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Remarkable Preservation of Undigested Muscle Tissue Within a Late Cretaceous Tyrannosaurid Coprolite from Alberta, Canada

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Exceptionally detailed soft tissues have been identified within the fossilized feces of a large Cretaceous tyrannosaurid. Microscopic cord-like structures in the coprolitic ground mass are visible in thin section and with scanning electron microscopy. The morphology, organization, and context of these structures indicate that they are the fossilized remains of undigested muscle tissue. This unusual discovery indicates specific digestive and taphonomic conditions, including a relatively short gut-residence time, rapid lithification, and minimal diagenetic recrystallization. Rapid burial of the feces probably was facilitated by a flood event on the ancient coastal lowland plain on which the fecal mass was deposited.

INTRODUCTION

Fossilized soft parts help flesh out our understanding of the anatomy and physiology of ancient vertebrates. Proteinaceous tissues readily decompose, but extraordinary examples of soft-tissue preservation have been recovered from a small number of exceptional fossil Lagerstätten. Fossilized muscle tissues from fish (Dean, 1902; Martill, 1988; Voigt, 1988; Schultze, 1989; Martill, 1990), amphibians (Willems and Wuttke, 1987; Voigt, 1988), and dinosaurs (Kellner, 1996; Briggs et al., 1997; Dal Sasso and Signore, 1998) have been reported. Even more remarkable is the fact that entire organs in a tiny dinosaur (Dal Sasso and Signore, 1998) and finely detailed tissues such as fish gill filaments (Martill and Harper, 1990), pterosaur wing epidermis (Martill and Unwin, 1989), and nucleated frog epidermal cells (Voigt, 1988) also have been fossilized.

These reported examples of preserved soft tissues were observed in the flattened carcasses of small vertebrates—usually in small sporadically distributed patches. Now remarkably preserved muscle and connective tissues have been identified in a different and highly unusual taphonomic setting—within the fossilized feces of a large Cretaceous theropod. Such extraordinary preservation provides information about diet, digestion, and diagenetic conditions. Only one other early paper has reported traces of muscle tissue in much smaller coprolites attributed to carnivorous dinosaurs (Bertrand, 1903) or crocodilians (Abel, 1935), but photographic documentation of the tissues was not provided.

METHODS

Three-dimensional soft-tissue impressions on uncoated fragments of the coprolite were imaged with a LEO 982 field emission scanning electron microscope at the United States Geological Survey in Menlo Park. Gold-coated specimens also were observed on a Philips 525M scanning electron microscope at the University of Colorado, Boulder. Bulk chemical analyses of elements in the coprolite and associated sediment were conducted by Chemex Labs Inc. by XRF (x-ray fluorescence spectroscopy) analysis of metaborate fusions of powdered samples. Percentages of total carbon (through infrared analysis of pyrolysis products) and inorganic carbon (by volumetric measurement of off-gassed carbon dioxide) also were measured, so the amount of total organic carbon in the samples could be calculated.

Thin sections of the coprolite were prepared by embedding fragments of the specimen in epoxy or polyester resin. Slices of the embedded samples were cut with a slow-speed diamond saw, mounted to frosted glass slides with epoxy, ground thin with abrasive silicon carbide paper, and polished with an aluminum oxide polishing compound or diamond paste.

A thin section of the sample (slide DP-1Dh, polished only with aluminum oxide) was carbon-coated and analyzed to determine the cellular-scale distribution of elements within the coprolite. The elemental composition of the sample at specific sites was determined with energy-dispersive (ED) spectrometers on the LEO 982 field emission microscope. Wave-dispersive (WD) spectrometers on a JEOL 8900 microprobe were used to generate 250 μm x 250 μm elemental maps of carbon, phosphorus, calcium, iron, and sulfur of the same carbon-coated slide with a 50 nA beam current at 15 keV.

Geologic Setting and Specimen Description

The coprolitic mass (TMP 98.102.7; Fig. 1) was discovered *in situ* near Onefour, Alberta, Canada, in the middle of the Campanian Lethbridge Coal Zone, a 20 meter-thick, transgressive succession that forms the top of the largely non-marine Dinosaur Park Formation in southern Alber-

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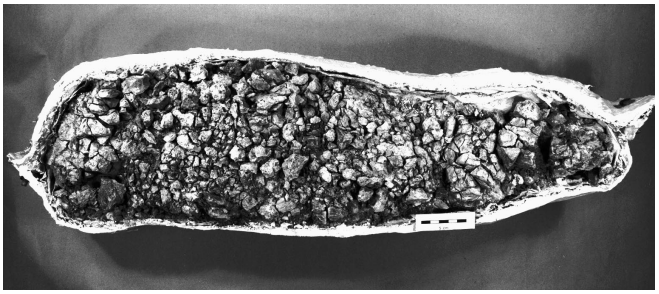

FIGURE 1—Large, phosphatic coprolite from southeastern Alberta. The highly fractured nature of the specimen allows scrutiny of the interior of the lithified fecal mass. Scale bar in cm increments.

ta (Eberth and Hamblin, 1993). The specimen was situated on the contact between a very fine-grained sandstone and an overlying massive carbonaceous shale. The sandstone underlies the specimen and exhibits abundant Cretaceous rooting whereas there is no evidence of rooting in the carbonaceous shale that buried the coprolite. Although the lithified mass is highly fractured, it was recognized as a coprolite by its general appearance, color, and numerous included bone fragments. The presence of lichen on parts of the specimen are a sign of prolonged modern exposure, but the coprolite was discovered before extensive disruption by recent erosion and weathering, and was collected in a plaster jacket to preserve its general morphology.

The large oblong coprolite is approximately 64 cm long and up to 17 cm wide. The uneven topography on the underside reflects a variable thickness ranging from 8 to 16 cm, and suggests that the mass was deposited in a viscous state on uneven terrain. Based on dimensions, the volume of the specimen is conservatively estimated to be approximately 6 liters. The interior of the specimen is brown (Munsell soil color 10YR 5/3), while the exterior has weathered to nearly white (Munsell soil color 10YR 8/1). Bulk chemical analyses (Table 1) show that the coprolite contains high concentrations of phosphorus (32.9%) and calcium (47.7%) relative to the associated sediment (0.96% and 2.09%, respectively; Table 1). The comminuted bone contents, phosphatic composition, and consolidated form demonstrate that the mass is a large carnivore coprolite, similar to the probable *Tyrannosaurus* feces found in the Maastrichtian of Saskatchewan (Chin et al., 1998). The great size and faunal and stratigraphic context of the Onefour specimen also indicate that it was produced by a large tyrannosaurid dinosaur. Three tyrannosaurids are known from the Dinosaur Park Formation: *Daspletosaurus*, *Gorgosaurus*, and *Aublysodon* (Eberth et al., 2001).

This coprolite is unusual because it bears numerous, distinctive three-dimensional structures that are interpreted as fossilized soft tissue. The structures are microscopic to macroscopic (up to 4.4 cm^2) in size, are distributed throughout the coprolitic ground mass, and are characterized by two distinct surface patterns. A fluted pattern is formed by multiple layers of long, parallel fibers, approximately 30 μm in diameter (Fig. 2B). A second surface pattern has a reticulated configuration created by a web of ridges (Fig. 2D, E). This reticulated pattern often is coated with a patchy covering of loosely consolidated, dark-brown material. Although the fluted and reticulated patterns are morphologically distinct, it is apparent that they represent associated tissues because the fluted pattern can be observed through windows demarcated by the raised reticulated network (Fig. 2D, E). Further three-dimensional organization of these structures is revealed by larger patches of preserved tissues that show the parallel fibers (forming the fluted pattern) bundled into narrow rope-like structures (up to 1 mm in diameter) that are encased by the reticulated network (Fig. 2A).

TABLE 1—X-ray fluorescence data of the weight percentage of oxides found in bulk powdered samples of the coprolite and the associated sediment.

XRF analysis	Coprolite	Sediment
Al_2O_3	0.94	12.17
CaO	47.7	2.09
Cr_2O_3	<0.01	<0.01
Fe_2O_3	1.25	3.29
K_2O	0.03	2.09
MgO	0.19	0.89
MnO	0.19	0.03
Na_2O	0.70	1.71
P_2O_5	32.9	0.96
SiO_2	3.35	66.7
TiO_2	0.03	0.50
Loss on ignition	11.61	8.74
Total	98.9	99.1

Petrographic analysis of the three-dimensional structures in this specimen reveals surprising internal detail. A thin section through parallel fibers forming the fluted pattern confirms that cross sections of these fibers correspond to small, rounded structures that are visible in transmitted light. These structures are circular to polygonal in cross-section (Fig. 3A), and appear to be elongate cells. Longitudinal sections verify that they have a long, cord-like morphology, and exceptionally well-preserved examples display fine striations that are orthogonal to their long axis (Fig. 3B).

In thin section, it is apparent that the individual cell-like structures are defined by dark-brown outlines (Figs. 3A–C), which are quite pronounced in some areas (Fig. 3C). Scanning electron microscope ED spectra and microprobe-generated elemental maps indicate chemical differences that correspond to morphological differences. ED spectra of the finely crystalline material in the coprolitic ground mass and cell centers (Fig. 4C) show large phosphorus and calcium peaks and a small fluorine peak that are indicative of a calcium phosphate such as fluorapatite (Reed, 1996; p. 124); the oxygen peak characterizing this sample suggests that this apatite is carbonate fluorapatite. This is similar to an ED spectrum of a bone fragment in the coprolite (Fig. 5A).

In contrast, ED spectra of the demarcating brown material (Fig. 4D) show a high percentage of carbon relative to other elements. Since all spectra were derived from the same carbon-coated slide, the much larger carbon peak characterizing the dark-brown material is significant. The relatively low percentage of oxygen is inconsistent with inorganic carbon compounds (carbonates), and thus indicates organic carbon. ED analyses of small, dark, tubular structures (e.g. Fig. 6A) in the coprolitic ground mass show comparable spectra that also indicate a significant organic carbon component (Fig. 5B).

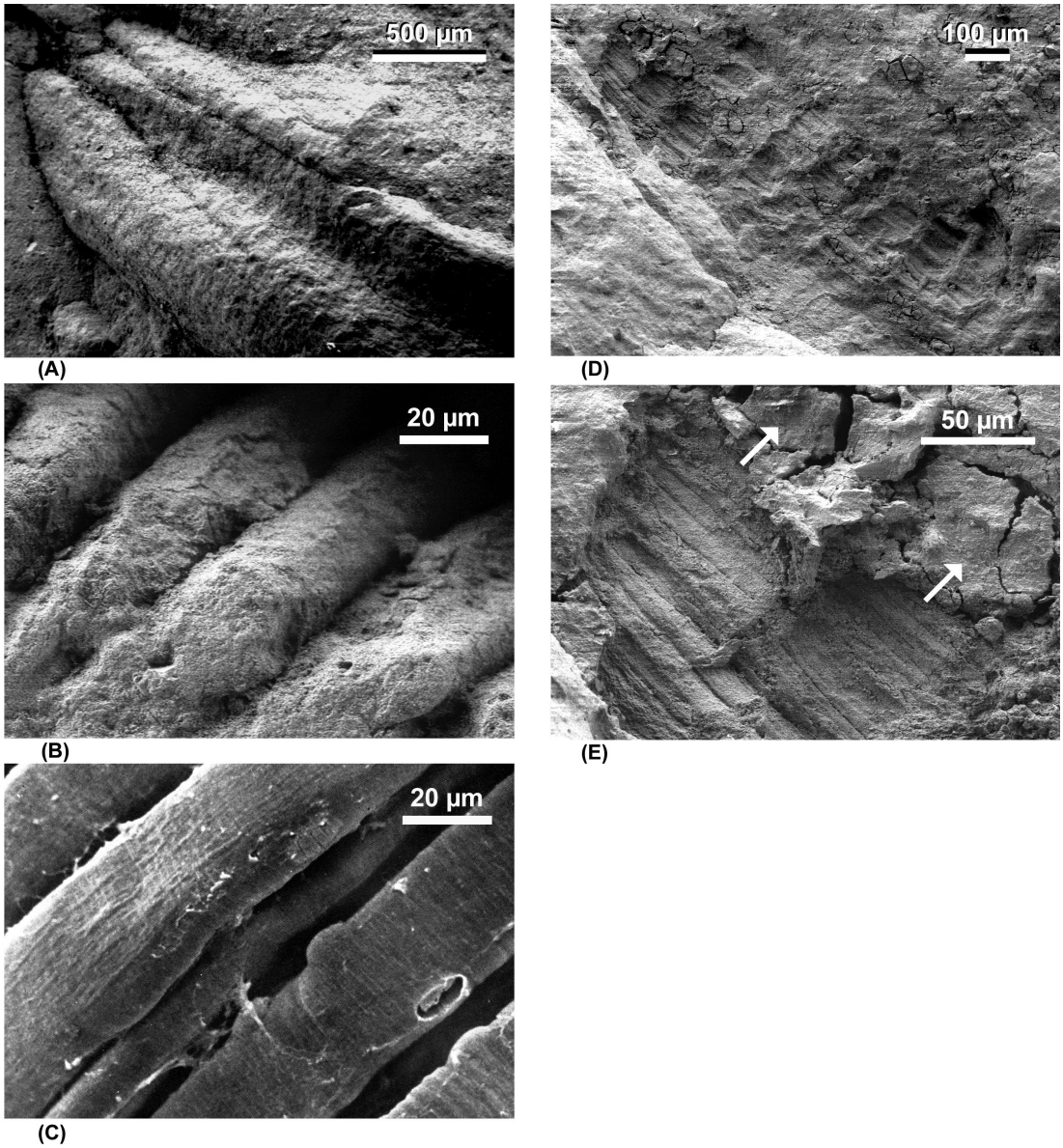

FIGURE 2—SEM photomicrographs showing three-dimensional structure of fossil soft tissues in the coprolite (A, B, D, E) compared with fixed muscle tissue (C). (A) Bundles of tightly packed fluted structures interpreted as fossil muscle cells. Bundles are comparable to muscle fascicles (fragment DP-18D). (B) Close-up of fossilized muscle cells (fragment DP-22Aae). (C) SEM photomicrograph of rat skeletal muscle fiber cells (portion of photo by J. Vial, published in Motta, et al., 1977). (D, E) Distinctive reticulated pattern with elongate muscle fiber cells evident in windows framed by reticulated network (fragment DP-9F). (E) Close-up of upper left corner of (D). Arrows indicate pieces of dark, flaky material. These uncoated coprolite fragments were imaged with a LEO 982 field emission scanning electron microscope.

The microprobe-generated elemental maps of phosphorus (Fig. 4A) and calcium reflect the high concentrations and widespread distribution of calcium phosphate in the sample (the phosphorus elemental map functions as a proxy for calcium phosphate). The elemental map of carbon (Fig. 4B) shows that this element is confined largely to the dark-brown material. This restricted carbon distribution is consistent with the approximately 2.05% total organic carbon found in a bulk sample of the coprolite (Table 2).

TISSUE IDENTIFICATION

It often is difficult to identify dietary components within coprolites because the included digestive residues lack anatomical context and have been degraded by digestion and diagenesis. The size, morphology, and organization of the soft-tissue structures in this specimen, however, are consistent with cells in fresh muscle tissues. Muscle tissues are composed of long, cord-like fiber cells grouped into numerous fascicles. Layers of fibrous connective tissue help maintain muscle structure by investing individual muscle cells, fascicles, and entire muscles. SEM micrographs show that the cord-like, fossilized cells in the coprolite (Fig. 2B) have similar size and morphology as unfossilized muscle-fiber cells (Fig. 2C). A longitudinal view of the fossil cells in thin section reveals subcellular striations (Fig. 3B) that resemble the myofibrillar banding of unfossilized

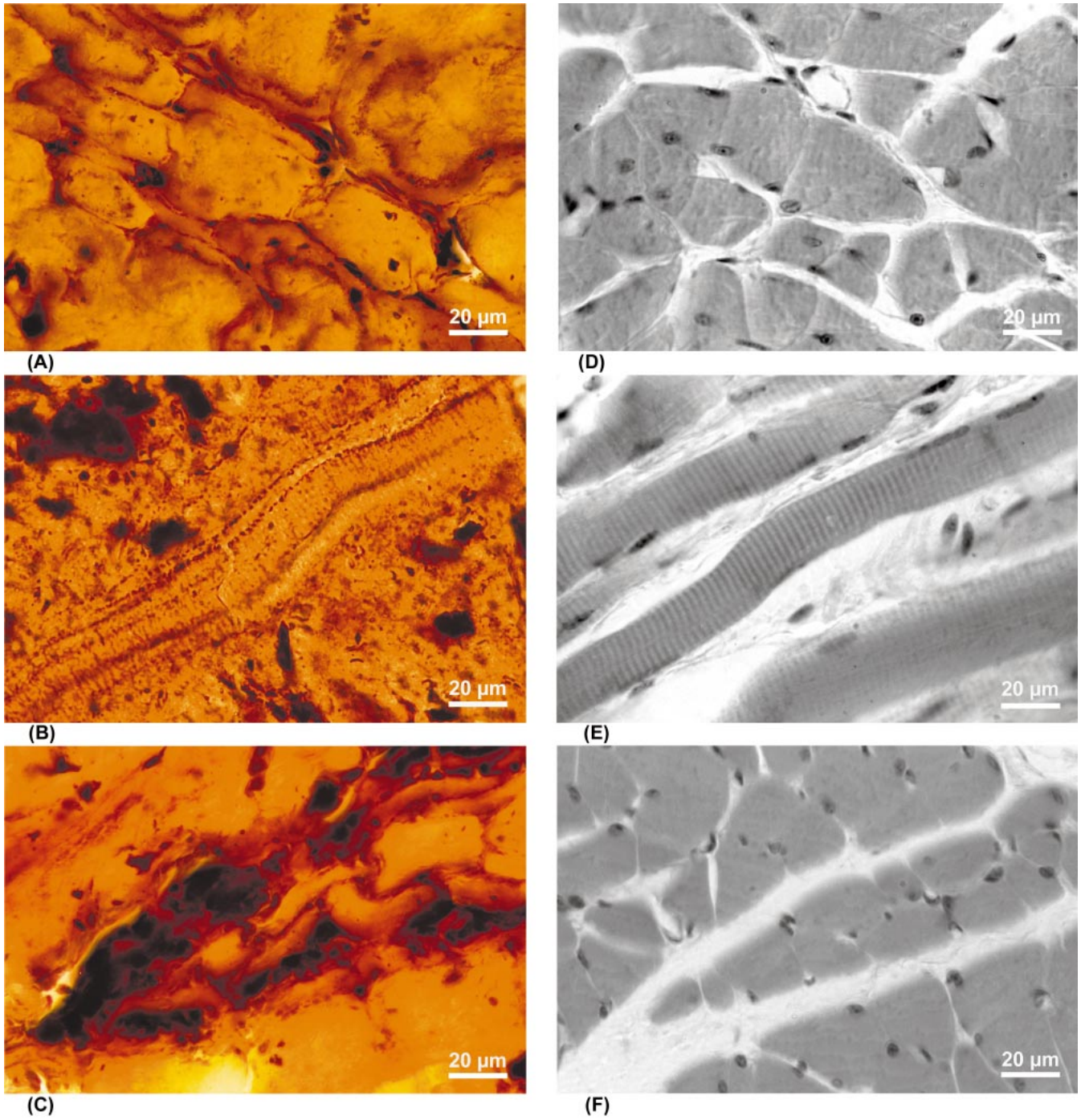

FIGURE 3—Photomicrographs of thin sections of coprolite (A–C) and fixed skeletal muscle tissue from a monkey pharynx (D–F) showing similar morphology. (A) Cross section through fossil cells (Slide DP-1Dc) showing thin membranes and polygonal morphology. (B) Longitudinal section of fossil cells (Slide DP-22Aa-j) with striations equivalent to myofibrillar banding in fixed muscle cells (E). (C) Fossil cells (Slide DP-1Dd) surrounded by pronounced area of dark material that may represent a perimysial boundary between muscle fascicles. (D) Cross-section through monkey muscle cells. (E) Longitudinal view of monkey muscle cells. (F) Fascicle of monkey muscle cells surrounded by thick layer of light-colored perimysium.

muscle fibers (Fig. 3E). Similar striations have been found in the fossil muscle tissue of a Devonian shark (Dean, 1902). The dark material that delineates individual cells (evident in thin section; Fig. 3A) is comparable to endomysial connective tissue (Fig. 3D), and the more pronounced dark layers (Fig. 3C) are consistent with perimysial or epimysial connective tissues surrounding fascicles or whole muscles (Fig. 3F). The bundling of numerous fibers into rope-like structures (Fig. 2A) is also commensurate with the assembly of muscle fibers in fascicles. These

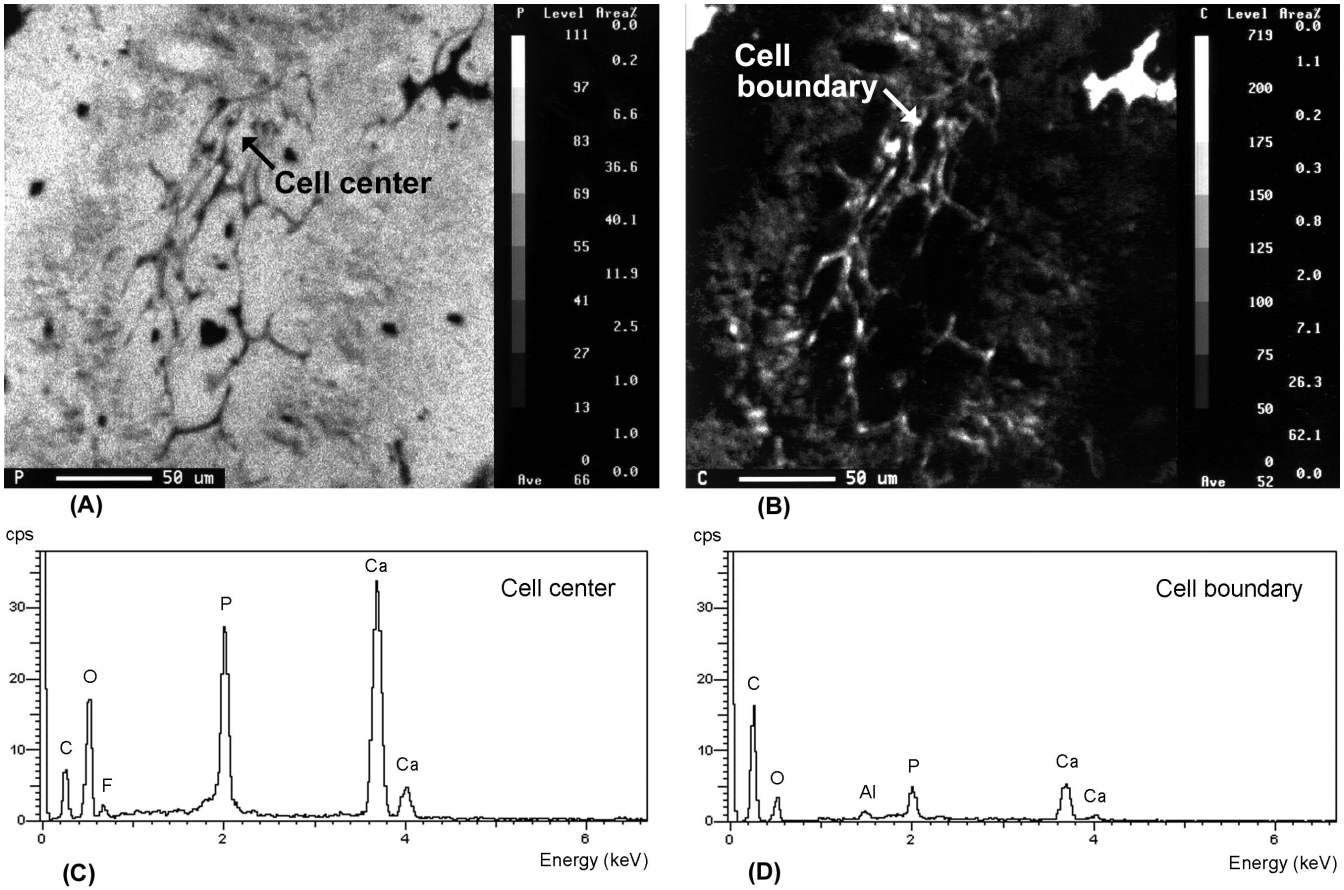

FIGURE 4—Elemental composition of lithified cells from Onefour coprolite (slide DP-1Dh). Microprobe-generated wave dispersive (WD) elemental maps showing relative distribution of phosphorus (A) and carbon (B) in cross section of muscle cells. Arrows indicate sampling areas represented by SEM electron dispersive (ED) spectra (C, D). Map A shows that phosphorus is widely distributed in the ground mass and cell centers. The large phosphorus and calcium peaks and the smaller carbon, oxygen, and fluorine peaks in the ED spectrum of the cell center (C) suggest a calcium phosphate like carbonate fluorapatite. Map B shows that carbon is concentrated locally—most notably in the cell boundaries. The ED spectrum of the cell boundary (D) shows a high percentage of carbon relative to other elements that is inconsistent with inorganic carbon compounds.

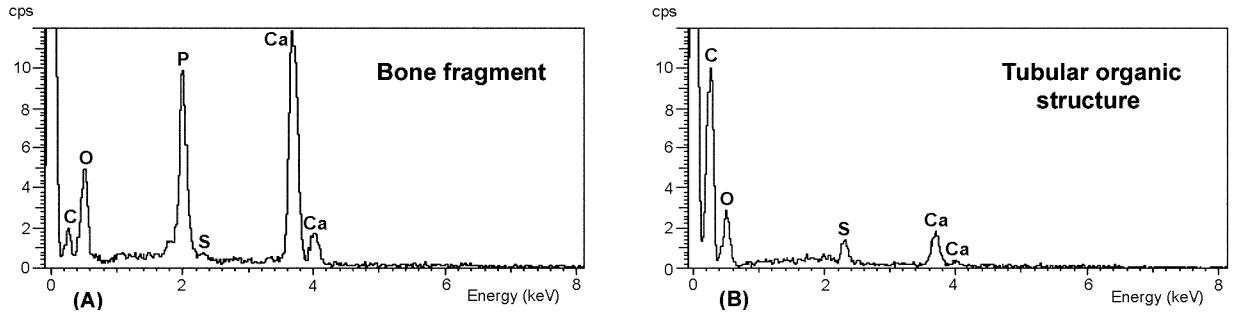

FIGURE 5—SEM electron dispersive (ED) spectra of a bone fragment (A) and an organic tubular structure (B) in the coprolitic ground mass. Note that the elemental composition of the bone (from Slide DP-1Dh) is very similar to that of the lithified cells (Fig. 4C), in that the relative percentages of carbon, oxygen, phosphorus, and calcium are comparable (the relative intensity is lower, but this may reflect different sampling conditions). The ED spectrum of the tubular structure (Slide DP-22Aa-o) confirms that it is predominantly carbon, though lesser amounts of oxygen, sulfur, and calcium are present. The absence of significant iron (the primary iron peak occurs around 6.2 keV) indicates that the dark material constrained within the tubular structures cannot be pyrite framboids, which have been implicated in some cases as resembling fossilized blood cells (Martill and Unwin, 1997).

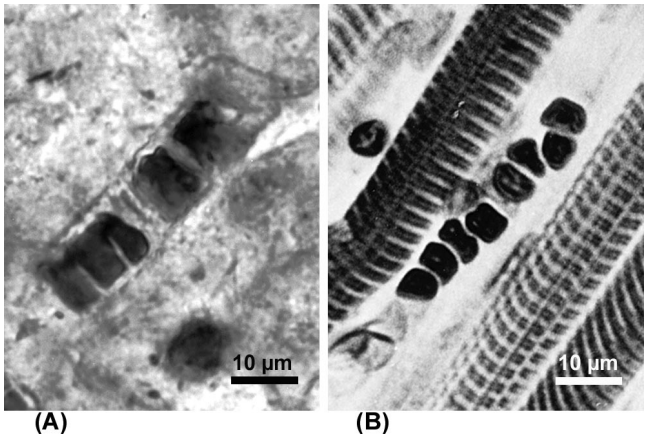

FIGURE 6—Photomicrographs of coprolite thin section (A) and a fixed capillary (B). (A) Fossil structure (Slide DP-22Aa-k) shows tiny blocks of organic material that appear to be constrained within a narrow tube. (B) Longitudinal view of a capillary surrounded by skeletal muscle cells shows a similarly blocky aspect (photo by M.H. Ross, published in Ross et al., 1995).

observations provide substantial morphological evidence that the cord-like structures in the coprolite are fossilized muscle cells, and hereafter, they are referred to as such.

The fossilized muscle tissue in the Onefour coprolite is very similar to fossil muscle tissues found in ancient compressed carcasses. Muscle tissues are among the most commonly preserved soft tissues in the fossil record (see Dean, 1902; Willems and Wuttke, 1987; Martill, 1988; Voigt, 1988; Schultze, 1989; Martill, 1990; Kellner, 1996; Briggs et al., 1997; Dal Sasso and Signore, 1998), and have been identified on the basis of anatomical context and morphology. Reports of fossil muscle tissues usually describe three-dimensional, phosphatic muscle fibers that are comparable to the muscle fibers forming the fluted pattern in the coprolite. In a few cases, myofibrillar banding has also been evident in scanning electron micrographs (Schultze, 1989; Martill, 1990; Wilby and Briggs, 1997), thin sections (Dean, 1902), or cellulose film transfers (Voigt, 1988).

The degree of fidelity of soft-tissue preservation appears to be determined by the microstructure of the phosphatized tissues. Wilby and Briggs (1997) observed distinct microfabrics associated with lithified muscle tissues. Microbial microfabrics are composed of 1–2 μm spheres formed by lithified microorganisms. Substrate microfabrics are formed by much smaller phosphatic crystallites (which may be smaller than 30 nm) and can record subcellular detail more faithfully. High magnification (up to 8,800 times) of fossilized muscle tissues in the Onefour coprolite revealed an absence of lithified microbe pseudomorphs, indicating that at least some of the muscle tissues in the fecal ground mass were preserved by substrate microfabric lithification.

The identification of the muscle cells provides histological context that sheds light on other morphologically distinct tissues in the coprolite. Because the three-dimensional reticulated pattern frequently overlays groups of fossil muscle fibers (Fig. 2D, E), this pattern appears to indicate exterior surfaces of muscle fascicles. Thus, the raised reticulated networks may represent fossil traces of perimysium and/or associated adipose tissues. Intramuscular fat can be deposited at perimysial boundaries (Nishimura et al., 1999), and it is worth noting that adipose tissues stripped of lipid components exhibit a honeycombed structure (Nishimura et al., 1999; Kessel and Kardon, 1979) that bears a resemblance to the reticulated pattern in the coprolite.

TABLE 2—Measured percentages of total carbon and inorganic carbon and extrapolated percentages of total organic carbon in bulk powdered samples of the coprolite and the associated sediment.

Carbon analysis	Coprolite	Sediment
Total C	3.05	0.36
Inorganic C	1.00	<0.05
Total organic carbon	2.05	<0.36

In addition to fossilized muscle cells, numerous fragments of other miscellaneous tissues are evident in thin sections of the specimen. Although most fragments are not identifiable, a number of distinctive elongate structures show tiny (~ 10 μm diameter) bits of dark material that appear to be constrained within narrow tubes (Fig. 6A). ED spectra of these structures indicate a largely organic composition (Fig. 5B), and they probably represent fragments of undigested prey tissues. The size and morphology of the structures resemble longitudinal views of red blood cells in capillaries (Fig. 6B). Muscle tissues contain extensive networks of blood vessels, so numerous fragmented capillaries would be present in comminuted muscle tissue. Although very rare, probable fossil red blood cells have been previously reported. Maat (1991) discovered morphologically diagnostic blood cells in scanning electron micrographs of human bone fragments from the Hellenistic Period (around 2200 years old). Voigt (1939, p. 55) found a cylindrical body containing microscopic (12 to 15 μm diameter), brown, "plate-shaped structures" in the vicinity of fossil muscle tissue in an Eocene lizard, and interpreted the structures as fossil red blood corpuscles. Schweitzer and Horner (1999) examined thin sections of *Tyrannosaurus* bone and discovered globular microstructures (~25 μm diameter) in vascular channels that have morphological similarities to red blood cells. This observation is particularly intriguing because several chemical analyses indicated the presence of heme compounds in the bone tissues in which the microstructures were found (Schweitzer et al., 1997b).

The histological structure of the bone fragments in the coprolite is highly unusual in having conspicuous acellular areas, distinctive branching canaliculi, and numerous Sharpey's fibers. Such bone tissue is most similar to that observed in pachycephalosaur skulls (J. R. Horner & M. B. Goodwin, unpublished data, 2001). Because bone fragments from different areas of the specimen have the same distinctive histology, it is reasonable to surmise that most of the bone and soft tissues present in the feces were largely derived from one prey animal.

IMPLICATIONS FOR TYRANNOSAURID DIGESTION

Animal flesh is highly digestible (measured as assimilation efficiency; Castro et al., 1989), but several factors may contribute to incomplete digestion. Because degradation depends upon adequate exposure to digestive com-

pounds, digestion is a function of the volume of flesh ingested, degree of comminution, and gut residence time. Thus, large chunks of meat passing rapidly through a gut may be incompletely digested. Indeed, undigested muscle fibers have been found in the feces of healthy dogs (Canfield and Fairburn, 1983) and gorging animals (Curio, 1976). Size considerations, feeding behavior, and/or digestive physiology may have contributed to incomplete digestion of the muscle tissue in the Onefour coprolite. As immense carnivores with poor dental occlusion, tyrannosaurids routinely would have swallowed sizable masses of flesh with reduced surface-to-volume ratios, so it is likely that intact chunks of muscle tissue passed through the digestive tract on a regular basis. Incomplete digestion may also reflect a relatively short gut-residence time. Gorging may result in rapid digesta transit, and is frequently observed in large extant predators (Curio, 1976) that feed sporadically. Tyrannosaurids probably contended with comparable episodes of fluctuating prey availability.

Another possibility is that tyrannosaurids typically processed meals relatively rapidly. The bone fragments in the coprolite are fairly small (most are < 1 cm long), and probably would have been completely digested during an extended stay in the digestive tract. Extant crocodilians and snakes commonly retain food long enough to decalcify most ingested skeletal elements (e.g., Skoczylas, 1978; Fisher, 1981a, 1981b). In contrast, identifiable bone fragments often are found in extant mammal feces (e.g., Johnson and Hansen, 1979; Emmons, 1987). Although gastrointestinal problems can increase the frequency of defecation, the compact morphology of this specimen is not indicative of diarrhea. The discovery and analysis of additional specimens will help determine whether the Onefour coprolite represents a typical fecal deposit from a healthy tyrannosaurid. Even so, it is worth noting that comparably sized, undigested bone fragments in a Maastrictian tyrannosaurid coprolite (Chin et al., 1998), and etched Campanian bones interpreted as tyrannosaurid stomach contents (Varricchio, 2001) support the prospect that tyrannosaurids did not retain food in their guts for long periods of time. These considerations suggest a different digestive behavior than that of extant crocodilians and snakes.

DIAGENESIS

Feces and other labile organic tissues usually degrade soon after deposition, so the preserved soft tissues within the coprolite indicate distinctive conditions that were conducive to exceptional preservation. The depositional and stratigraphic settings of the Lethbridge Coal Zone provide clues to the taphonomic history of the specimen. This interval has been interpreted as a system of rapidly aggrading, lower coastal-plain facies influenced by frequent changes in relative sea level (Eberth, 1996). The rooted sandstone unit on which the coprolite was found was probably either a levee deposit or late-stage fill in a small, abandoned channel. The overlying carbonaceous shale reflects a facies difference that indicates a change in fluvial deposition. This suggests that the fecal mass was deposited on a sandy soil among deeply rooted plants, and subsequently was buried by sediments deposited during a flood event.

Rapid burial of organic remains can set up conditions conducive to fossilization. Long-term preservation of soft tissue usually occurs when authigenic mineralization exceeds rate of decay (Allison, 1988). Labile tissues have been fossilized by morphological replication with calcium phosphate, and some specimens show such fine anatomical detail that extremely rapid lithification has been posited (Martill and Harper, 1990). Briggs and Kear (1993, 1994) induced mineralization of fresh invertebrate muscle tissues in a laboratory within two weeks. Their work suggests that decaying tissues contain sufficient phosphorus for phosphatization. Indeed, rapid decomposition appears to be prerequisite for lithification of soft tissues. Actualistic experiments by Sagemann et al. (1999) demonstrated that microbial activity during anaerobic decay of shrimp carcasses produced steep chemical gradients (oxygen, sulfide, and pH) that were conducive to the exceptional preservation of soft tissues by phosphatization. Other experiments have established that specific bacteria can play a key role in phosphate precipitation. Some bacteria (including the coliform bacterium, *Escherichia coli*) appear to facilitate phosphatization by contributing phosphorus-liberating phosphatases (Hirschler et al., 1990).

These observations suggest a mechanism for the rapid lithification of the Onefour coprolite. Rapid burial would have provided protection from bioturbation (Allison, 1988) and coprophagy, and generated a closed, anoxic environment where anaerobic decomposition predominated and steep chemical gradients formed. Phosphorus and calcium available in the ingested bone and flesh probably were released by digestion and by the metabolic activities of high concentrations of fecal bacteria. These microbially mediated chemical conditions apparently favored the early diagenetic phosphatization of patches of undigested muscle tissue within the feces.

Long-term preservation of the fossilized soft tissues in the coprolite was dependent on the relative stability of the depositional environment over the succeeding millennia. Significant changes in groundwater chemistry might have facilitated recrystallization, which could have destroyed the delicate morphology of the phosphatized tissues and oxidized the included organic matter. Recrystallization of the phosphatic ground mass in the tyrannosaurid coprolite from the Frenchman Formation (Chin et al., 1998) may be responsible for the absence of recognizable soft tissues in that specimen.

Microprobe analyses of other three-dimensional fossils have demonstrated that organic carbon is commonly localized around cells that have been permineralized with silica. Such organic material appears to represent refractory substances that were relatively resistant to diagenetic alteration (Boyce et al., 2001). In the Onefour coprolite, the distribution of refractory organic residues also corresponds with cellular morphology. Indeed, in this case, the configuration of the organic matter is largely responsible for the histological detail.

The nature of the organic residues in the coprolite can be inferred from the composition of muscle cells and connective tissues. Although contractile proteins are the most abundant components of muscle fibers, connective tissues predominantly are composed of collagen. The largely phosphatic composition of the interiors of the fossil muscle cells (Fig. 4) suggests that lithification of areas once occupied by contractile proteins preserved the three-dimen-

sional morphology of the muscle fibers. Increased microbial activity (Sagemann et al., 1999) within the labile muscle fibers may have promoted rapid phosphatization. The dark residual organic material in the muscle tissue largely is confined to the exterior of the muscle cells—sites previously occupied by endomysial and perimysial connective tissues. The structure and histological setting of collagen probably help account for the persistence of organic remnants of connective tissues in the Onefour coprolite. Collagen is a large protein that is relatively resistant to degradation (Jope, 1980; McNulty et al., 2002). Furthermore, the preservation of ancient collagen appears to be enhanced by association with (Ostrom et al., 1990) or enclosure within (DeNiro and Weiner, 1988) biomineralized crystals, and such conditions likely explain the recovery of collagen-like substances from Mesozoic bones (Wyckoff, 1980; Armstrong et al., 1983; Ostrom et al., 1990; Gurley et al., 1991; Schweitzer et al., 1997a). Incorporation of macromolecules in structural tissues has been shown to increase the preservation potential of other tissues as well (Briggs, 1999). Thus, rapid lithification of the muscle cells probably served to retard degradation of associated connective tissues, and residues of endomysial connective tissues now delineate some of the lithified muscle cells.

The fragile brown coating that is evident on the three-dimensional reticulated pattern may indicate residues of perimysial connective tissues. It is also possible that this material is diagenetically altered adipose tissue, since saturated fatty acids are resistant to degradation as well (Killops and Killops, 1993).

CONCLUSIONS

The large tyrannosaurid coprolite recovered from the Dinosaur Park Formation is quite remarkable because it bears morphological and organic evidence of undigested soft tissues. The distinctive morphologies of the preserved structures indicate that they are the fossilized remains of muscle cells, connective tissues, and possible capillary fragments. These tissues are the major components of animal flesh and the surprising quality of their preservation reveals clues about their digestive and diagenetic history.

It is apparent that the ingested flesh was poorly comminuted and/or passed through the gut of the fecal producer relatively rapidly. Additional finds may help determine whether this represents a digestive pattern that was typical of tyrannosaurid theropods. The exceptional preservation of the soft tissues also indicates that the fecal matter lithified rather quickly. Phosphatization probably facilitated by rapid burial and chemical reactions generated by the metabolic activity of bacteria. After early diagenetic mineralization preserved cellular structures in the coprolite, the absence of significant recrystallization over time prevented subsequent destruction of the morphology of the phosphatized tissues.

The consequence of this fortuitous sequence of events is a coprolite that provides a glimpse of the tissues of a Cretaceous prey animal—possibly a pachycephalosaurid dinosaur. As such, it contributes significant baseline information on dinosaurian histology. This discovery also reveals that well-preserved carnivore coprolites can provide an unexpected source of rare, fossilized soft tissues.

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REFERENCES

ABEL, O., 1935, Vorzeitliche Lebensspuren: Verlag von Gustav Fischer, Jena, 645 p.

ALLISON, P.A., 1988, Konservat-Lagerstätten: cause and classification: Paleobiology, v. 14, p. 331–344.

ARMSTRONG, W.G., HALSTEAD, L.B., REED, F.B., and WOOD, L., 1983, Fossil proteins in vertebrate calcified tissues: Philosophical Transactions of the Royal Society of London B, v. 301, p. 301–343.

BERTRAND, C.E., 1903, Les Coprolithes de Bernissart. I. partie: Les Coprolithes qui ont ete attribues aux Iguanodons: Mémoire du Musée royal d'histoire naturelle de Belgique, Brussels, v. 1, p. 1–154.

BOYCE, C.K., HAZEN, R.M., and KNOLL, A.H., 2001, Nondestructive, *in situ,* cellular-scale mapping of elemental abundances including organic carbon in permineralized fossils: Proceedings of the National Academy of Sciences, v. 98, p. 5970–5974.

BRIGGS, D.E.G., 1999, Molecular taphonomy of animal and plant cuticles: selective preservation and diagenesis: Philosophical Transactions of the Royal Society, London, B, v. 354, p. 7–17.

BRIGGS, D.E.G., and KEAR, A.J., 1993, Fossilization of soft tissue in the laboratory: Science, v. 259, p. 1439–1442.

BRIGGS, D.E.G., and KEAR, A.J., 1994, Decay and mineralization of shrimps: PALAIOS, v. 9, p. 431–456.

BRIGGS, D.E.G., WILBY, P.R., PÉREZ-MORENO, B.P., SANZ, J.L., and FREGENAL-MARTÍNEZ, M., 1997, The mineralization of dinosaur soft tissue in the Lower Cretaceous of Las Hoyas, Spain: Journal of the Geological Society, London, v. 154, p. 587–588.

CANFIELD, P.J., and FAIRBURN, A.J., 1983, Effect of various diets on faecal analysis in normal dogs: Research in Veterinary Science, v. 34, p. 24–27.

CASTRO, G., STOYAN, N., and NYERS, J.P., 1989, Assimilation efficiency in birds: a function of taxon or food type?: Comparative Biochemistry and Physiology, v. 92A, p. 271–278.

CHIN, K., TOKARYK, T.T., ERICKSON, G.M., and CALK, L.C., 1998, A king-sized theropod coprolite: Nature, v. 393, p. 680–682.

CURIO, E., 1976, The Ethology of Predation: Springer-Verlag, New York, 250 p.

DAL SASSO, C., and SIGNORE, M., 1998, Exceptional soft-tissue preservation in a theropod dinosaur from Italy: Nature, v. 392, p. 383–387.

DEAN, B., 1902, The preservation of muscle-fibres in sharks of the Cleveland Shale: The American Geologist, v. 30, p. 273–278.

DENIRO, M.J., and WEINER, S., 1988, Organic matter within crystalline aggregates of hydroxyapatite: a new substrate for stable isotopic and possibly other biogeochemical analyses of bone: Geochimica et Cosmochimica Acta, v. 52, p. 2415–2423.

EBERTH, D.A., 1996, Origin and significance of mud-filled incised valleys (Upper Cretaceous) in southern Alberta, Canada: Sedimentology, v. 43, p. 459–477.

EBERTH, D.A., CURRIE, P.J., BRINKMAN, D.B., RYAN, M.J., BRAMAN, D.R., GARDNER, J.D., LAM, V.D., SPIVAK, D.N., and NEUMAN, A.G.,

2001, Alberta's dinosaurs and other fossil vertebrates: Judith River and Edmonton Groups (Campanian–Maastrichtian): *in* Hill, C.L., ed., Mesozoic and Cenozoic Paleontology in the Western Plains and Rocky Mountains: Museum of the Rockies, Occasional Paper No. 3, p. 47–75.

EBERTH, D.A., and HAMBLIN, A.P., 1993, Tectonic, stratigraphic, and sedimentologic significance of a regional discontinuity in the upper Judith River Group (Belly River wedge) of southern Alberta, Saskatchewan, and northern Montana: Canadian Journal of Earth Sciences, v. 30, p. 174–200.

EMMONS, L.H., 1987, Comparative feeding ecology of felids in a neotropical rainforest: Behavioral Ecology and Sociobiology, v. 20, p. 271–283.

FISHER, D.C., 1981a, Crocodilian scatology, microvertebrate concentrations, and enamel-less teeth: Paleobiology, v. 7, p. 262–275.

FISHER, D.C., 1981b, Taphonomic interpretation of enamel-less teeth in the Shotgun local fauna (Paleocene, Wyoming): Contributions from the Museum of Paleontology, University of Michigan, v. 25, p. 259–275.

GURLEY, L.R., VALDEZ, J.G., SPALL, W.D., SMITH, B.F., and GILLETTE, D.D., 1991, Proteins in the fossil bone of the dinosaur, *Seismosaurus*: Journal of Protein Chemistry, v. 10, p. 75–90.

HIRSCHLER, A., LUCAS, J., and HUBERT, J.-C., 1990, Bacterial involvement in apatite genesis: FEMS Microbiology Ecology, v. 73, p. 211–220.

JOHNSON, M.K., and HANSEN, R.M., 1979, Estimating coyote food intake from undigested residues in scats: The American Midland Naturalist, v. 102, p. 363–367.

JOPE, E.M., 1980, Ancient bone and plant proteins: the molecular state of preservation: *in* Hare, P.E., Hoering, T.C., and King, K., eds., Biogeochemistry of Amino Acids: John Wiley and Sons, New York, p. 23–33.

KELLNER, A.W.A., 1996, Fossilized theropod soft tissue: Nature, v. 379, p. 32.

KESSEL, R.G., and KARDON, R.H., 1979, Tissues and Organs: A Text-Atlas of Scanning Electron Microscopy: W.H. Freeman and Company, San Francisco, 317 p.

KILLOPS, S.D., and KILLOPS, V.J., 1993, An Introduction to Organic Geochemistry: Longman Scientific and Technical, Essex, England, 265 p.

MAAT, G.J.R., 1991, Ultrastructure of normal and pathological fossilized red blood cells compared with pseudo-pathological biological structures: International Journal of Osteoarchaeology, v. 1, p. 209–214.

MCNULTY, T., CALKINS, A., OSTROM, P., GANDHI, H., and GOTTFRIED, M., 2002, Stable isotope values of bone organic matter: artificial diagenesis experiments and paleoecology of Natural Trap Cave, Wyoming: PALAIOS, v. 17, p. 36–49.

MARTILL, D.M., 1988, Preservation of fish in the Cretaceous Santana Formation: Palaeontology, v. 31, p. 1–18.

MARTILL, D.M., 1990, Macromolecular resolution of fossilized muscle tissue from an elopomorph fish: Nature, v. 346, p. 171–172.

MARTILL, D.M., and HARPER, L., 1990, An application of critical point drying to the comparison of modern and fossilized soft tissues of fishes: Palaeontology, v. 33, p. 423–428.

MARTILL, D.M., and UNWIN, D.M., 1989, Exceptionally well preserved pterosaur wing membrane from the Cretaceous of Brazil: Nature, v. 340, p. 138–140.

MARTILL, D.M., and UNWIN, D.M., 1997, Small spheres in fossil bones: blood corpuscles or diagenetic products?: Palaeontology, v. 40, p. 619–624.

MOTTA, P., ANDREWS, P.M., and PORTER, K.R., 1977, Microanatomy of Cell and Tissue Surfaces: An Atlas of Scanning Electron Microscopy: Lea and Febiger, Philadelphia, 189 p.

NISHIMURA, T., HATTORI, A., and TAKAHASHI, K., 1999, Structural changes in intramuscular connective tissue during the fattening of Japanese Black cattle: effect of marbling on beef tenderization: Journal of Animal Science, v. 77, p. 93–104.

OSTROM, P.H., MACKO, S.A., ENGEL, M.H., SILFER, J.A., and RUSSELL, D.R., 1990, Geochemical characterization of high molecular weight material isolated from Late Cretaceous fossils: Organic Geochemistry, v. 16, p. 1139–1144.

REED, S.J.B., 1996, Electron Microprobe Analysis and Scanning Electron Microscopy in Geology: Cambridge University Press, Cambridge, 201 p.

ROSS, M.H., ROMRELL, L.J., and KAYE, F.I., 1995, Histology: A Text and Atlas, Williams and Wilkins, Baltimore, 875 p.

SAGEMANN, J., BALE, S.J., BRIGGS, D.E.G., and PARKES, R.J., 1999, Controls on the formation of authigenic minerals in association with decaying organic matter: an experimental approach: Geochimica et Cosmochimica Acta, v. 63, p. 1083–1095.

SCHULTZE, H.-P., 1989, Three-dimensional muscle preservation in Jurassic fishes of Chile: Revista Geológica de Chile, v. 16, p. 183–215.

SCHWEITZER, M.H., and HORNER, J.R., 1999, Intravascular microstructures in trabecular bone tissues of *Tyrannosaurus rex*: Annales de Paléontologie, v. 85, p. 179–192.

SCHWEITZER, M.H., JOHNSON, C., ZOCCO, T.G., HORNER, J.R., and STARKEY, J.R., 1997a, Preservation of biomolecules in cancellous bone of *Tyrannosaurus rex*: Journal of Vertebrate Paleontology, v. 17, p. 349–359.

SCHWEITZER, M.H., MARSHALL, M., CARRON, K., BOHLE, D.S., BUSSE, S.C., ARNOLD, E.V., BARNARD, D., HORNER, J.R., and STARKEY, J.R., 1997b, Heme compounds in dinosaur trabecular bone: Proceedings of the National Academy of Sciences, v. 94, p. 6291–6296.

SKOCZYLAS, R., 1978, Physiology of the digestive tract: *in* Gans, D., and Gans, K.A., eds., Biology of the Reptilia, Volume 8: Academic Press, London, p. 589–717.

VARRICCHIO, D.J., 2001, Gut contents from a Cretaceous tyrannosaurid; implications for theropod dinosaur digestive tracts: Journal of Paleontology, v. 75, p. 401–406.

VOIGT, E., 1939, Fossil red blood corpuscles found in a lizard from the Middle Eocene Lignite of the Geiseltal near Halle: Research and Progress: Quarterly Review of German Science, v. 5, p. 53–56.

VOIGT, E., 1988, Preservation of soft tissues in the Eocene lignite of the Geiseltal near Halle/S: *in* Franzen, J.L., and Michaelis, W., eds., The Eocene at Lake Messel, An International Symposium: Courier Forschungsinstitut Senckenberg v. 107, Senckenbergische Naturforschende Gesellschaft, Frankfurt, p. 325–343.

WILBY, P.R., and BRIGGS, D.E.G., 1997, Taxonomic trends in the resolution of detail preserved in fossil phosphatized soft tissues: Geobios, v. 20, p. 493–502.

WILLEMS, H., and WUTTKE, M., 1987, Lithogenesis of lacustrine dolomites and "soft part preservation" of tetrapods by microbial replacement in the Lower Rotliegends (Permian, Saar-Nahe-Basin, SW-Germany): Neues Jahrbuch für Geologie und Paläontologie Abhandlungen, v. 174, p. 213–238.

WYCKOFF, R.W.G., 1980, Collagen in fossil bones: *in* Hare, P.E., Hoering, T.C., and King, K., eds., Biogeochemistry of Amino Acids: John Wiley and Sons, New York, p. 17–22.

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