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

## III. THE EFFECTS OF BURROWING

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SUMMARY

When *Bufo marinus* burrows, the skin becomes intimately surrounded by substrate but the nares always remain exposed to the surface air. Upon entering into a state of dormancy the animal hypoventilates and this together with the loss of the skin as a respiratory site results in a rise in arterial blood $P_{CO_2}$ despite a probable decline in metabolism. Even though lung ventilation falls, the toad regulates blood pH and the respiratory acidosis is progressively compensated for by a progressive increase in plasma [$HCO_3^-$] along the course of an elevated $P_{CO_2}$ isopleth. At steady state, the acidosis is fully compensated for by a new equilibrium ratio of $HCO_3^-$ to $P_{CO_2}$ at the same pH as the non-burrowed animal. Arousal from the dormant state at this time results in a marked lung hyperventilation and a sharp decline in body $CO_2$ stores.

### INTRODUCTION

Many amphibious vertebrates avoid prolonged periods of dehydration by burrowing into a moist substrate and entering upon a state of dormancy. The African lungfish *Protopterus* possesses the ability to form a subterranean aestivation cocoon in which it has been reported to remain dormant for several years (Smith, 1961). Several recent studies on lungfish have described a complex series of metabolic, respiratory and osmoregulatory adjustments that attend aestivation periods of a year or more (DeLaney, Lahiri & Fishman, 1974; DeLaney, Shub & Fishman, 1976; DeLaney *et al.* 1977; Johansen *et al.* 1976).

During seasonal periods of drought (3–4 months), a number of desert-dwelling frogs and toads form aestivation cocoons which serve to reduce evaporative water losses across the skin (Lee & Mercer, 1967). In contrast to the lungfish, however, many of these burrows are soil-filled thus eliminating an open channel to the surface for breathing (Mayhew, 1965; McClanahan, 1967; Lee & Mercer, 1967; Seymour, 1973). Other anurans such as *Bufo marinus* create more shallow burrows so that the nares are exposed to the surface for pulmonary gas exchange. Burrowing into a moist

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substrate in this fashion enables these animals to avoid at least short periods of unfavourable conditions on the surface.

Most $CO_2$ is excreted passively across the skin of the resting hydrated toad, but the levels of $P_{CO_2}$ in the lung and therefore in the blood are controlled by the pattern and frequency of ventilations. Exposure to hypercapnic conditions has clearly shown that *Bufo marinus* can regulate arterial blood $P_{CO_2}$ and consequently $pH_a$ by adjusting the $P_{CO_2}$ difference between arterial blood and inspired gas via changes in breathing (Macintyre & Toews, 1976; Boutilier *et al.* 1979*a*). Moreover, when extrapulmonary $CO_2$ excretion is reduced as the skin dries during dehydration, the lungs take on an increased importance in $CO_2$ elimination and act to regulate $P_{a,CO_2}$ within a narrow range of normal resting values (Boutilier *et al.* 1979*b*). When *Bufo marinus* burrows, the gaseous environment surrounding its skin may be altered by the low convection of gases in soil. In view of the above considerations, we examined the blood acid-base balance and pulmonary ventilation in the burrowed toad.

## MATERIALS AND METHODS

*Bufo marinus* were obtained commercially. Upon arrival, they were kept at room temperature ($25 \pm 2$ °C) in moistened aquaria for at least 2 weeks before the experiments began.

Ten animals (250–500 g) were used in the burrowing studies. For arterial blood sampling, a polyethylene catheter (P.E.60) was chronically implanted in the femoral artery. The pH, $P_{CO_2}$, $P_{O_2}$ and total $CO_2$ content ($C_{CO_2}$) of arterial blood were measured at the experimental temperature (25 °C) using Radiometer microelectrode assemblies and display meters. Plasma bicarbonate concentrations were calculated with the Henderson–Hasselbalch equation using $pK_1'$ (6·05) and $\alpha_{CO_2}$ (0·033 m-mol.$l^{-1}$.mm $Hg^{-1}$) estimates which were experimentally derived for *Bufo marinus* plasma at 25 °C. Whole blood [$HCO_3^-$] was estimated using the formula:

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

Further details of the above procedures have been given elsewhere (Boutilier *et al.* 1979*a*). Buccal pressure was recorded from a water-filled polyethylene catheter (P.E.100) chronically implanted in the roof of the mouth as previously described (Boutilier *et al.* 1979*b*).

Sand, the burrowing medium in these experiments, was added to a large glass aquarium to provide an overall depth of 25 cm. Water was added below its surface through a stationary glass pipette and the moisture level continually adjusted by observation through the sides of the chamber, to leave a 10 cm curface layer of dry sand. When placed on the dry surface, the toads would invariably spend 1–2 h above ground and then burrow down to the moist sand by digging with their hind legs. Burrowing activity continued for some 10–15 min until usually only the head and a small portion of the back was visible. In this state, the cutaneous area most responsible for skin gas exchange was now intimately surrounded by moist sand but the nares always remained exposed to the surface air. Buried animals would sometimes shift positions in the burrow overnight but for the most part showed no visible body

movements and remained in a state of dormancy with their eyelids closed for several days.

The protocol for all burrowing experiments was to take blood samples (in replicate if possible) and respiratory recordings after the animals were allowed to settle in the aquarium and were resting quietly on top of the sand. After these surface samples, the animals were allowed to burrow and further samples were taken at regular intervals (at least once daily) for the duration of the experiments. The animals usually spent some 5–8 days in the burrow before the arterial catheters became plugged and the experiments had to be terminated.

## RESULTS

### *Acid-base changes during burrowing*

Fig. 1 shows the relationships between the measured values of arterial blood pH, $P_{CO_2}$ and bicarbonate as a function of the time in which *Bufo marinus* remained dormant in their sand burrows. These data were recorded from serial blood collections taken at regular intervals during the course of individual experiments on ten toads and are plotted as means ± the standard error (S.E.). In six animals, determinations of all parameters were possible at each sampling interval over the period shown in Fig. 1.

$P_{a,CO_2}$ progressively increased (up to twofold) over the first day and a half of burrowing and thereafter remained approximately constant for the duration of the experiments (Fig. 1). Blood pH declined in concert with the increasing levels of $P_{a,CO_2}$ over the initial 36 h period but then rose continuously for an additional 36 h until reaching a value near that of the non-burrowed animals. Coincident with the $pH_a$ and $P_{a,CO_2}$ disturbances over the first 3 days was a continuous rise in blood $[HCO_3^-]$ which subsequently reached a plateau after 72 h. Beyond 72 h and for the duration of the experiments, no further acid-base changes were apparent as long as the animals remained undisturbed and buried. In the latter stages of the experiments (4–6 days), it became increasingly difficult to obtain blood samples without causing some form of disturbance to the otherwise dormant toad. Such disturbances were inevitably related to re-establishing blood flow in catheters which had become plugged. When toads were aroused in this fashion, it resulted in a marked hyperventilation and a sharp decline in body $CO_2$ stores (see below and Fig. 2). Recorded values from those blood samples taken during bouts of hyperventilation were not included in the calculations of the means in Fig. 1 since these data describe only a transient and short-lived acid-base disturbance.

For each sampling interval shown in Fig. 1, plasma bicarbonate concentrations were calculated from the measured values of $pH_a$ and $P_{a,CO_2}$. The $HCO_3^-$ concentrations thus calculated were then plotted as a function of pH in a Davenport diagram (Fig. 2). This diagram clearly illustrates two distinct stages of acid-base adjustment which occur when toads burrow and become dormant: (1) an uncompensated respiratory acidosis and (2) a subsequent compensatory phase.

Over the first 36 h of burrowing, a progressive increase in arterial blood $P_{CO_2}$ caused $pH_a$ to decline and plasma $[HCO_3^-]$ to rise. This is illustrated as the shift

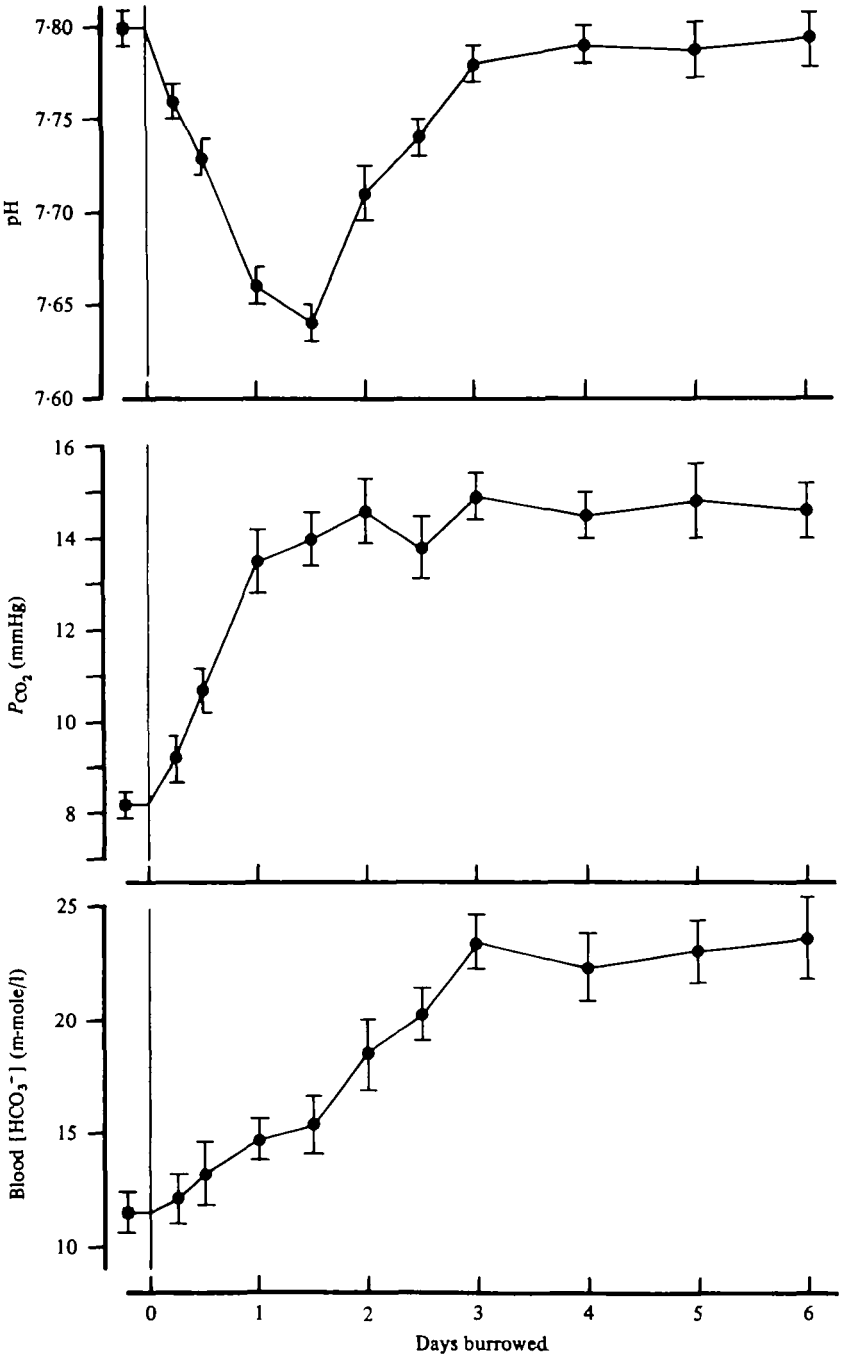


Fig. 1. Mean (±S.E.M.) measurements of arterial blood pH, $P_{CO_2}$ and [$HCO_3^-$] before (■) and as a function of time following burrowing (●) of *Bufo marinus* ($N$ = 6–10). Vertical line at time zero indicates the onset of burrowing. Temperature: 25 °C.

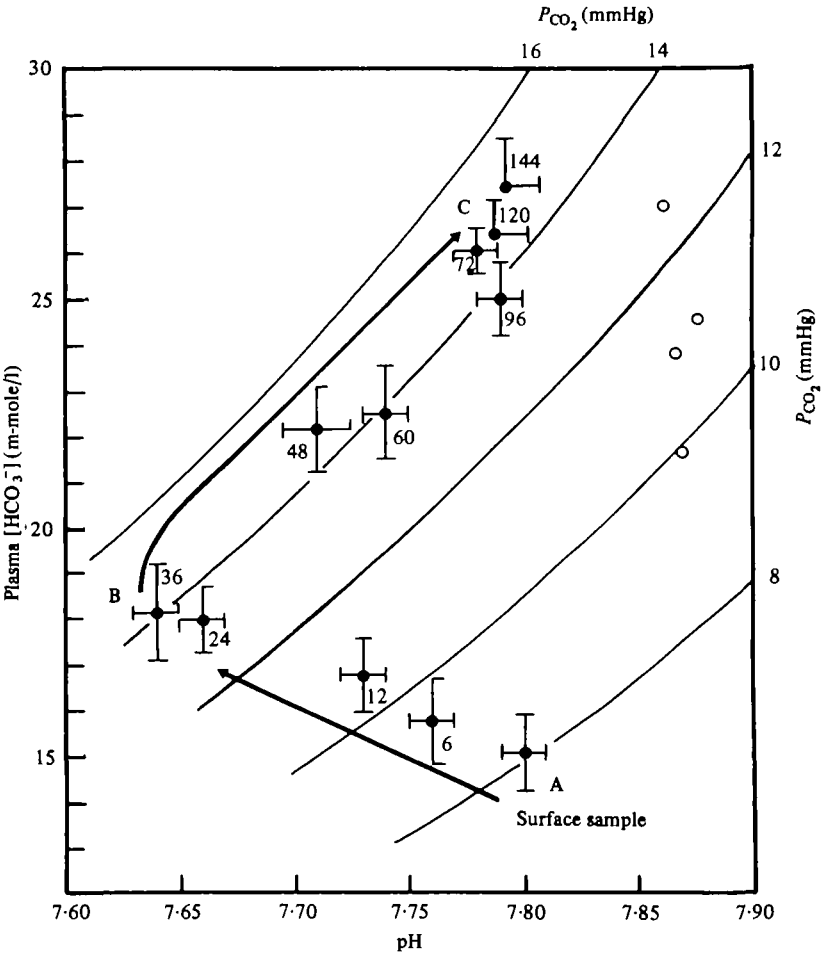


Fig. 2. Davenport diagram showing mean (±S.E.M.) changes in plasma [$HCO_3^-$] estimates and pH measurements during burrowing of *Bufo marinus* ($N$ = 6–10). Arrows indicate direction of the major movements (A–C) as described in text. Numbers adjacent to the points represent time burrowed in hours. Curved lines are calculated $P_{CO_2}$ isopleths. Open circles = individual hyperventilation values from a single animal. Temperature: 25 °C.

from A to B in Fig. 2 and represents a gradual movement along an *in vivo* whole body buffer line. The rise in $P_{a,CO_2}$ could not have resulted from an increase in $P_{I,CO_2}$ since the burrow was only shallow and the nares were always in direct contact with surface air (i.e.: no increase in respiratory dead space). Thus, during the initial respiratory acidosis, the $P_{a,CO_2}$ to $P_{I,CO_2}$ difference gradually widened until stabilizing at approximately twice the gradient seen in the non-burrowed animals (point B, Fig. 2). Over the next 36 h, the respiratory acidosis was progressively compensated for by a gradual increase of plasma [$HCO_3^-$] along the course of an elevated $P_{CO_2}$ isopleth until the $pH_a$ disturbance brought about by burrowing was completely restored (B–C, Fig. 2).

Blood samples taken beyond 72 h indicated that the blood acid-base status was

maintained at this new steady state for the duration of dormancy. The equilibrium was occasionally upset, however, by transient hyperventilation brought on by disturbances to the animal during blood sampling. Arousal for more than a few min invariably led to a marked respiratory alkalosis along an elevated whole body buffer line (Fig. 2). Usually within 30 min following the sample, the hyperventilation subsided and the toads appeared dormant once again. If subsequent samples were possible without causing the animal to hyperventilate, it was clear that the equilibrium at point C was re-established.

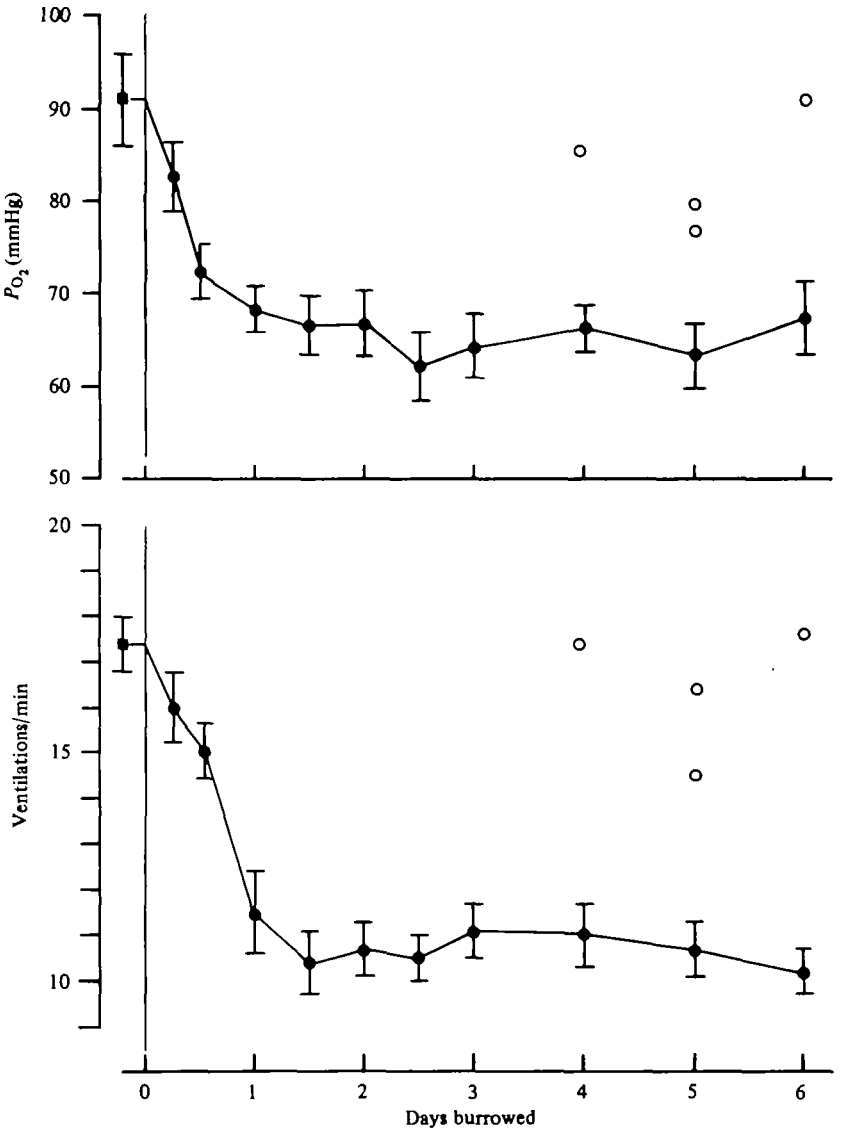


Fig. 3. Mean (±S.E.M.) measurements of arterial blood $P_{O_2}$ and ventilation frequency before (■) and as a function of time following burrowing (●) of *Bufo marinus* ($N$ = 6–10). Vertical line at time zero indicates the onset of burrowing. Open circles = individual hyperventilation values from a single animal. Temperature: 25 °C.

*Blood gases and respiratory frequency*

Arterial blood $P_{O_2}$ measurements were recorded from each blood sample taken during burrowing and their means ($\pm$ S.E.) are plotted as a function of time in Fig. 3. Also shown is the corresponding ventilation frequency at each interval which was extracted from records taken 30 min prior to and following each blood collection.

Over the first 36 h of burrowing, $P_{a,O_2}$ and ventilation frequency progressively declined (Fig. 3) and $P_{a,CO_2}$ always increased as described above (Figs 1 and 2). For the duration of the burrowing period, the blood gases and respiratory frequency were not significantly different from those values recorded after 36 h. As noted earlier, individual data in the longer term were selectively omitted from the means when hyperventilation during arousal caused rapid transients in the blood gas composition. Hyperventilation values and the corresponding $P_{a,O_2}$ levels of a single experiment are shown in Fig. 3.

## DISCUSSION

In resting hydrated *Bufo marinus*, cutaneous $CO_2$ elimination accounts for 76% of the total $V_{CO_2}$ at 25 °C (Hutchison, Whitford & Kohl, 1968) and is governed by the relationship between cutaneous perfusion and external medium flow (Piiper & Scheid, 1975). The substrate which surrounds the skin of the buried toad must, in comparison to water, offer only a limited capacity for the convection of gases and therefore must restrict transcutaneous respiration. In this state, it seems likely that the lung would increase its importance to overall gas exchange and become the principal organ for $CO_2$ removal. When *Bufo* are deprived of adequate cutaneous moisture, this too restricts skin gas exchange and the toads respond by increasing ventilation to regulate their arterial blood $P_{CO_2}$ (Boutilier *et al.* 1979*b*). In contrast, burrowing results in a decline in ventilatory rate which causes $P_{a,O_2}$ to fall and $P_{a,CO_2}$ to rise (Figs. 1 and 3).

Considering the $CO_2$ sensitivity which exists in the respiratory control system of *Bufo marinus* (Macintyre & Toews, 1976; Boutilier *et al.* 1979*a*) it is surprising that the elevated $P_{a,CO_2}$ in the burrowed toad does not stimulate greater pulmonary ventilation. However, the lowered levels of arterial blood $P_{O_2}$ and the fall in breath frequency together suggest that metabolism may have been reduced during burrowing. Hypoventilation and a concomitant bradycardia have been previously reported in several species of fossorial toads and are thought to reflect the animals' lowered metabolic demands during dormancy (McClanahan, 1967; Whitford, 1969; Seymour, 1973). It appears that *Bufo* responds in the same overall fashion since arousal from the dormant state always led to an increase in ventilations and marked reversals of the blood gas concentrations (Figs. 2 and 3). Even so, the fall in ventilations together with a reduced efficiency for extrapulmonary $CO_2$ excretion led to a rise in $P_{a,CO_2}$ and the development of a marked respiratory acidosis. A similar hypoventilatory hypercapnia has been reported to occur when the intertidal crab, *Carcinus maenas*, is transferred from water to air breathing (Truchot, 1975). Perhaps the most striking parallel with *Bufo*, however, is that of the African lungfish *Protopterus* (DeLaney *et al.* 1977); when forming its aestivation burrow, the gills and skin are lost as sites for extrapulmonary $CO_2$ excretion. While buried, the lungfish, like the burrowed toad, does not appear

to regulate $P_{a,CO_2}$ by changes in breathing and a respiratory acidosis develops despi[te] a reduction in metabolism.

Even though lung ventilation falls (Fig. 3) the burrowed toad regulates its arterial blood pH by adjusting the circulating levels of plasma [$HCO_3^-$]. The respiratory acidosis is progressively compensated for by a rise of buffer base concentration (principally $HCO_3^-$) at a near constant $P_{CO_2}$ in the blood (B–C, Fig. 2). Presumably, this compensation involves either the mobilization of fixed carbonates (Sulze, 1942; Simkiss, 1968) or the excretion of hydrogen ions from the plasma, but the underlying mechanisms of this active process in *Bufo* have not been investigated. When steady-state conditions are reached (point C, Fig. 2) the acidosis is fully compensated for by a new equilibrium ratio of $HCO_3^-$ to $P_{CO_2}$ at the same pH as the non-burrowed animals. An increase in breath frequency during arousal (Fig. 3) disrupts this new steady state and results in a marked decline in $P_{a,CO_2}$ which causes pH to rise (Fig. 2). A similar hyperventilatory and blood acid-base response occurs when aestivating lungfish are aroused in their subterranean cocoon (DeLaney *et al.* 1974, 1977). There have also been reports of dormant fossorial toads (*Scaphiopus*) reacting to mild ambient disturbances with bursts of breathing movements (McClanahan, 1967; Seymour, 1973). These data give further evidence that the dormancy seen in amphibious vertebrates is only shallow in comparison to the deep torpor of mammalian hibernation (Hudson & Bartholomew, 1964). In periodic burrowers such as the toad, a light dormancy would allow for rapid arousal soon after moisture conditions on the surface became favourable for emergence. We have in fact observed dormant *Bufo marinus* to emerge from its sand burrow within a few minutes after the surface sand has been wetted with a light sprinkling of water.

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