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23. High ground-water P<sub>CO<sub>2</sub></sub> values were also measured in the Toolik Lake watershed in summer 1990 (up to 46,500 ppmv). Such high P<sub>CO<sub>2</sub></sub> values are attributed in part to permafrost conditions; ground waters are usually confined by permafrost to shallow (50 cm) organic-rich soil layers, where they can accumulate CO<sub>2</sub> produced by plant and microbial respiration.
24. The <sup>14</sup>C activity of an A.D. 1950 sample is expressed as 100% modern. Dissolution of marine carbonate outcrops would tend to depress the <sup>14</sup>C activity of CO<sub>2</sub> in the river, but such rocks are scarce in the Kuparuk basin. Carbon-14 activities are from Beta Analytic (#31808 and #31809).
25. The basin area is 143 km<sup>2</sup>, and the surface area of the river is 0.12 km<sup>2</sup> (11). Ground-water P<sub>CO<sub>2</sub></sub> is taken as 8315 ppmv. We assumed that the river output had an average DIC concentration of 308 μmol liter<sup>-1</sup> (11). This value includes CO<sub>2</sub>(aq) plus bicarbonate and therefore is conservative with respect to the contribution of CO<sub>2</sub> from the terrestrial landscape.
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## Putative Skeletal Neural Crest Cells in Early Late Ordovician Vertebrates from Colorado

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Two vertebrates from the Colorado Formation are known to have acellular bone and dentine, tissues found in heterostracan fishes and proposed as the primitive exoskeleton. High-resolution optical microscopy and scanning electron microscopy indicate that a third vertebrate is represented by a quite distinct exoskeleton of denticles, with many cell and cell process spaces throughout, tissue that resembles that of osteostracans more than heterostracans. Cellular bone coexists with cellular dentine in this Ordovician vertebrate, demonstrating that these skeletal tissues are as old as acellular bone. Both are proposed to come from neural crest because denticles are considered homologous with teeth.

THE EARLIEST STRATIGRAPHIC occurrence of bone tissue of a type containing interconnected cells (osteocytes situated in lacunae) is central to any discussion of the evolution of bone with a role in mineral homeostasis (1), or in protection or insulation for sensory organs, or for mechanical support (2). It has also been proposed that the embryonic cell origin of the exoskeleton is from the neural crest and is a vertebrate innovation (3–6). Early vertebrate fossils with well-preserved histology are rare, but those from the Harding Sandstone in Colorado, dated as early Late Ordovician and known for almost 100 years, are the earliest of this kind (7–13). Two jawless fishes (agnathans) are recognized in these fossils (known only by their generic names, *Astraspis* and *Eriptychius*) based on differences in the shape and histology of their tubercles (odontodes fused to bony plates). However, it is accepted that the ex-

tensive exoskeleton in both consists of acellular bone (aspidin, with no evidence of cell lacunae), a feature of heterostracan fishes.

In contrast, uncertainty and controversy have accompanied the few descriptions of cellular bone in skeletal fragments of another vertebrate in this fauna (10, 14–17). With greater resolution of tissue microstructure, it can be seen that cell lacunae with interconnections are present in the skeletal remains of the undetermined third vertebrate (10). This exoskeleton is quite distinct in this character (the type of bone and dentine) from that of *Astraspis* and *Eriptychius*. Earlier reports (14, 15, 18) had suggested the presence of cellular bone but had either not illustrated it, or had not said to which part of the skeleton it belonged. However, it is emphasized here that the tissue with cell spaces is in the denticle of the exoskeleton. Such dermal denticles are considered to be homologous with teeth (19) and to share developmental origins. It is generally accepted that the mesenchymal component of the teeth (in gnathostomes) originates from

neural crest cells (20) and that it produces the dentine and probably the bone of attachment. New information on these tissues in this early vertebrate will, therefore, contribute to understanding the role of neural crest in the evolution of bone and dentine.

The record of bone with cells now appears to be as old as that of bone without cells, 450 million years before the present (BP). In addition, the characteristic histology of dentine and bone could also indicate the presence of an osteostracan, making this their earliest recorded occurrence (21). Although the oldest vertebrates recorded are *Arandaspis* and *Porophoraspis*, in the Stairway Sandstone of central Australia (early Llanvirn, 470 × 10<sup>6</sup> BP) (12), they are solely impressions in the sandstone, with no skeletal remains. Another older fossil, *Sacabambaspis* from the Anzaldo Formation, Bolivia (early Caradoc) (22), slightly younger than *Arandaspis*, retains skeletal material, but it has poorly preserved histology.

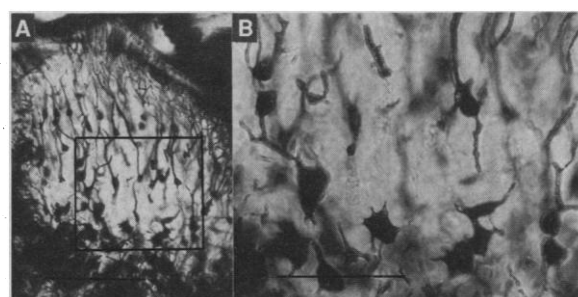
Newly prepared ground sections (60 to 80 μm) of bone-bed material from the Harding Sandstone Formation, near Canon City, Colorado, were examined with phase contrast and Nomarski optics. Consecutive, polished, etched section surfaces were examined in the scanning electron microscope. The formation has a high density of vertebrate skeletal remains, the dominant species are *Astraspis desiderata* Walcott and *Eriptychius americanus* Walcott (7–11, 16). However, there are also many examples in the sections of a denticulate exoskeleton with a histology, distinct from that of *Astraspis* and *Eriptychius*. This exoskeleton is characterized by two things: (i) the tissue of the denticles has many clearly defined lacunae (spaces for cell bodies), and interconnecting canaliculi (spaces for extensive cell processes) (Fig. 1,

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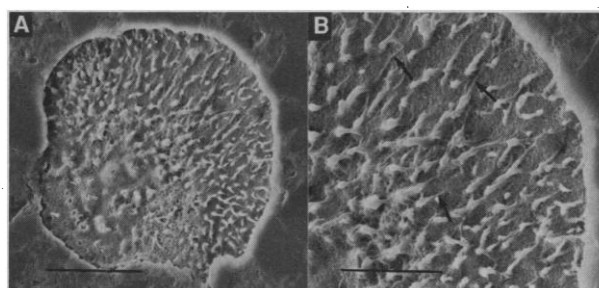
A–D), implying the presence of a network of cells within the mineralized tissue; (ii) the denticles are always found separate from any plates (tesserae) of dermal bone, implying that the exoskeleton consisted only of separate denticles (odontodes). This is different from the exoskeleton of *Astraspis* and *Eriptychius*, in which the dentine tubercles (odontodes), with few or no odontoblast tubules, are superimposed on acellular bone tesserae (10, 11). The details of the histology in this third vertebrate are considered to be highly significant both for the evolution of skeletal types in early vertebrates, in particular for the debate about whether dentine or bone is the more primitive vertebrate tissue (6, 13, 23), and for phylogenetic relations amongst agnathans.

The denticles are small (range, 0.18 to 0.42 mm), the main body slanted on the base, with additional crests or ridges as part of a sculptured, refractile outer layer, separated by a distinct undulating junction from the body of the dentine (Fig. 1, A–C). These denticles consist of three distinct tissue types; basal tissue with cell lacunae with many branches and irregular interconnecting canaliculi (Figs. 1, C and D; 2B); central tissue with pear-shaped lacunae, each with polarized tubules directed toward the surface but interlinked by many branches as a network of fine tubules (Figs. 1, A and B; 2A; 3, A and B); and the superficial, refractile sculptured layer with tubules for cell processes terminating from the tubules in the layer below (Figs. 1, A–C; 2A). These three tissues are interpreted as bone with osteocytes, mesodentine with odontocytes, and either enameloid or enamel. This particular tissue combination is one described for

**Fig. 2. (A)** Brightfield of part of one denticle shows cell spaces and tubules containing a dark infilling material. There are polarized cells in the middle dentine, a network of tubules in the outer dentine, and irregular cell spaces and canaliculi in the basal tissue. Scale bar, 50  $\mu$ m. **(B)** The boxed area in (A) showing that odontocytes at the top were linked through canaliculi with osteocytes in the basal tissue. Scale bar, 25  $\mu$ m.



**Fig. 3. (A and B)** Scanning electron micrographs of polished, etched (1% HCl/min) section surface, cut obliquely through the outer, middle region of a denticle, very similar to that in Fig. 2. The greater etch depth of the biomineral leaves the infilling matrix of the cell derived spaces proud of the biological matrix. This shows the large number of spaces for cell processes, and the narrow diameter of a few of them (arrows). Scale bars in (A), 30  $\mu$ m; in (B), 25  $\mu$ m.

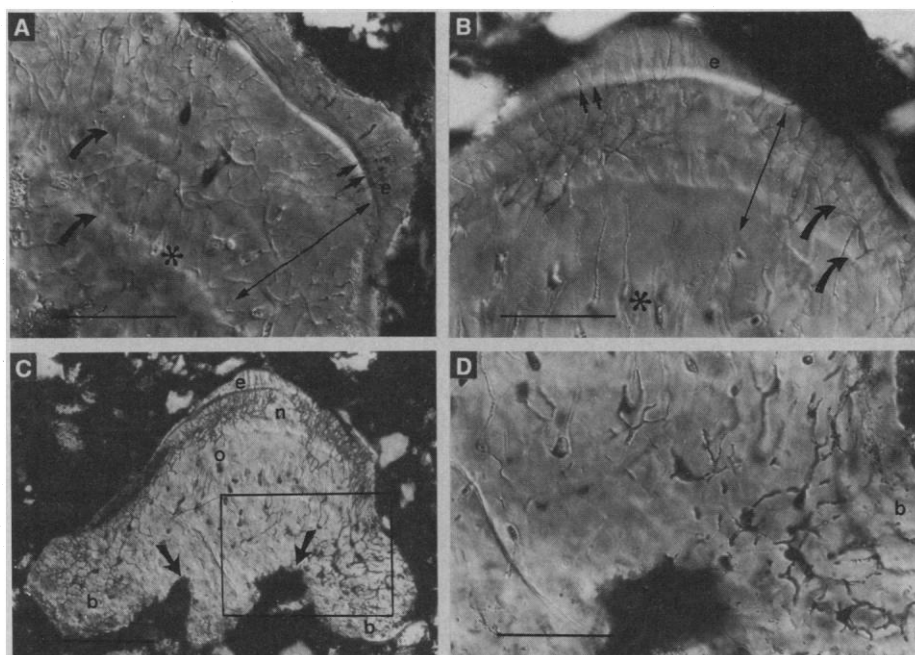


the tubercular ornament in some of the earliest genera of osteostracans, tremataspids, from the Late Silurian [Lower Ludlow, (9, 24)]. However, in these younger forms the dentine tubercles are supported by an extensive plate of spongy bone, itself also with enclosed cells. The resolution of cellular detail achieved in this study confirms that this type of exoskeleton undoubtedly represents that of another vertebrate, distinct from *Astraspis* and *Eriptychius*, and it corresponds with that described by Denison as "vertebrate indeterminate A" (10).

Current ideas on the earliest time at which

species possibly related to the osteostracan agnathans lived are challenged by these histological observations on fossils that occur in the early Late Ordovician. There are other early vertebrates, thelodontids and sharks, with an exoskeleton of separate denticles, considered by some as the micromeric condition that represents the primitive condition for vertebrates (25, 26); however, the thelodonts, of which the earliest occur in the Early Silurian, do not have osteocyte lacunae in the basal tissue, nor odontocytes in the dentine. Other examples are of gnathostomes and are also represented in the Early

**Fig. 1. (A–D).** Photomicrographs with Nomarski differential interference contrast show relief effect in one focal plane (section number H9-1, UMDS). The whole denticle is shown in an orthovertical section (C) with two pulp chambers open at the base (arrows) and basal flanges of osteocytic bone (b). The main body is of mesodentine (o) with cell spaces for odontocytes and a network of fine tubules toward the margins (n). Extensions of the tubules end in the enameloid (e) shown in (A), the superficial part of one denticle, as expanded terminal ends, and in (B) the main crest of the denticle in (C), as finer terminations. A strong demarcation exists between the enameloid and the dentine (double arrow, A); below is a network of fine tubules (double-headed arrow) in the mesodentine, arising from polarized cells below (odontocytes, asterisk). Centripetal growth zones are suggested by the phase differences at the curved arrows (B). The cell spaces and interconnections in the tissue at the basal lateral margins are more irregular and resemble osteocyte lacunae and irregular canaliculi, in a tissue similar to the bone of attachment (b). In (C) the box outlines the field shown in (D). Scale bars in (A, B, and D), 50  $\mu$ m; in (C), 100  $\mu$ m.



Silurian, by placoid scales of primitive sharks, also without cell spaces in the basal tissue and none in the dentine (28), and by scales of acanthodians, in which some bone has cell lacunae and the dentine also has spaces interpreted as those for odontocytes, and a network of fine tubules for interconnecting cell processes [mesodentine, (28)]. New discoveries of early vertebrate tissues are needed before the relationships of the Ordovician vertebrates to the other groups can be resolved.

The developmental basis for the evolution and diversification of the early vertebrate skeletal tissues is discussed by Smith and Hall (6), who look at the role of neural crest cells and the conservation of causal developmental sequences in odontogenesis and skeletogenesis throughout the groups. It is considered to be highly significant that the tissues described here, cellular bone and cellular dentine, are in juxtaposition within the denticle, of what is considered to be an early example of a primitively micromeric skeleton. These observations accord with the hypothesis that the first exoskeletal tissues of both the cranium and the trunk evolved as products of neural crest-derived cells (6).

The following proposals arise from the data reported here: (i) bone tissue with cell lacunae similar to that reported in Early Silurian osteostracans and gnathostomes occurs in the early Late Ordovician, much earlier than has been generally acknowledged; (ii) it is just as probable that the direction of evolutionary change in the exoskeleton was from cellularity to acellularity, as from the acellular tissue to the cellular—the latter has recently been the more accepted view; (iii) the presence in the same denticle, of mesodentine with odontocyte lacunae and attachment bone with osteocyte lacunae, suggests that the cells derived from neural crest gave rise to both dentine with cells and bone with cells, as the first vertebrate exoskeletal tissues. It follows that neither dentine nor bone gave rise to the other in development or evolution. Developmental data, on the neural crest-derived tooth tissues, shows that dentine and attachment bone develop in close association, and the two processes may be so tightly linked that one did not evolve without the other (6).

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## Altered Perception of Species-Specific Song by Female Birds After Lesions of a Forebrain Nucleus

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Female birds that do not normally sing possess brain nuclei associated with song production in males. To determine whether one song nucleus, the caudal nucleus of the ventral hyperstriatum (HVc), acts in conspecific song perception, courtship responses of female canaries to canary and white-crowned sparrow songs were compared before and after HVc lesions. Bilateral lesions of a portion of the HVc resulted in copulation solicitations to heterospecific song as well as conspecific song. Control females continued to respond only to conspecific song. This suggests that the HVc is critical for conspecific song perception in females. Because female canaries do not normally sing, neurons in female HVc must develop response selectivity by a mechanism different from that proposed for male birds in the motor theory of song perception.

**S**UCCESSFUL REPRODUCTION IN SEXUAL animals depends on mating with members of the same species. In many species, females are able to recognize conspecific males on the basis of their species-specific courtship signals. Numerous studies have documented the selectivity of neurons in sensory brain regions for conspecific signal features (1). The role played by brain nuclei in the behavioral recognition of conspecific mating signals by females, however, has received scant attention.

Song production in male songbirds is controlled by a network of brain nuclei (2). In many species females possess the same network of song nuclei as conspecific males, albeit they are of smaller size (3). The presence of these nuclei in the brains of females that do not normally sing is puzzling. Why should these females possess such song nuclei at all? No function for these song nuclei in females has yet been demonstrated empirically.

One possibility is that these song nuclei

play a role in song perception in females (4). In males of several songbird species, neurons in several of these nuclei receive and respond to input from the auditory system (5). Neurons in male song nuclei respond more selectively to conspecific song stimuli than do neurons in purely auditory nuclei. For example, the caudal nucleus of the ventral hyperstriatum (HVc) of males is critical for song production. The HVc receives auditory input from field L, the primary forebrain auditory nucleus. Multineuron clusters in HVc respond preferentially to an individual bird's own song (6). Neurons in field L do not respond selectively to an individual's song, but show more generalized response properties (6).

Previous studies have not shown whether HVc or other song nuclei play a role in conspecific song perception in female birds, as they do in males. To examine this hypothesis, I tested the behavioral responses of female roller canaries (*Serinus canarius*) to playback of conspecific and heterospecific songs, before and after selective lesions of HVc. I found that lesions of part of HVc eliminated the discrimination between these

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