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Isotopic and Molecular Preservation in Cretaceous Fossils

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Key Words: carbon isotopes, dinosaur bone, Hell Creek Formation, molecular preservation, primary isotopes, stable isotopes

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Abstract

Molecular, and even whole tissue preservation, including bone collagen sequences and blood vessels from Cretaceous bone samples, are described in a growing body of technical literature. Separate studies also describe primary isotopes from many of the same geological formations as those that have fossils with molecular preservation. To investigate what might have led to the co-occurrence of molecular and isotopic preservation, we collected and analyzed fifteen stable carbon isotope results from three bone fractions (organic collagen, inorganic bioapatite, and a mixture of both in "bulk"), each isolated from six Cretaceous dinosaur bone specimens. Assessments of preservation evaluated the endogeneity of our isotope ratios. Broad offsets between bioapatite and collagenous extracts were inconsistent with secondary isotope ratios (i.e., contamination), and largely consistent with biological ratios (i.e., ratios expected in fresh bone). Our results include the first stable carbon isotope ratios from collagen extracts of dinosaur bone. Co-occurrence of primary carbon isotope ratios and biomolecules suggest two aspects of their diagenetic history. Firstly, it appears that any ancient hydrothermal fluids failed to facilitate the total decay of organics, nor to totally randomize carbon isotopes. This result is consistent with a relatively recent burial. Lastly, given that the well-characterized decay of bone collagen even under ideal conditions limits it to fewer than a million years at 10°C, these fossils likely experienced a much more brief burial history than mainstream sources cite.

Simple Summary

This scientific paper investigates why dinosaur fossils from Cretaceous System strata (like those from the Hell Creek and Lance Formations) sometimes contain both original biological materials and likely original carbon-isotope ratios. The researchers collected and analyzed six dinosaur bone specimens. They measured carbon isotopes from different parts of the bone: organic collagen, inorganic bioapatite, and a mixture of both. Their analysis showed carbon isotope ratios that reflect to some degree those ratios the dinosaurs had when they died. The study authors found consistent differences in isotope signatures between the collagen and bioapatite parts within each bone. This consistency suggests that most of the measured isotopes did not result from contamination, but from the dinosaurs, even though the difference between collagen and bioapatite was larger than what is typically seen in modern animals. The study concludes that the co-occurrence of preserved biomolecules and original isotope ratios suggest a unique burial history. This preservation, especially the presence of fragile collagen, implies that these fossils likely experienced a much shorter burial time than commonly believed, and this matches the idea that a relatively recent global flood buried these creatures.

Introduction

Primary fossil biomaterials, including proteins, carbohydrates, lipids, and nucleic acids, encounter limited reception in certain circles despite dozens of reported techniques that independently confirm biomolecular and/or morphological preservation (Thomas and Taylor, 2019). Investigation of the mineral fraction of bone offers a new route to evaluate this fossil biochemical controversy and related diagenetic options. We therefore describe stable isotope analyses of the mineral fraction (bioapatite) of Cretaceous samples and compare these with two preparations that include organic fractions (mostly collagen) from the same fossil bone samples (Brock et al., 2012).

Reports abound of molecular preservation in fossils. The number of such publications, and especially the specificity of molecular identification, has increased significantly in recent years, as our review revealed (Thomas, 2019). Several of the more impactful molecular preservation reports, including those that describe the vertebrate-specific protein collagen, originate in the Hell Creek Formation (HCF), which outcrops mostly in Montana. These sources of molecular and/or morphological preservation in, for example, *Tyrannosaurus rex* (Acera et al., 2007), *Triceratops horridus* (Armitage and Anderson, 2013), and *Edmontosaurus* (Tuinstra et al., 2025) offer new views of Cretaceous paleobiology (Hartman et al., 2014). Some HCF samples have even furnished enough molecular integrity for collagen protein sequencing (Acera et al., 2007; Schroeter et al., 2017). Literature also reveals a growing list of geologic strata that contain molecular preservation in fossils. These include, from North America, the HCF-equivalent Lance Formation (Fm). Underlying these, the Cretaceous Judith River Fm., Dinosaur Park Fm., and the Niobrara Chalk Fm. with outcrops in Kansas (Lindgren et al., 2010) also contain material with published organics. Elsewhere, the Ciply Phosphatic Chalk of Belgium (Lindgren et al., 2011), the Jurassic Lower Lufeng Fm. of China (Reisz et al., 2013), and the Triassic Lower Gogolin Fm. in Poland (Surmik et al., 2016), among others, retain molecular preservation. These strata, commonly labeled Mesozoic, also belong to the Zuni Megasequence. Clarey (2020, pp. 284–285) interpreted these as deposits from near the high-water mark of Noah's Flood. Reports of biomolecular and morphological tissue preservation do not include stable isotope data. To our knowledge, this paper is the first in this regard.

However, stable isotope ratios, including $^{13}C/^{12}C$ ratio measurements from bioapatites, have been used to convey meaningful paleodietary data from Cretaceous strata (Fricke et al., 2008). Results are typically reported in $\delta^{13}C$, where

$\delta^{13}C = (R_{sample}/R_{standard} - 1) \cdot 1000‰$,

where $R = {}^{13}C/{}^{12}C$, and

the standard for carbon isotope values derive from the Vienna Pee Dee Belemnite (VPDB). (Faure and Mensing, 2012)

Research questions about original isotope ratios for ancient samples supply meaningful data only if they reflect at least some primary isotopes. Establishing the presence of primary carbon is a necessary step for $\delta^{13}C$-based reconstructions to evaluate either the hypothesis of primary Cretaceous organics or diagenetic scenarios. Secondary isotope alteration of bone bioapatite can occur by metamorphism, hydrothermal action, or groundwater percolation. These processes would facilitate dissolved carbonate interactions as well as chemical decay of organics (Lee Thorp and van der Merwe, 1987). Within-bone indicators of secondary isotopes include:

- mismatches between stable isotope ratios found in fossil versus modern bone,
- randomized between-species or within-bone fraction offsets, and
- homogeneity between fossil bones of different taxa within a site or microsite.

Thus, primary bioapatite and biomolecular carbon sources should show

- isotopic heterogeneities consistent with modern biological patterns,
- bone fraction and taxon-specific isotopic offsets, and
- bone fractionation patterns between taxa or microsites that resemble those from comparable modern bone samples and ecosystems. (Tütken, 2011)

If dinosaur bone bioapatite and bone protein remnants preserve primary carbon isotope ratios, then both fractions should contain largely original carbon. We therefore evaluate (1) biological ratios and (2) bone fraction ratios for six Cretaceous fossils.

Finally, similar diageneses may accompany any co-occurrence in fossils of primary protein remnants with primary carbon isotopes. Such co-occurrence must be established prior to investigating possible diagenetic causes. We therefore took two steps toward investigating the co-occurrence of primary molecules and isotopes. First, we collated disparate literature that might reveal patterns of co-occurrence. Second, we assessed the possibility of primary carbon isotopes in Hell Creek and Lance Formation bone fossils. One assessment compared newly acquired stable carbon isotope ratios from dinosaur bone bioapatite against 73 isotope ratios taken from published dinosaur tooth enamel and dentine. In another assessment, we compared 15 isotope ratio measurements from three within-bone fractions in our six bone samples. If indeed hydrothermal fluids have erased isotopic signals, then have they also erased organics since the bones were buried?

Background: Stable Carbon Isotope Ratios

Many factors influence stable isotope ratios in modern biologies and ecologies. Marine environments are enriched in ^{13}C compared to terrestrial environments (Hoefs, 1987, p. 35). Terrestrial plants that use the more common C3 photosynthetic pathway are more depleted in ^{13}C than C4 plants. Animal bones, therefore, reflect isotopic signals that reflect their various diets. Trophic levels, taxon or age-specific bodily processes such as hibernation or weaning (Bochrens et al., 1994), or a particular mix of dietary components such as marine and terrestrial sources (Lee-Thorp et al., 1989) can generate $\delta^{13}C$ offsets in differing body tissue fractions, including the biomineral and organics fractions of teeth and bones. These biological differences provide isotopic offsets to which fossil data can be compared.

Several studies have explored links between stable carbon isotope ratios and biome variances within extant (Grey and Jones, 1999) and extinct ecosystems (Kohn and Cerling, 2002). Unfortunately, no purely objective test exists to verify the primary origin of isotope ratios, especially for fossil samples. Differences in geologic settings, such as matrix permeability and lithology, diagenetic processes such as hydrothermal circulation and possible crystallite reorganization during permineralization, metamorphic processes, and time since burial, likely have effects on isotopic exchange in different tissue components. Although they are not strictly empirical, several strategies have been developed to help assess the degree of primary versus secondary sourcing of isotopes. After applying appropriate sample cleaning and treatment protocols, these strategies include comparing isotope ratios from different taxa in one location, comparing different isolated fractions from one body part, and comparing fossil isotope fractionations with biological fractionations. Such comparisons are preferably done with known parallels in extant biomes (Fricke et al., 2008).

Experiments show that $\delta^{13}C$ values in bone or tooth apatite are higher (enriched) compared to collagen sampled from the same organism. Krueger and Sullivan (1984) modeled this offset as a product of dietary carbohydrate carbons entering bone apatite and dietary proteins entering bone collagen. If undisturbed, ^{13}C content in organic versus inorganic bone fractions preserves food source signals plus isotope partitioning effects. Such differences between $\delta^{13}C$ values in apatite and collagen may be observed even after presumed diagenetic processes have occurred. As an example, Lee-Thorp et al. (1989) found that bone collagen in herbivore remains from southern Africa and Malawi represents a fractionation of ~+5‰, and herbivore bone apatite ~+12‰, relative to the vegetation source.

Stable carbon and oxygen isotopes in apatite of dinosaur and other fossil reptile femurs and teeth, plus several shells and other bones, supplied sufficient data to begin reconstruction of East Asian dinosaur environments, including a regional paleoclimate (Amiot et al., 2015). Tütken's (2011) $\delta^{13}C$ analysis of sauropod biominerals and extant plus extinct plant matter concluded that sauropods from the Morrison Fm. and the Tendaguru Beds of Tanzania preferred terrestrial C3 plants. Stable isotope analysis of an organic fraction of a 10-cm-long, sauropod coprolite revealed a mean $\delta^{13}C$ value of -24.1‰, also consistent with preservation of C3 plant matter (Ghosh et al., 2003). Closer to the context of this present study, Fricke and Pearson (2008) reconstructed dinosaur and garfish niche partitioning in the HCF using $\delta^{13}C$ and $\delta^{18}O$ ratios, respectively. Similarly, Fricke et al. (2008) interpreted fossil ^{13}C signatures from Canada's Cretaceous Dinosaur Park, Two Medicine, and Judith River Formations as primary.

If similar assessments of our samples likewise conclude some degree of primary sourcing and are found also to include molecular preservation, then it would become appropriate to propose similar causal explanations of both fossil features. Thus, Cretaceous specimens from geologic formations containing fossils with already published descriptions of original biochemistry were obtained and evaluated for $\delta^{13}C$ ratios.

Geologic Setting and Materials

The HCF shows siliciclastic lithology containing laminar and cross-bedded sandstones, mudstones, and ironstones, as well as coal seams and coal lenses of various purities. Figure 1 shows the general location of the HCF material used herein. Cretaceous fauna are generally preserved in its impure sandstones. These characteristic lithologies and paleofaunas also outcrop in the Williston Basin and Bighorn Basin in Wyoming to comprise the Lance Formation, for which reason the two formations are considered equivalent (See references in Clarey, 2015). Figure 2 correlates the main strata included in this study. It reveals, for example, that the Lance and Hell Creek Formations correlate in lithology, paleontology, and relative positioning both with each other and with the Edmonton Group that outcrops further north. If fossils in these strata retain both original organics and original isotopic ratios, then the correlations of their strata offer a third observation that a model of origins should explain.

In many areas, for example, the HCF type locality in Flag Butte, north of Jordan, Montana, these equivalent formations contain a K-Pg boundary signature of two thin lignite beds near an iridium anomaly (Hartman

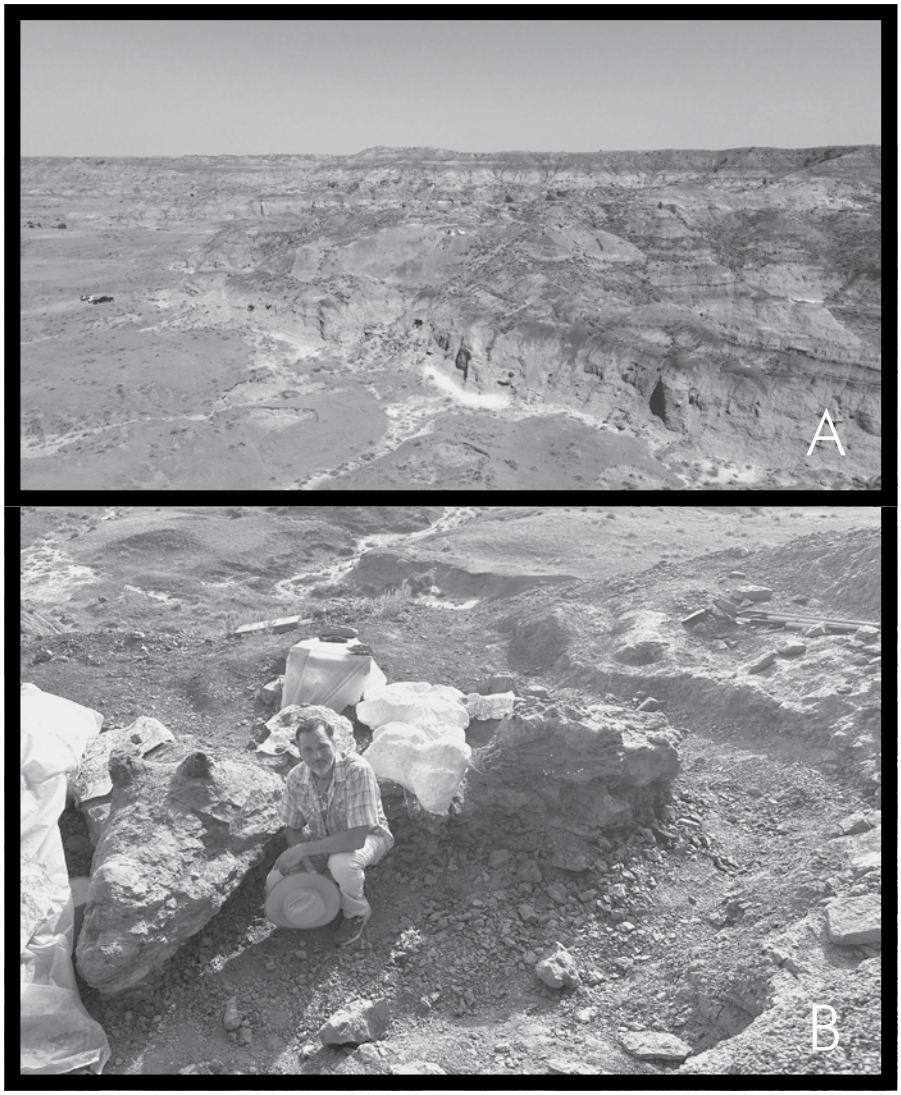


Figure 1. Geologic context for samples used in this study. (A) Drone image of the Montana private ranch from which all GDFM plus HTCH06 (See Table II) specimens were excavated. Abundant Hell Creek Formation badlands expose various intermixed sediment types. (B) Author (Thomas) beside an *in situ Triceratops* on the same ranch, illustrating Hell Creek excavation settings.

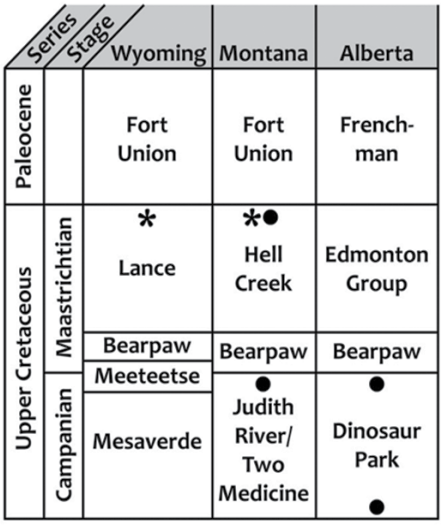


Figure 2. Correlation chart of geologic formations. Asterisks mark approximate positions of fossils sampled in this study. Dots mark positions of fossils sampled by Fricke and Pearson (2008) and Fricke et al. (2008). Formations and Stages adapted from Rogers et al. (1993) and Finn (2010).

et al., 2014). Fossils from the HCF in this study included two *Triceratops horridus* femora and one hadrosaur femur collected from a private ranch near Glendive, Montana, 7.6 m below the narrow K-Pg coal seam marking the upper boundary of the HCF at that location. These fossils belong to the Glendive Dinosaur and Fossil Museum (GDFM) repository. A small portion of the *Triceratops* brow horn described in Armitage and Anderson (2013) as HTCH06 was tested alongside a separately catalogued fragment of the same horn core, GDFM 12001. The sandstone layer from which these samples were extracted, approximately 3 m below the surface, was poorly cemented.

The HRS fossils from the Lance Fm. used in this study came from a private ranch near Roxson, Wyoming, and belong to the Hansen Research Station (HRS), with full repository data catalogued and freely available at https://fossil.swau.edu/. An unidentified dinosaur limb bone fragment from Rose quarry and a *Lambaeosaurus* long bone fragment from the Southwest quarry are two of well over twenty thousand dinosaur and other bones and fragments from the poorly cemented and poorly sorted Maastrichtian Lance sandstone lenses. Historically, the Lance has been interpreted as a vast collection of ancient fluvial deposits (Clemens, 2014), but more recently, a subaqueous debris flow has been well-defended (Snyder et al., 2020). The latter interpretation is more friendly to the Creation/Flood model, which holds to a single, recent, and total water coverage of the globe.

Here we present additional carbon isotope signatures from Hell Creek fossils and new results from two Lance Formation dinosaur bone fossils. We

Table I. Literature survey reporting geologic formations with both primary stable carbon isotopes and endogenous biomolecular remnants.

	Geologic formation and Megasequence	Fossil feature with primary ^{13}C signature	Organic material
1	Green River, Tejas	Stromatolite laminae (Frantz et al., 2014)	Lizard keratin (Edwards, 2011)
2	Messel, Tejas	Intraleaf structures (Grein et al., 2010)	Insect cuticle (McNamara et al., 2012)
3	Hell Creek, Zuni	Dinosaur tooth enamel, gar scale enamel (Fricke et al., 2008)	*T. rex* bone collagen (Asara et al., 2007, v. 316), hadrosaur blood vessels (Ullmann et al., 2020), and Edmontosaurus collagen (Tuinstra et al., 2025)
4	Dinosaur Park, Zuni	Dinosaur tooth enamel (Fricke et al., 2008)	Theropod bone collagen (Bertazzo et al., 2015; Schweitzer et al., 2005)
5	Judith River, Zuni	Dinosaur tooth enamel (Fricke et al., 2008)	Dinosaur bone amino acids (Ostrom et al., 1990) Brachylophosaur collagen, elastin, etc. (Schweitzer et al., 2009)
6	Jehol Biota, Zuni	Dinosaur, crocodile, and turtle tooth and bone apatites (Amiot, 2015)	*Psittacosaurus* sp. probable integument proteins (Vinther, 2016), feather proteins (Slater et al., 2023)
7	Morrison, Zuni	Lacustrine carbonates (Dunagan and Turner, 2004)	Seismosaur protein (Gurley et al., 1991)
8	Emu Bay Shale, Sauk	Cyanobacteria (Hall et al., 2011)	*Gloeocapsomorpha prisca* (cyanobacterium) N-alkanes (Hall et al., 2011)
9	Gunflint Chert, pre-Sauk	Microfossil components (Willifort et al., 2013)	Cyanobacteria amide functional groups (Alleon et al., 2016)

compare these signatures to published results on fossils from HCF, Judith River, and Dinosaur Park Formations. We hypothesize that since original organics should preserve primary isotope indications, strata that have fossils with original organics are more likely than other strata to also contain fossils with primary isotope ratios.

Fossil sites were selected based on a high likelihood of verifying both primary, largely unaltered organic material (OM) and primary carbon isotopes as described in the literature. Lithology and paleofauna identify formation name equivalences shown in Figure 2. The Dinosaur Park Formation from eastern Montana and southeastern Alberta, Canada, would stratigraphically underlie the Hell Creek and Lance Formations if present in one geographic location. All three formations, plus Judith River, have already shown remarkable preservation of primary carbon sources. Fossil site selection included consideration of similarities in lithology (i.e., rock type and depth from surface to fossil) (Asara, 2007) with the assumption that lithology may contribute to both molecular and isotopic preservation.

Results and Discussion

Literature Search for Molecular and Isotopic Co-occurrence

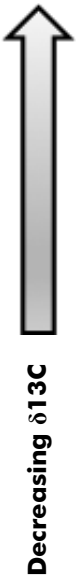
As a first step toward investigating any co-occurrence of primary molecules and isotopes in fossils, we compared literature describing primary stable carbon isotopes to reports of primary OM from the same geologic settings. Table I shows nine previously published co-occurrences of primary carbon and OM. They represent, from early Flood to late Flood deposits, pre-Sauk, Sauk, Zuni, and Tejas, from three continents. This observation suggests that whatever factors contributed to the preservation of both isotopes and biomolecules, they did so throughout depositional history and on a possibly worldwide basis. More co-occurrences will probably emerge from additional literature searches and data collections.

Sample Treatments

Seven Upper Zuni dinosaur bones were collected and portioned into 15

Table II. $\delta^{13}C$ measurements of stable carbon isotope ratios in ‰ (parts per thousand) for three chemically separated fractions of six Cretaceous System dinosaur bones identified in the table: 1) GDFM 08.027, 2) GDFM 04.001, 3) GDFM 03.001, 4) GDFM 12.001, 5) HRS 08267, 6) HRS 19114, and 7) GDFM 08.027. HTCH 06 represents a fragment of the same *Triceratops* horn core as GDFM 12001. Bone collagen extraction as per Arslanov and Svezhentsev (1993) yields mostly collagenous residue mixed with trace proteinaceous remnants. HRS samples were sourced from Wyoming's Lance Formation, and all others from Montana's Hell Creek Formation. "GDFM" samples are curated at Glendive Dinosaur and Fossil Museum, "HCTC" is taken from the literature (Armitage and Anderson, 2013), "HRS" samples are curated by Hansen Research Station, "UGAMS" sample numbers derive from the University of Georgia Accelerator Mass Spectrometer facility, and GX sample numbers derive from Geochron Laboratories.

(Numbered bone) Taxon, Description	Apatite $\delta^{13}C$	Bulk $\delta^{13}C$	Collagen $\delta^{13}C$		Lab Number
Triceratops femur GDFM 08.027			-23.8		UGAMS-03228b
Hadrosaurid femur GDFM 04.001			-22.7		UGAMS-01937
Triceratops 1 femur GDFM 03.001			-20.1		GX-32372
Hadrosaurid femur GDFM 04.001		-18.4			GX-32678
Triceratops horn GDFM 12.001		-17.1			UGAMS-11752
Triceratops 1 femur GDFM 03.001		-16.6			GX-32647
Hadrosaurid femur GDFM 04.001		-16			GX-32739
Hadrosaurid femur GDFM 04.001		-15.7			UGAMS-01936
Triceratops 1 femur GDFM 03.001	-7.24				UGAMS-17386
Hadrosaurid femur GDFM 04.001	-6.4				UGAMS-01935
Triceratops horn HCTH 06	-5.51				UGAMS-17387
Triceratops femur GDFM 08.027	-4.7				UGAMS-03228a
Triceratops horn GDFM 12.001	-4.3				UGAMS-11752a
Dinosaur HRS 08267	-2.6				UGAMS-20476
Lambeosaurus HRS 19114	-2.4				UGAMS-20477



samples. Taxa included eight samples from *Triceratops horridus*, one unknown taxon, and two hadrosaurids including a *Lambeosaurus lambei*. From these, 15 $^{13}C/^{12}C$ ratio measurements from commercial laboratories are reported in $\delta^{13}C$ and shown in Table II. $\delta^{13}C$ results from three different chemical preparation procedures were performed in order to isolate and compare within-bone fractions. One preparation isolated bone protein remnants (mostly collagen), a second isolated bioapatite carbon, and a third "bulk bone" extraction results in carbon from a mixture of bioapatite plus protein. Stable carbon isotopes from each fraction were compared to end members from corresponding biological bone fractions, expectations from diagenesis, and previously published data.

Archaeological practice has, over several decades, established decontamination procedures for bone radiocarbon analyses (Wood et al, 2010). In particular, acid plus alkaline washes (Arslanov and Svezhentsev, 1993) have demonstrated effectiveness in removing organics—humic substances for example—that may have adsorbed onto microsurfaces of the organic fraction, and thus in isolating primary collagen. Similarly, established protocols (Cherkinsky, 2009) were used to remove contaminants from the mineral fractions of our dinosaur samples. The caption for Table II lists the six specimens investigated and their extracted fractions, as well as the names of fossil repositories and laboratories used in this study.

The commercial laboratories were directed to follow updated versions of the collagen extraction protocol of Arslanov and Svezhentsev (1993), for ex-

ample, adding ultrafiltration (Brock et al., 2013). Our samples were measured beginning in 2007 and ending in 2016, thus prior to the very recent dispersion of hydroxyproline dating protocols across commercial and academic AMS facilities. In any case, this basic Arslanov method recovered no collagen in four of our seven specimens. The three that did contain collagen showed high levels of decay, with average yields of only 0.275% by weight. This falls below the minimum that most labs require for carbon dating, but since the present study is unconcerned with age-dating, the extraction of any collagenous remnants is of use for the present comparison purpose. Further, the consistency of our results across samples supports the validity of our data. For that matter, Cretaceous System collagen found here is consistent with the reports of collagen sequence from these fossils as noted above.

Evaluation of Primary Sourcing

To assess the degree of primary versus secondary carbon isotope ratios, results were first compared to known biological end members. One near proxy in the literature that compared bioapatite to collagen within the same ancient bone came from mammalian bones that showed an ~+7‰ offset (Lee-Thorp et al., 1989). Cherkinsky et al. (2023) compared collagen with bioapatite fractions from dozens of the same samples. Thirteen of various taxa excavated from arid or semiarid climates showed an average $\Delta\delta^{13}C_{apat\text{-}coll}$ of +8.39‰. Six excavated from underwater averaged +10.82‰. The authors argued that apatite dates are more reliable from arid settings, with collagen being the more reliable fraction from wet environments. In any case, our average of +17.7‰ exceeds even their 10.82, and this deserves explanation, which we attempt below.

A second assessment of our results included a comparison of our $\delta^{13}C$ measurements with those of Fricke and Pearson (2008) and Tütken (2011). Fricke and Pearson totaled 67 Hell Creek $\delta^{13}C$ enamel and dentine bioapatite measurements from ceratopsian and hadrosaurian teeth found at several microsites. They ranged from approximately -1.0‰ (their -0.4‰ outlier was removed from the data set) to -9.4‰, finding a roughly Gaussian distribution with a mean of -5.2‰. We combined Fricke and Pearson's dinosaur enamel results into one large bin. This permitted a generalized comparison between their tooth and the present bone bioapatites. Zooming out in this way also enabled visualization of the large offsets between our bone bioapatite and organic components, as shown in Figures 3 and 4. We found a lower depleted minimum for collagen of -20.1‰ and for bone bioapatite of -2.4‰, giving a $\Delta\delta^{13}C_{apat\text{-}coll}$ of +17.7‰, shown in Figure 3. Our data in Figure 4 show an upper depleted maximum for collagen of -23.8‰ and for bone bioapatite of -7.24‰, giving a $\Delta\delta^{13}C_{apat\text{-}coll}$ of +16.56‰.

Our seven dinosaur bone bioapatite $\delta^{13}C$ results, shown as the same black bars on both figures 3 and 4, ranged in Table II from -2.4‰ to -7.24‰ with a mean of -4.3‰. Fricke and Pearson (2008) took steps to demonstrate primary isotope ratios in enamel bioapatites—steps we mirrored and show in the next section. Figure 3 shows that our bone bioapatite results correspond to Fricke and Pearson's (2008) Hell Creek tooth bioapatite $\delta^{13}C$ range. Also, reports discussed below show that some burial environments appear to better preserve bone apatites than tooth apatites. Therefore, the overlap between our bone and their tooth $\delta^{13}C$ could signify that similar diageneses helped preserve primary isotopes in certain Hell Creek fossils.

Comparison of $\delta^{13}C$ in Tooth Versus Bone Bioapatite

No procedure can empirically separate diagenetic (i.e., secondary) from primary (original) carbonate from ancient or fossil bone, since burial history always remains unobserved. However, fossils contain clues to reasonable inferences. For example, Fricke and Pearson (2008) argued that diagenetic alteration would have homogenized isotope ratios from various taxa. In order to discern whether or not isotope ratios preserve at least some original signals, "it is simply enough to know that isotopic offsets and differences in correlation coefficients [e.g. $\delta^{13}C_{apat\text{-}coll}$] among animals can be preserved only if isotopic alteration does not obscure original isotopic information" (Fricke and Pearson, 2008). Their results revealed exactly these isotopic offsets and differences in correlation coefficients. Next, they found that the offset between dietary $\delta^{13}C$, inferred from organic matter extracts from sedimentary matrix, and herbivore enamel $\delta^{13}C$ from eight microsites ranging from upper Campanian to upper Maastrichtian and from western Montana to North Dakota consistently held to ~18‰. Since no known diagenetic process would manufacture such a consistent signal across such time and space, they concluded that their Hell Creek fossil carbonates retain and express primary isotopic information.

The mean apatite value from Fricke and Pearson (2008) of -4.7‰ and the bone apatite mean value of -5.9‰ differ by only 1.2‰. (Inclusion of one outlier would raise this offset to 2.1‰.) Similar arguments made by Fricke and Pearson (2008) for the primary origin of their enamel $\delta^{13}C$ signals (grey bars in Figure 3) should apply to the present bone apatite $\delta^{13}C$ values (black bars in Figure 3). In sum, the considerable overlap of our bone apatite within their tooth $\delta^{13}C$ range, as revealed in Figure 3, suggests that Hell Creek bone $\delta^{13}C$

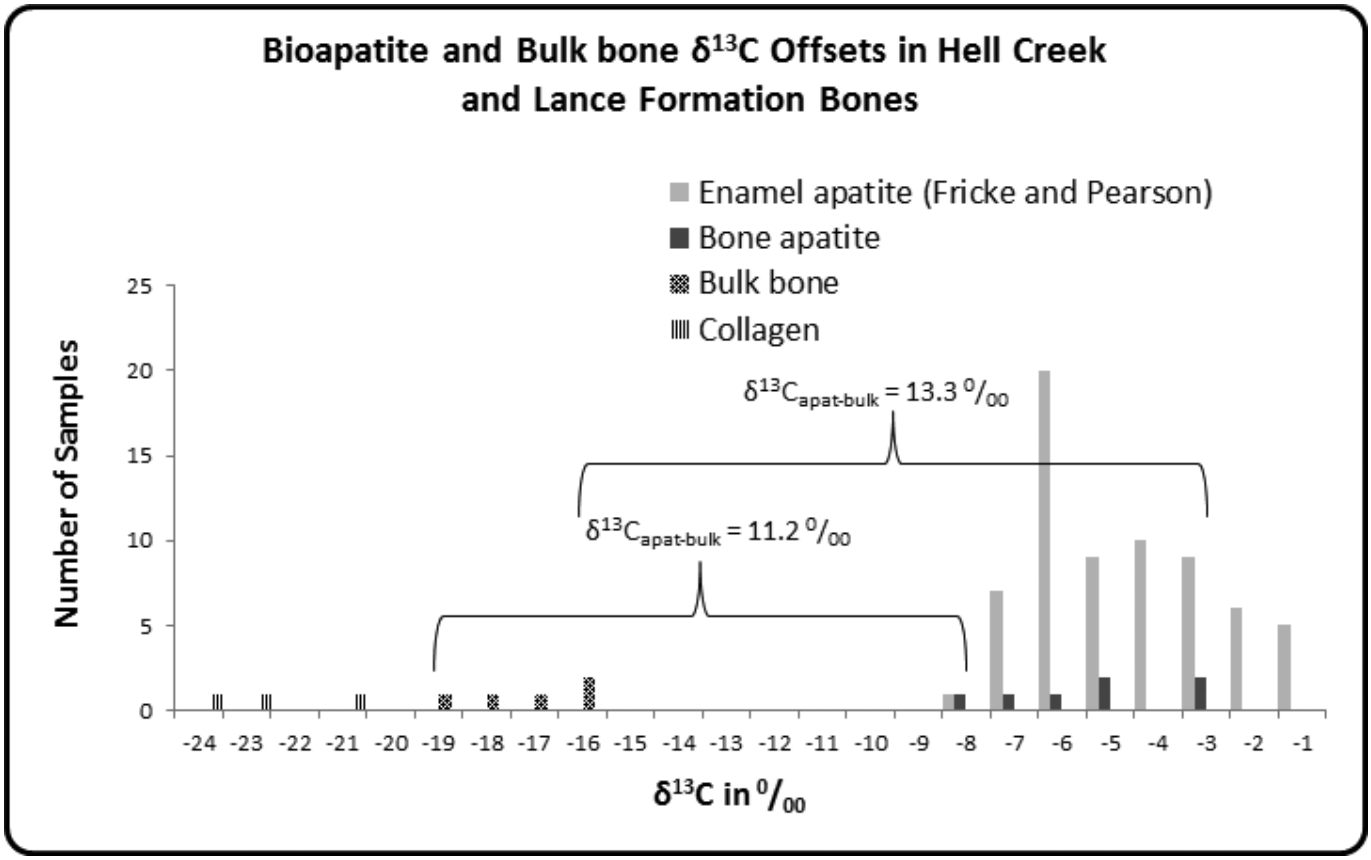


Figure 3. $\delta^{13}C$ measurements from Table II plotted with those from Fricke and Pearson (2008). Light gray bars show 67 measurements of Hell Creek ceratopsian and hadrosaur enamel and dentine bioapatite reported by Fricke and Pearson (2008). Black bars show seven measurements of hadrosaur and ceratopsian bone bioapatites. Grey bars show that one hadrosaur and two ceratopsian collagen extracts contain heavily depleted $\delta^{13}C$ levels relative to bioapatite, inconsistent with contamination through isotopic exchange (see text). Hatched bars show five results from bulk bone extracts that contain a mixture of mineral and organic bone fractions. Brackets indicate upper and lower end member offsets between apatite and bulk bone fractions.

may also preserve original isotopic signal.

Tütken (2011) found an overlap in 64 Jurassic System sauropod long-bone bioapatite and 19 tooth enamel results. We chose to plot Fricke and Pearson's but not Tütken's data in our figures because the former came from bone and the latter from enamel, and we wished to remove any variables that enamel might introduce into an already variable-rich analysis. Sauropods had a mean enamel $\delta^{13}C$ of -8.0 ± 1.2‰ (ranging from -9.1 to -4.1‰), and a mean bone bioapatite of -7.1 ± 1.4‰ (ranging from -10.9 to -4.7‰). We note, thus, that Tütken's (2011) argument for endogeneity of signal based on overlapping ranges matches our argument above. However, his argument challenges the widespread belief that tooth enamel preserves both its original protein and isotope ratios with higher fidelity than does bone.

Bone or Teeth?

The smaller crystal size of bone apatite is thought to be linked to increased susceptibility to recrystallization and diagenetic alteration. Bone bioapatites occur as flattened rods of roughly 30Å X 400Å, whereas enamel crystallites range one or two orders of magnitude larger (Wopenka and Pasteris, 2005). However, Zazzo and Saliege (2011) and Hollund et al. (2015) found evidence of isotopic exchange in archaeological tooth enamel in certain contexts. One rigorous assessment of isotope data from about 150 bone and tooth samples concluded that "the information provided by the carbon isotopic analysis of bone apatite is

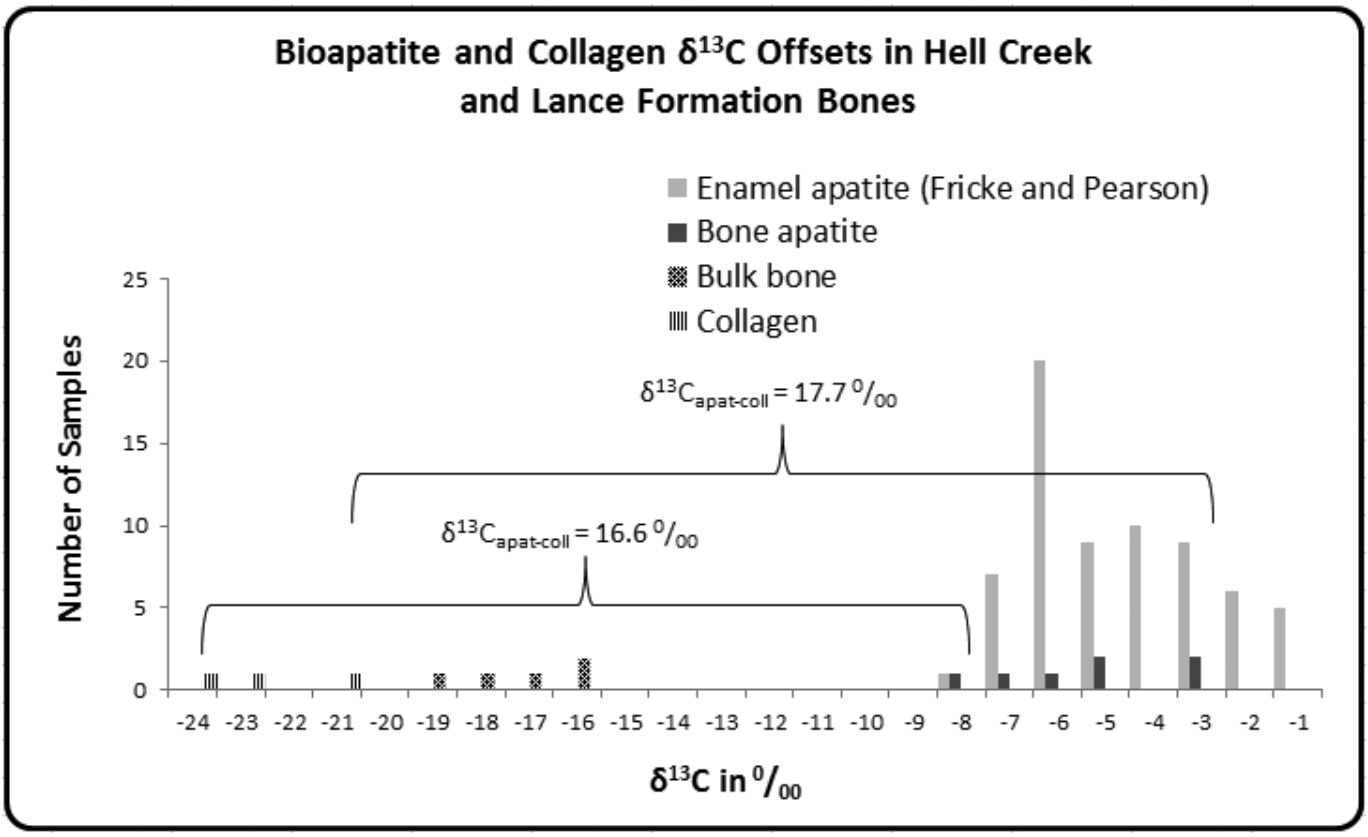


Figure 4. $\delta^{13}C$ measurements from Table II plotted with those from Fricke and Pearson (2008), as in Figure 2. Brackets indicate upper and lower end member offsets between apatite and collagen (apat-coll) bone fractions.

potentially as reliable as in enamel at least for the past 40,000 yr," a result that "seems to challenge more than 30 yr of bone and enamel diagenesis research" (Zazzo, 2014). This refers to carbon years, not calendar years. His archaeological samples of that "age" overlap 23/43 similar "ages" reported from Flood fossils (Thomas and Nelson, 2015). Thus, it appears reasonable to extend Zazzo's reliability from archaeological to paleontological bone.

Other studies lend additional support to the possibility of some preserved isotopic signal in fossil bone. For example, Lee-Thorp and van der Merwe (1987) observed that the increase of diagenetic $\delta^{13}C$ signal is "initially rapid, then slowing" toward paleontological timescales. Elsewhere, Lee-Thorp (2002) speculated regarding the variable alteration effects of burial conditions on archaeological bone: "Could the crucial difference be that the [heavily altered] specimens used in the former studies are on a pathway to destruction rather than fossilization, whereas those from Die Kelders, Klasies and Border Cave are buried in environments conducive to fossilization with minimal chemical alteration?" She observed that minimal isotopic alteration implies that recrystallization has not incorporated exogenous carbonates in the crystal structure. Instead, "Structural changes may have merely involved the rearrangement (Ostwaldian ripening) and/or incorporation of in situ 'raw material'" (Lee-Thorp, 2002). Similarly, Cherkinsky et al. (2023) made a data-derived case that isotopic exchange occurs in wetter climates. The $\delta^{13}C$ data for bone apatite presented in the current study and published reports may indicate that protection in archaeological environments "conducive to fossilization" could also apply to paleontological settings. However, these idealized conditions already approximate those used in collagen decay kinetics measurements, which, after all, represent "an optimal burial environment" (Buckley and Collins, 2011). Our results, along with others such as Tütken (2011), align with the hypothesis that paleontological bone sources may preserve more primary isotopic information than typically suspected.

Collagen and Bioapatite Comparison

The non-random and internally consistent pattern of dinosaur bone fraction offsets shown in Figure 2 matches the hypothesis that these bones preserve primary $\delta^{13}C$ ratios. Otherwise, an unlikely scenario would have to be invoked whereby a single diagenetic process altered separate bone fractions to produce the observed non-random heterogeneity between collagen and bioapatite. Similarly, bulk bone preparations supply a cross-check of the degree to which an isotope signal is primary versus secondary in origin. Bulk bone incorporates both OM and mineral bone components. Therefore, primary bulk $\delta^{13}C$ values should indicate a mixture of the isotope ratios from each fraction. Their positions in Figure 2 show values between bioapatite and collagen, as expected if the bone retains both primary isotope values and primary OM.

However, our observed dinosaurian $\delta^{13}C_{apat\text{-}coll}$ value (17‰) exceeds offsets based on modern biological bone values. The nearest proxy to our $\delta^{13}C_{apat\text{-}coll}$ offset that we could discover in the literature was biological bone fractions for (Lee-Thorp et al. 1989). They reported a ~+7‰ offset in mammalian herbivores. Our extinct reptilian herbivores exceed this by ~10‰—a significant difference. One way to explain this difference would be to invoke isotopic exchange, but that would fail to explain the consistent offsets between bone fractions, as already seen. We therefore consider instead paleobiological explanations for this difference. Fricke and Pearson (2008) offered several possibilities to account for biological carbon enrichment, including:

- Extinct hadrosaurian and ceratopsian digestive systems differed from extant mammals and birds in ways that enabled them to acquire sufficient nutrients from the tough, fibrous plant matter in their diets. Their unknown metabolic systems may have selectively incorporated heavy carbon isotopes that then enriched their bioapatites.
- Different environments, including uniquely antediluvian microhabitats, and unique dinosaurian behaviors, including competition for available resources, may have affected dietary uptake of $\delta^{13}C$.
- Marine influences could favor an abundance of C3 plants enriched in $\delta^{13}C$. Mainstream literature invokes slow changes in sea level along widespread tidal flats, whereas creation literature cites marine mixing from Flood processes. The former scenario predicts sloping shorelines that are not apparent in the strata. The latter scenario has room to speculate that pre-Flood dinosaurs may have lived near ancient seas and consumed more C3 plants back then.
- Available food may have comprised leaves exposed to direct sunlight. This canopy effect causes up to 10‰ higher $\delta^{13}C$ values compared to understory foliage. Other authors have suggested or measured additional factors that influence carbon isotope fractionation.
- Wang et al. (2014) suggested that the higher partial pressure of CO_2 indicated by Cretaceous System fossils, possibly associated with a greenhouse effect, may affect carbon isotope ratios in plant tissues.
- Fernandes et al. (2012) found that body size may positively correlate with $\delta^{13}C_{bioapat\text{-}diet}$, with pigs showing a larger apatite-diet offset than rats, and rats larger than mice. Possibly, the larger dinosaur body sizes also increased their $\delta^{13}C_{bioapat\text{-}collagen}$ ratios.
- Tütken (1987) noted known $\delta^{13}C$ differences that arise from soil moisture, water use efficiency, plant growth cycles, different plant organs, and substrate salinity.
- Jim et al. (2004) discovered a controlled isotope diet that generated a +11‰ offset between bone apatite and collagen in mice. They wrote, "On diets in which the $\delta^{13}C_{prot\text{-}engy}$ is negative, e.g. …where protein is C3 and energy is C4, $\delta^{13}C_{coll\text{-}bchol}$ [bone collagen-bone cholesterol offset] will be small (~+3‰) and both $\delta^{13}C_{apat\text{-}bchol}$ [bone apatite-bone cholesterol offset] and $\delta^{13}C_{apat\text{-}coll}$ [bone apatite-bone collagen offset] will be large (~+14 and +11‰, respectively…)."

This final scenario would explain Hell Creek herbivorous dinosaur apatite-collagen offsets if not for the fact that C4 flora are not known in Cretaceous deposits. However, possibly some dinosaurian plant sources and/or metabolisms led to uptake of protein from C3 plant sources. For example, seagrasses from inland salt marshes might be invoked, although the only known Cretaceous seagrass fossil is in the Netherlands (Voigt, 1981). In any case, a combination of some or all of these eight factors could have caused part or all of the observed 17‰ apatite-collagen offset. These paleobiological considerations do not rule out the possibility of some level of isotope exchange, but they do permit our data to remain consistent with the hypothesis of primary within-bone isotopic heterogeneities.

Cretaceous Bone Protein

Given the relative rarity of bone collagen preservation in Mesozoic sources, little to no recovery was initially expected. However, tiny amounts were recovered in three of the seven Hell Creek dinosaur bones included in this analysis. The alternative scenario to the recovery of primary proteins would be difficult to defend: i.e., that exogenous protein remnants were transported to the interior of these bones and survived

Table III. Isotopic and organic remnants correlate within the Hell Creek and Lance Formations, including the present results listed here.

	Geologic formation and Megasequence	Fossil feature with primary ^{13}C signature	Organic material
1	Hell Creek, Zuni	Dinosaur tooth enamel, gar scale enamel (Fricke et al., 2008) Dinosaur bone apatite (Thomas et al, 2019; this study)	*T. rex* bone collagen (Asara et al., 2007, v. 316), hadrosaur blood vessels (Ullmann et al., 2020), and Edmontosaur collagen (Tuinstra et al., 2025)
2	Lance, Zuni	Dinosaur bone apatite (Thomas et al, 2019; this study)	Dinosaur bone collagen (Pawlicki et al., 1966; Schweitzer, 2011; Bertazzo et al., 2015; Lee et al., 2017)

the now-rigorous industry standards for decontamination and purification to finally masquerade as endogenous collagen. On the other hand, recovery of proteinaceous vestiges aligns with the many other descriptions of dinosaur bone protein remnants reviewed above and listed in Table I. Table III shows our results alongside others from the same geologic formations and in a similar format as results summarized in Table I.

A Flood Model Explanation

On the whole, the literature demonstrates primary isotope ratios that co-occur with primary fossil organics in at least nine geologic settings, including certain Upper Cretaceous sites in Western North America. The $\delta^{13}C$ results reported here appear to add a tenth co-occurrence—this one in the Lance Fm. These observations are consistent with the suggestion of Zazzo (2014) that certain taphonomic and diagenetic conditions favor preservation of stable isotopes in bone. Future research might find that some or all of those conditions also favor molecular preservation. For example, for bone samples with apparently primary stable isotope ratios, one could predict that original organics could also be found. However, time since decay presents another qualifying factor, since primary biomaterials must be relatively young. A younger age assignment, for example, the thousands of years that attend the Flood model, would harmonize the foregoing observations:

- Broad extent of geologic formations that contain these fossils
- Rapid deep burial of fossils with apparently primary organics and isotopes
- Original (at least in part) stable carbon isotope ratios in Cretaceous System fossils
- Traces of original biomolecules that include collagen in the same
- Diagenesis that favors preservation, namely, a relative rarity of hydrothermal percolation and relatively short time available for isotopic exchange
- Relatively short half-life of bone collagen (Buckley and Collins, 2011)

The Flood models would have produced large-scale, hydrological catastrophic effects such as these broadly spread sedimentary formations. Flood models also assign most of Earth's fossiliferous sediments to a single year of rapid and successive deposition instead of billions of years. This aspect of the model is consistent with the observation that even fossils from the lowest layers contain some large degree of primary isotopes and organics. The Flood also supplies a time since burial that is three orders of magnitude more brief than mainstream expectations, which accommodates primary isotopes and organic remnants.

Conclusions

Literature examples of unaltered biological material are increasing with descriptions of apparently original molecular and even whole tissue preservation in fossil bone, including bone collagen sequence and blood vessels from Cretaceous System samples. This study adds to that trend by presenting the first report of $\delta^{13}C$ values taken directly from collagen extracts of dinosaur bone, shown in rows 1–3 of Table II. Separate studies also describe co-occurrences of primary isotopes and molecular preservation. The present study compares these heretofore disparate reports for the first time, suggesting a common cause for this observation. Next, we assessed both the co-occurrence already described in fossil bone from Hell Creek Fm. and similar co-occurrence in the equivalent Lance Fm.

In one assessment, primary $\delta^{13}C$ from dinosaur teeth was compared to dinosaur bone bioapatites from

HCF, showing congruent signals. A second assessment found broad offsets between bioapatite and collagenous extracts. Since secondary replacements should have erased such offsets, they are better explained as primary (i.e., original bone) rather than secondary (i.e., isotopic exchange) isotope signals. However, some degree of isotopic alteration cannot be eliminated in light of the challenge in accounting for the large (~+17‰) collagen-apatite offset, which would be rare in modern biological scenarios. Therefore, a further literature search was undertaken to explore paleobiological scenarios. Half a dozen of them could have produced either all or some of these large offsets that our data reveal. Recovery of sufficient collagen from which *any* isotope data could be measured, and which occurred in three of seven samples, seems to require recent burial.

A third and final assessment of isotopic endogeneity involved carbon isotope ratios from bulk bone extracts. They revealed $\delta^{13}C$ values between those of the collagen and apatite end-members, as expected if the bulk extraction indeed represents a mixture of isotopes from those two endogenous fractions. These offsets are consistent with the hypothesis that HCF and Lance Fm. bone samples preserve mostly primary isotopes. The results of these three assessments, therefore, suggest that molecular and isotopic preservations also co-occur in Lance Fm. fossils.

These conclusions lead to two outcomes. Firstly, the co-occurrence of primary carbon isotope ratios and molecular preservation can inform future investigations into preservation modes that preserve both primary isotopes and molecules. In particular, bone samples that preserve both original molecular and original isotopic remnants must have undergone a diagenetic history that favored preservation, for example, relative rarity of hydrothermal percolation. Lastly, given the biochemical barrier to the survival of original organics over deep time, even under ideal diagenetic scenarios, as well as the increasing likelihood of isotopic homogenization over time, the persistence of organics and isotopic heterogeneity found in our samples is consistent with the recent deposition of Cretaceous System fossils as deposits from Noah's Flood.

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