Dermal bone in early tetrapods: a palaeophysiological hypothesis of adaptation for terrestrial acidosis

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The dermal bone sculpture of early, basal tetrapods of the Permo-Carboniferous is unlike the bone surface of any living vertebrate, and its function has long been obscure. Drawing from physiological studies of extant tetrapods, where dermal bone or other calcified tissues aid in regulating acid–base balance relating to hypercapnia (excess blood carbon dioxide) and/or lactate acidosis, we propose a similar function for these scultured dermal bones in early tetrapods. Unlike the condition in modern reptiles, which experience hypercapnia when submerged in water, these animals would have experienced hypercapnia on land, owing to likely inefficient means of eliminating carbon dioxide. The different patterns of dermal bone sculpture in these tetrapods largely correlates with levels of terrestriality: sculpture is reduced or lost in stem amniotes that likely had the more efficient lung ventilation mode of costal inspiration, and in small-sized stem amphibians that would have been able to use the skin for gas exchange.

Keywords: early tetrapods; basal tetrapods; dermal bone; acidosis; hypercapnia

1. INTRODUCTION

The first tetrapods of the Late Devonian (385–365 Ma) were semi-aquatic forms, possessing lungs inherited from their fish ancestors [1]. The transition to life on land would have imposed several new physical constraints, including the absence of water’s buoyancy to counteract gravity, the risk of dehydration and the need to perform all respiratory gas exchange via the lungs or skin. The emergence of tetrapods has been related to low atmospheric oxygen levels in the later Palaeozoic (14–16% O₂ [2]), which would have severely limited oxygen availability in water owing to its low solubility [1]. Lungs would have been advantageous under these conditions, even for an aquatic animal gulping air; even relatively hypoxic air has many times more oxygen than a similar volume of normoxic water. However, the respiratory physiology of extant vertebrates strongly suggests that a terrestrial lifestyle would have been disadvantageous for these early tetrapods (both stem forms basal to the extant tetrapod lineages, and early members of the stem amniote and amphibian lineages) with regard to excretion of CO₂, even though Late Devonian and Early Carboniferous CO₂ levels were relatively low (0.3–0.35% [2]). Possible solutions to CO₂ excretion in early tetrapods have been extensively debated [3–8]: here we propose a novel mechanism by which these tetrapods might have tolerated CO₂ accumulation on land, relating to their integumental structure.

The integument of early tetrapods differed from that of earlier tetrapodomorph fish and of extant tetrapods, by the dermal bones of skull and pectoral girdle that were often highly scultured (or ornamented) and/or thickened; there is as yet no entirely satisfactory explanation for this morphology [9–12]. Physiological studies on extant tetrapods lead us to the hypothesis that mineralized tissues, particularly scultured dermal bones, may have functioned to buffer CO₂ and metabolic acids that would have accumulated in these early tetrapods during prolonged excursions onto land. We detail below the physiological problems entailed in CO₂ loss on land. Additionally, we note that terrestrial tetrapods would face greater exertion (and hence greater generation of metabolic CO₂) during locomotion on land than in the water, due to the higher costs of terrestrial locomotion [13].

This paper is speculative in its approach: at present it is impossible to confirm or refute our hypothesis with histological studies of fossil vertebrates, although future work on extant vertebrates may yield anatomical or chemical signatures that can be applied to fossils. Meanwhile, we demonstrate here that our hypothesis is strongly supported by the observed dermal morphology in the diversity of early tetrapods in correspondence with their presumed ecologies (terrestrial or aquatic), as determined by other anatomical features.

2. THE VERTEBRATE INTEGUMENT

Early tetrapods possessed various types of dermal mineralized elements [6,14–18]. The heavy dermal cover of some of these tetrapods, bearing a sculptured surface of pits and ridges, may be considered to be osteoderms, similar to those seen today in some reptiles and frogs [19]; but these can be distinguished from thinner,
overlapping dermal scales in pockets of the dermis [20] that are an ancestral tetrapod feature [16,17,21]. Dermal bone does not block interaction between the dermis and the superficial integument because blood vessels run through it to the epidermis [22].

Many early tetrapods possessed dermal bones of the skull and pectoral girdle with an external (superficial) surface heavily sculptured by pits, ridges and furrows. The microanatomy of these dermal bones shows that they were extensively vascularized, with blood vessels opening to the bone surface within the sculptural pits and furrows [9,12]. The osteoderms of many early tetrapods have basically the same structure [18]. This extensive dermal cover is often referred to as ‘dermal armour’.

Various hypotheses exist for the presence of sculptured dermal bones in early tetrapods: cutaneous respiration [9,10,23], strengthening adaptation [24], protection from desiccation [25], mechanical protection of the soft-tissue dermis including vessels and nerves [11,12] and thermoregulation [12,26]. The fact that this dermal sculpture is always associated with a high degree of vascularization indicates that this morphological feature certainly had a physiological significance. In contrast to sculptured bones, the smooth or weakly sculptured dermal bones of other early tetrapods are normally less vascularized in the superficial (external) part, and bear a lower number of vascular openings on their surface. We believe that our hypothesis adds a new perspective on the evolution of dermal sculpturing that does not compete or conflict with those previously proposed.

3. CARBON DIOXIDE ELIMINATION IN VERTEBRATES

(a) Extant vertebrates

There is a profound difference between modern fishes and most modern tetrapods in CO₂ elimination and acid–base balance, both in how CO₂ is excreted and in how decreases in body fluid pH are managed. A fundamental shift in these physiological processes must have occurred early in tetrapod evolution, at least by the common ancestor of modern amphibians and amniotes. Our interest here is in how this change might have occurred, and whether early tetrapods used mechanisms that were different from those seen in extant forms as an intermediate condition.

Most fishes have little difficulty eliminating CO₂ [27], and teleosts rely heavily on the gills for physiological mechanisms of pH compensation from acidosis caused by excessive CO₂ accumulation [28]. Because there is approximately 15 times more O₂ in air than a similar volume of water (estimated at 25°C, 1 atm), relatively large gill ventilatory volumes are required (i.e. a high convection requirement) to extract enough oxygen to meet metabolic needs. The result is that nearly all of the CO₂ is washed out of the venous blood at the gills such that a typical fish has CO₂ partial pressure (Paco₂) of the blood of around 2–5 mmHg. Conversely, because of the abundance of oxygen in air, reptiles (i.e. ectothermic amniotes) ventilate their lungs at a comparatively low rate (i.e. a low convection requirement) in comparison with the rate of gill ventilation: thus they have higher blood Paco₂ values between 10 and 30 mmHg, depending on temperature (see [29] for review).

Extant amphibians (lissamphibians) are usually bimodal breathers, relying on buccal pumping to ventilate their lungs as well as a thin, highly vascularized skin for gas exchange, which augments CO₂ elimination. As a result, their blood PCO₂ values are lower than reptiles but higher than fishes, usually between 5 and 15 mmHg. The relative importance of the lungs and skin for eliminating CO₂ depends on factors such as temperature, metabolic rate and season; some small salamanders lack lungs, with all metabolically produced CO₂ eliminated transcutaneously [30]. Extant lungfishes, while they do eliminate some CO₂ by aerial respiration, principally rely on the gills and skin for this purpose even if some species (i.e. the lepidosirenid lungfishes Protopterus and Lepidosiren) require the lung for oxygen uptake [31].

(b) Limits on carbon dioxide elimination in early tetrapods

It is unlikely that the early tetrapods were lissamphibian-like bimodal breathers: they probably had a skin more like that of modern amniotes, albeit not as heavily keratinized as in most reptiles [32], and thus their skin was unlikely to have served as an important avenue for eliminating CO₂. Additionally, the larger body size of many taxa (in comparison with modern amphibians; up to 1 m in length) would result in greater CO₂ accumulation because of surface-area to volume constraints.

Packard [7] proposed three alternative hypotheses for CO₂ elimination in early tetrapods. First, that these tetrapods retained their gills and excreted CO₂ upon returning to the water [6]. Gills are retained in Devonian tetrapods [1] and many aquatic anamniote tetrapods in the Permo-Carboniferous and Triassic also retained fish-like internal gills [33]. Thus, all gill-retaining tetrapods appear to have been primarily aquatic forms: retention of gills does not seem to have been an option for the more terrestrial taxa.

Second, that early tetrapods had amniote-like high rates of ventilation to ‘breathe off’ more CO₂; however, their ribs were immobile and costal ventilation would have been impossible [8]. A third hypothesis, that the ability to tolerate high levels of CO₂ evolved before the transition to land (see also [34,35]), will be discussed later.

(c) Defending blood pH when carbon dioxide elimination is impaired

When the ability of an animal to eliminate CO₂ is impaired, CO₂ accumulates in the animal as carbonic acid, which dissociates to form HCO₃⁻ and H⁺ to decrease body fluid pH. While the actual handling of the CO₂ by different tissues can vary, the end result is a net accumulation of protons in the body fluids, including the extracellular fluid. If the accumulated CO₂ originates from the respiratory processes occurring at the cellular level, such as insufficient ventilation, it is known as respiratory acidosis. A regulated increase in blood HCO₃⁻ in response to any kind of acidosis is referred to as compensatory metabolic alkalosis.

As mentioned previously, air-breathers have higher blood PCO₂ levels than water-breathers because of the lower convection requirement of their respiratory medium (air). In order to prevent this CO₂ from acidifying their blood, tetrapods have evolved the ability to maintain higher blood HCO₃⁻ levels. In amniotes, regulation
is accomplished by the kidney, while in amphibians regulation also occurs in the skin and urinary bladder [36]. Lungfishes, which can breathe in both water and air, routinely experience respiratory acidosis when gill ventilation is compromised, such as when aestivating. However, the African lungfish (Protopterus) cannot fully compensate its blood pH and shows only small changes in blood HCO$_3^-$ during aestivation [37]. The South American lungfish (Lepidosiren) shows incomplete pH compensation and no increase in blood HCO$_3^-$ when subjected to aquatic hypercapnia, even when its gills can be ventilated [38]. Thus, extant lobe-finned fish appear to have limited physiological capacities for acidosis regulation.

Modern amphibians, which breathe primarily air but also may exchange gases across the skin when submerged in water, are remarkably similar to lungfishes in that they also show either incomplete or no change in blood HCO$_3^-$, and show incomplete pH compensation with respiratory acidosis [36]. This leads us to conclude, by the application of the extant phylogenetic bracket, that early tetrapods likely shared these physiological limitations with modern air-breathing fish and amphibians, and thus also had a limited capacity to defend their extracellular pH in the face of limited CO$_2$ elimination.

Any early tetrapod with limits on CO$_2$ elimination from basal metabolism alone would have even more difficulty doing so when metabolic rate, and therefore CO$_2$ production rate, was elevated, such as during activity. The situation would be compounded by the higher energetic costs of activity on land, compared with water [13], which would have resulted even greater CO$_2$ accumulation and respiratory acidosis. It is also likely that the higher metabolic demands on land might have frequently exceeded the ability of the cardiorespiratory system to deliver oxygen to active muscles, so that terrestrial locomotion probably resulted in lactic acid production from anaerobic metabolism. With a limited capacity to compensate extracellular pH under conditions favouring respiratory and probably metabolic acidoses, early tetrapods would have needed to rely on some other buffering means to minimize acidosis and maintain activity.

**d) Mineralized tissue as a pH buffer in vertebrates**

Overwhelming evidence from recent physiological studies supports a role for mineralized tissue in the buffering of metabolic and respiratory acidosis in ectothermic tetrapods, including frogs [39–41], turtles [42,43] and caiman [44]. Terrestrial frogs subjected to hypercapnia will mobilize calcium carbonate deposits in their endolymphatic lime sacs [39,40], and they also use this source to buffer lactic acidosis from anoxia and exercise [41]. Endochondral bone can also buffer lactic acidosis in amphibians [41] and turtles [45], although some bones, such as the pelvis, scapula and fibula, have been shown to be less effective [45]. Even the turtle’s skull, which is dermal bone, does not buffer lactic acidosis as effectively as the shell. A lesser degree of vascularization is most likely the limiting factor in each of these cases.

Turtles show non-renal increases in plasma concentrations of calcium, magnesium and bicarbonate with hypercapnia, which is direct evidence that bone buffers respiratory acidosis in reptiles [42]. The mechanisms by which bone buffers acidosis in reptiles and amphibians appear to involve passive exchange of ions between the bone mineral matrix and the extracellular fluid. Protons generated metabolically or from carbonic acid (from carbon dioxide) exchange with calcium and magnesium in bone, causing the latter to accumulate in the extracellular fluid.

There have been no studies in reptiles and amphibians that suggest a clear histological signal for detecting previous bone buffering episodes, and it is debatable whether one might be able find that would not have a high rate of false negatives. Studies in turtles show that bone contains so much mineral that even under the most extreme lactic acidosis (plasma lactate = 150 mM), relatively large releases of extracellular calcium and magnesium from bone (46 mM and 12 mM, respectively), have no detectable effects on bone mineral content [46]. Thus, it remains unlikely that we would be able to detect a histological signal for this type of physiology in extinct tetrapods.

4. DISCUSSION: MINERALIZED-TISSUE-BUFFERED ACIDOSIS IN EARLY TETRAPODS

We propose that the sculptured dermal bones of early tetrapods, associated with their high degree of vascularization, functioned to buffer the respiratory acidosis that would have resulted from an increased duration on land. These animals likely would have lacked adequate means for CO$_2$ elimination, such as the capacity to achieve the high ventilation rates made possible by costal aspiration, the ability to lose significant amounts of CO$_2$ via the skin, or the kidney function necessary to increase blood HCO$_3^-$ concentrations to levels required to fully compensate for respiratory acidosis.

The physiology of some extant terrestrial frogs is especially illuminating here. Lissamphibians usually lack integumental dermal bone; yet the importance of buffering CO$_2$ is demonstrated by the use in frogs of both endochondral bone calcium deposits and calcium deposits in their endolymphatic sacs to compensate both respiratory acidosis, caused by elevated CO$_2$ levels, and lactic acidosis, caused by anoxic submergence and exercise. No such role has been proposed for the osteoderms seen in some frogs, but we note that such species [19] are primarily larger, terrestrial forms (H. Astley 2011, personal communication), which are precisely those forms that would experience difficulties in CO$_2$ loss.

The pattern of dermal sculpture and of possession of osteoderms in early tetrapods largely accords with our hypothesis that these integumentary structures would have been important for buffering acidosis on land, and that CO$_2$ loss was a significant problem for these animals. Below we detail various predictions that can be made if our hypothesis is correct, and if the data support the hypothesis.

It is important here to note that the lifestyle of early tetrapods is often difficult to assess (see discussion in [12]). Although the presence of lateral line sulci clearly shows that the animal was mainly aquatic, the absence of such sulci on the bone surface does not necessarily indicate that the lateral line organ was not present in the skin [47]. Further indicators of an aquatic lifestyle are ossified branchial arches and/or branchial dentition [33]. Terrestrial taxa usually have long and robust extremities, and the trunk and tail are rather short. In primarily aquatic taxa, in contrast, the trunk is often elongate, the limbs short and the tail is developed as a...
long propulsive organ. Furthermore, the overall degree of ossification of endochondral bones in the postcranial skeleton is often much higher than in aquatic taxa [48]. These criteria are used here to designate early tetrapod taxa as primarily 'aquatic', 'semiterrestrial' or 'terrestrial'.

The hypothesis that sculptured dermal bones are a response to terrestrial hypoxia, and that they had the function of buffering acidosis, can be tested by predictions as to the correlation of the degree of dermal sculpturing with an aquatic or terrestrial lifestyle.

**Prediction 1.** Terrestrial early tetrapods have a more pronounced dermal sculpture than primarily aquatic forms. (Note here that terrestriality was derived independently numerous times within these tetrapods, and that some formerly more terrestrial forms became secondarily more aquatic.)

**Observations.** In tetrapodomorph fishes like Eusthenopteron, Elpistostege and Panderichthys, dermal sculpture is weakly developed and consists of low tubercles and vermiculate ridges [49–51]. In the Devonian stem tetrapods, dermal sculpture is more pronounced than in their fish-like relatives and is rather irregular. Although these forms were primarily aquatic, they were certainly already capable of land excursions and periodically exposed to air [26]. In the basal-most post-Devonian tetrapods, the whatcheeriids, dermal sculpture is almost completely absent in Whatcheria, which can be interpreted as an aquatic animal [52]. In contrast Pederpes, which is regarded as more terrestrial than Whatcheria, bears dermal sculpture (although rather weakly developed) [53]. Within temnospondyls (stem amphibians), there is a pronounced trend for largely terrestrial forms like eryopids and dissorophids to have coarse, irregular sculpture [11,12]. In primarily aquatic temnospondyls like stereospondylomorphs, sculpture is not absent, but is normally not as pronounced (ridges and tubercles are lower), and is more regular than in terrestrial forms.

Tetrapods that do not fit this prediction include the various forms that appear to be aquatic (possessing lateral line sulci) yet that possess dermal armour. These include baphetids, colosteids (both stem tetrapods) and plagiosaurids (temnospondyls). Plagiosaurids, which bear pronounced tubercular dermal sculpture and a covering of osteoderms, were flattened-bodied bottom-dwelling forms, which also showed hypertrophic ossification throughout their entire skeleton that would have acted as ballast [54]. The colosteids also had morphological features such as general flattening, and heavy, thickened dermal scales, indicative of bottom dwelling.

**Prediction 2.** Dermal sculpturing would be reduced or lost in stem amniote taxa that showed evidence (from rib morphology) of some degree of costal ventilation (a much more efficient way of losing CO₂ than the buccal exchange, as in lissamphibians. Evidence of dermal sculpturing is seen only in Panoplosaurus, a particularly large microsaur [56]. However, the aquatic diplocaulids have a rather regular sculpture of pits similar to that of stereospondylomorphs [12].

The small terrestrial microsaur retained thin dermal scales, but sculation was lost in most of the amphibia, temnospondyls implicated in the origin of lissamphibians [57], and sculation was reduced in the nect-like amphibians temnospondyls [58]. These forms possibly also had a similarly thin skin for gas exchange as do lissamphibians. Note that the branchiosaurid *Apetosaurus* and the amphibian *Amphibamus grandiceps* have preserved postotic structures interpreted to be calcium-filled endolymphatic sacs, as in some living frogs [59,60], and similar structures can be found in small larvae of the seymouriamorph *Ariekanerpeton* (F. Witzmann 2011, personal observations).

In summary, dermal sculpture is indeed more pronounced in terrestrial early tetrapods. Dermal sculpture is reduced or lost only in those forms that would have been small enough to have been lissamphibian-like bimodal breathers, losing CO₂ through their skin, or in stem amniotes with evidence of costal ventilation.

However, the picture is not always consistent: while the majority of aquatic taxa had less pronounced sculptured dermal bone, a few showed more pronounced sculpturing (e.g. plagiosaurids, colosteids and baphetids), These formed were predominantly bottom-dwelling taxa, with heavily ossified skeletons, and dermal sculpture may have added to this ballast weight. Alternatively, these aquatic forms may have specialized for hypercapnic or hypoxic aquatic environments, as indicated by their flattened body forms, and their integumental features represented an adaptation for carbonic and metabolic acid buffering in this type of environment (see also [35]).

Dermal sculpture occurs also in a few terrestrial basal amniotes, such as most parareptiles (e.g. pareiasaurs and lanthanosphorids), and in the basal eureptile captorhinomorphs (e.g. romeriids [61]). It is possible that these forms were not yet as efficient at costal ventilation as later amniotes. Although derived terrestrial stem amniotes such as Diadectes appear to lack dermal armour, the post-cranial modifications in early amniotes (e.g. the more consolidated ankle joint) may have resulted in a greater degree of mobility on land, and hence a greater potential for acidosis, especially in larger forms such as these para-reptiles. However, sculptured dermal bones are lacking in the synapsids and in more derived eureptiles.

**5. CONCLUSIONS**

Studies on living tetrapods show that dermal bone has the physiological capacity to buffer acidosis caused by the build-up of CO₂ and lactic acid. We propose that a key function of the dermal bone sculpture associated with strong vascularization that is characteristic of early, basal tetrapods was to buffer CO₂-induced acidosis and perhaps also lactic acidosis induced by periods of anaerobic activity, thus allowing these animals to maintain homeostasis for longer periods of time while on land.

The longer an individual could survive in this new environment, the more it would be able to exploit
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terrestrial resources. The pattern of dermal sculpture in
the various early tetrapod taxa can be shown to corre-
spond with patterns of terrestrial versus aquatic habits,
with terrestrial forms generally having more pronounced
sculpture. Terrestrial forms with reduced sculpture were
either small stem lissamphibians, where CO2 loss could
be cutaneous, or stem amniotes with morphological evi-
dence of amniote-like costal ventilation.

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