, the P_{a,CO_2} to P_{I,CO_2} gradient was readjusted so as to approximate the gradient observed in the resting hydrated animal. The initial effects of recovery on arterial blood in dehydrated toads are similar in direction to those of hydrated animals (i.e.: the blood was back titrated along an elevated buffer line) but not nearly as complete. For example, dehydrated *Bufo* were unable to re-establish the pre-exposure P_{a,CO_2} to P_{I,CO_2} gradient (21·6 mmHg at D as against 11·5 mmHg at A) and thus pH_a. The persistent elevation of P_{a,CO_2}

point D) suggests that by this time, the restraints imposed by dehydration on cutaneous CO_2 removal were so severe that ventilation could not deal with the extra demands and therefore act to fully correct the respiratory acidosis. At this time, the toads had been dehydrated for approximately 48 h and in contrast to the animals subjected to a similar period of dehydration in air (Table 1) were unable to maintain a resting level of blood pH. This suggests then, that the intensity of dehydration was greatly accelerated by the hypercapnic exposure. In the hydrated state, there are further adjustments (over 24–48 h) which restore the circulating levels of plasma [HCO_3^-] toward normal (D–E) and these events are probably directed by renal mechanisms. Dehydrated animals made no further changes in blood acid-base balance beyond those described above and in effect were in a chronic state of respiratory acidosis during the entire recovery period.

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