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Bone chemistry and bioarchaeology

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Abstract

Isotopic analysis of bones and teeth is now routinely used for dating skeletons and archaeological sites, and for diet, climate, and habitat reconstruction. Techniques of radiocarbon dating of bones and teeth developed by Harold Krueger and others during the 1960s laid the groundwork for subsequent research on stable carbon, nitrogen, oxygen, and strontium isotope analysis. We first review salient points in the history of research in bone isotope biogeochemistry, focusing on Krueger's contributions. We then discuss the significance of contributions to this volume of the *Journal of Anthropological Archaeology* for the current state of research in dietary and environmental reconstruction in archaeology, bioarchaeology, and paleoanthropology. All papers in this volume include isotopic analysis of the carbonate phase of bone and/or tooth enamel apatite for dietary and/or environmental reconstruction. Harold Krueger was instrumental in developing methods of apatite purification for removing diagenetic phases, isotopic analysis, and interpretive models of paleodiets. Apatite isotopic analysis is now an important area of bone biogeochemistry research that provides powerful tools for reconstructing human behavior in the emerging anthropological discipline of bioarchaeology.

Keywords: Bone chemistry; Radiocarbon dating; Carbon isotopes; Nitrogen isotopes; Strontium isotopes; Bone chemistry; Paleodiet; Archaeology

Introduction

Bones and teeth lie at the emerging nexus of several subdisciplines within anthropology. This new interdisciplinary field of research, which is now called bioarchaeology (Larsen, 1997), encompasses the common interests and goals of anthropologists with training and skills in biological/physical anthropology and archaeology. The morphology, pathologies and chemistry of bones and teeth of humans and other animals retain a record of their evolutionary history, life history, growth and development, environment, diet, and behavior. Some bioarchaeologists now routinely analyze the chemical and isotopic composition of bones and teeth to reconstruct past diets, environments, and migration patterns.

The foundations of chemical bioarchaeology lie squarely in the domain of isotope geochemistry, particularly the radiocarbon dating of bone, which has been marked by persistent controversies over the validity and accuracy of results (Taylor, 1992). This controversy is unfortunate because bones are often the only organic remains recovered from archaeological sites available for dating. Harold W. Krueger (''Hal'') recognized that archaeology would benefit greatly by dating more sites, and spent most of his career developing methods of improving the accuracy of radiocarbon dates, and thus stable isotope and chemical analyses of bone. The papers on paleodietary research in this volume build upon his contributions, especially the analysis of bone and tooth apatite carbonate. These papers were presented at

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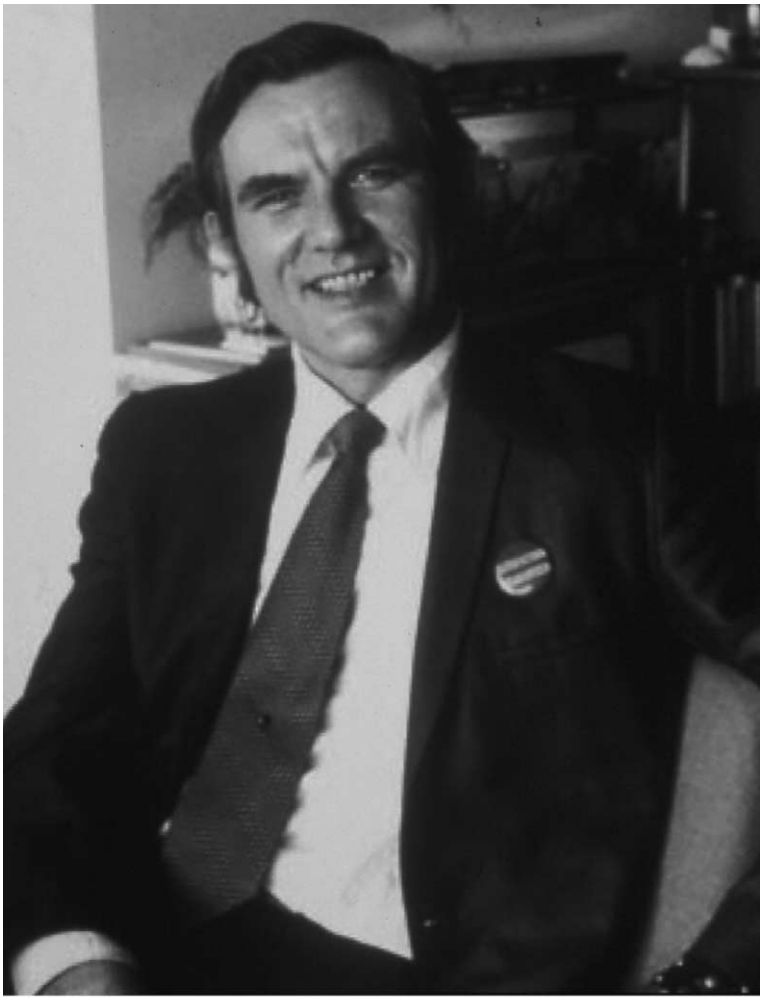

Hal Krueger

a symposium in honor of Hal Krueger held at the 66th Annual Meeting of the Society for American Archaeology (April 2001) in New Orleans, co-sponsored by the Society for Archaeological Science.

Radiocarbon dating of bone

Taylor (1992) has effectively reviewed the history of research on problems of radiocarbon dating of bone. Because techniques developed for reliable, accurate, and precise dating are also used for stable isotope and chemical analyses, it is appropriate to summarize this history here. Diagenetic alteration and contamination of bone and tooth enamel apatite are fundamental issues that are still under investigation, and we focus on these issues below.

The earliest bone radiocarbon dates were run on whole bone, without pretreatments to remove diagenetic organic contaminants such as humic and fulvic acids from decomposing soil organic matter, rootlets, fungal hyphae, etc., or to remove inorganic contaminants such as groundwater carbonates and sedimentary carbon. Because prehistoric bones have variable proportions of indigenous organic (collagen, other proteins, and lipids) and inorganic carbon (carbonate in the apatite mineral), and different proportions of diagenetic contaminants of different ages, whole bone dates were often grossly inaccurate, as judged by the standard of agreement with associated dates on charcoal. Isolation of the organic fraction, by removing the carbonate mineral phase, produced more accurate dates. Krueger (1965) proposed demineralization at low temperature and pressure, with relatively weak acids, to increase collagen yields. Although accuracy improved, "collagen" dates were sometimes younger than expected because the acid-insoluble residue comprised collagen and other proteins, plus varying amounts of soil organic and inorganic residues. Heating in weak acid dissolves collagen, and precipitates humates, permitting filtration of particulate contaminants and humates, and produces a cleaner protein extract called gelatine (Longin, 1970). Treatment with sodium hydroxide, which is a standard pretreatment step to remove humic acids from charcoal, improved accuracy considerably, but some humic contaminants remained bound to collagen (Stafford et al., 1988). With the development of accelerator mass spectrometry (AMS) methods of radiocarbon dating, small amounts of carbon from amino acids of collagen that were purified by chromatographic separation after high temperature hydrolysis with concentrated HCl could be dated with high precision and accuracy (Stafford et al., 1988). Hydroxyproline, an amino acid found only in collagen, is the gold standard for assessing collagen preservation, and for dating controversial finds such as extinct mammals at Paleoindian butchery sites.

The history of radiocarbon dating and stable isotope analysis of bone apatite carbonate is far more controversial. Indeed, Krueger himself was initially critical: "... dating of bone which show any form of significant ground-water alteration can be accomplished only by utilizing their collagen content. It is useless to date the carbonate fraction." (Krueger, 1965, p. 336). He subsequently developed a method of pretreatment of the apatite phase (calcium phosphate) of bone to remove the post-mortem carbonate contamination with weak acetic acid. This method is based on two properties of the bone mineral. First, carbonate (CO_3) is more soluble than apatite in acetic acid. Second, carbonate occupies two kinds of positions in the apatite mineral crystal structure. Adsorbed carbonate on apatite crystal surfaces is more soluble than structural carbonate, which is incorporated within the apatite crystalline lattice (Krueger, 1991; Lee-Thorp and van der Merwe, 1991). Greater solubility of adsorbed carbonate permitted removal of diagenetic carbonates from apatite, and often increased accuracy of radiocarbon dates. Krueger was one of the only radiocarbon specialists who routinely dated bone apatite. One notable improvement of the acetic acid pretreatment technique was reaction of apatite under vacuum to evacuate diagenetic carbonate gas before it could exchange with structural carbonate (Krueger, 1991).

Because strontium is chemically similar to and substitutes for calcium, differential solubility of carbonate and apatite is also the basis for recovering biogenic, in vivo amounts of strontium for Sr/Ca analysis of trophic levels, and of $^{87}Sr/^{86}Sr$ analysis of mobility and residence patterns (Price et al., 1994; Sealy et al., 1995; Sillen and LeGeros, 1991; Sillen et al., 1995).

Bone is porous and soft, and apatite crystals are very small in comparison to tooth enamel. Over long periods of time, or when bone is burned or recrystallized, irreversible exchange of structural carbonate and fixation of adsorbed carbonate occurs, rendering bone apatite useless for analysis. Mature tooth enamel is non-porous and hard, and crystals are large and dense, and thus far less susceptible to diagenesis (Balasse, 2002; Passey and Cerling, 2002; Wang and Cerling, 1994).

When diet reconstruction with apatite carbonate stable isotopes was first proposed by Sullivan and Krueger (1981) it was rejected by a number of researchers (Nelson et al., 1986; Schoeninger and DeNiro, 1982). Krueger and Sullivan (1984) subsequently demonstrated that some of the variability in apatite isotopic composition was due to trophic level differences rather than diagenesis, and to the methods used for sample preparation, which recrystallized the apatite, irreversibly binding the post-mortem contaminants (Krueger, 1991; Sillen and Sealy, 1995). With appropriate pretreatment procedures, and well-developed methods for identification of diagenesis (Lee-Thorp, 2000; Koch et al., 1997;

Kohn et al., 1999; Sponheimer and Lee-Thorp, 1999a), isotopic analysis of apatite of bone is now widely considered valid, especially where collagen is also preserved. Analysis of mature tooth enamel is now routine on teeth up to 50 million years old (Bryant et al., 1996; Cerling et al., 1997; Sponheimer and Lee-Thorp, 1999b).

Dietary and environmental reconstruction with stable isotopes

The early development of research on diet reconstruction with stable carbon isotopes is chronicled by van der Merwe (1982), and subsequent developments are reviewed by many others (Ambrose, 1993; Katzenberg, 2000; Koch et al., 1994; Pate, 1994; Schoeninger and Moore, 1992). Based on the observation that maize (a C_4 plant) often had anomalously young radiocarbon dates (high $^{14}C/^{12}C$), and that its $^{13}C/^{12}C$ ratios were also elevated compared to other plants (C_3 plants), Robert Hall (1967) proposed using stable carbon isotope analysis of bone for estimating maize consumption. This was first demonstrated in Late Woodland eastern North American societies by Vogel and van der Merwe (1977). The field has since diversified to include isotopic analysis of nitrogen isotopes in collagen (DeNiro, 1987), carbon and strontium isotopes in apatite (Krueger, 1991; Sillen et al., 1995), and strontium:calcium ratios and barium:calcium ratios in apatite (Burton and Price, 2000).

Environmental effects on carbon and nitrogen isotope ratios have been thoroughly investigated, in order to assess dietary and habitat influences on bone isotope ratios. For example, the canopy effect, leads to lower foodweb $\delta^{13}C$ values in closed, humid forest understories, and hot, open, dry habitats have comparatively high $\delta^{13}C$ and $\delta^{15}N$ values (Ambrose, 1991; Tieszen, 1991).

Sullivan and Krueger (1981) first proposed the use of carbonate stable carbon isotopes based on the linear correlation between collagen and apatite carbon isotopes. They observed an offset of about +12‰ between diet and apatite $\delta^{13}C$ values, and a smaller offset for carnivores. They proposed a sophisticated model to explain the apparent trophic level effect (Krueger and Sullivan, 1984), involving differences in the carbon isotope ratios of dietary macronutrients (fats, carbohydrates, and proteins), and isotope effects of amino-acid metabolism. Controlled diet experiments were conducted to test these models (Ambrose and Norr, 1993; Tieszen and Fagre, 1993), and refined models are described in this volume by Ambrose et al., Krigbaum, Lee-Thorp and Sponheimer, and van der Merwe et al., and by others (Ambrose et al., 1997; Hedges, 2003; Schwarcz, 2000).

In summary, the experimental studies show that when proteins and non-protein macronutrients have the same $\delta^{13}C$ values, collagen is enriched by +5‰ and apatite by ca. 9.5‰. In mammals (mainly ruminant herbivores) with symbiotic digestive microbes that produce large amounts of methane, apatite is enriched relative to diet by 13.5‰, but collagen enrichment is unchanged, so herbivores have higher apatite-collagen difference values. Experiments also show that the isotopic composition of collagen is controlled mainly by that of protein, while apatite faithfully reflects whole diet carbon isotope ratios. When the protein source has less ^{13}C than the bulk diet, large apatite-collagen difference values (>4.5‰) result. The typical diet of prehistoric farmers in eastern North America, which included ^{13}C-enriched foods with small amounts of protein, such as maize, combined with ^{13}C-depleted high protein resources such as deer and freshwater fish, produces large difference values. Conversely diets with ^{13}C-enriched protein and ^{13}C-depeleted non-proteins produce small collagen apatite difference values (<4.5‰). Bones of high latitude marine coastal foragers, consuming ^{13}C-enriched fish and shellfish plus ^{13}C-depleted terrestrial C_3 plants and C_3-feeding animals, have low difference values.

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These principles are illustrated by the results of the studies reported in this volume, which we now briefly discuss.

The growing corpus of stable isotope data from fossil tooth enamel apatite is steadily improving the paleoecological and paleobiological context of Plio-Pleistocene hominids. Schoeninger et al. present important new data for herbivore tooth enamel recovered from the Pliocene hominid site of Allia Bay in northern Kenya. ^{18}O data suggest greater amounts of rainfall in the Lake Turkana region 3.9 my, while ^{13}C data suggest more closed forest conditions but overall similar C_3 and C_4 mixed habitats characteristic of the region today. Results have important implications for generating hypotheses regarding the adaptive/dietary mechanism and ecological context associated with early hominids.

Diagenesis of bone apatite in Pleistocene and older fossils is considered inevitable. However, Lee-Thorp and Sponheimer show that some fossils, while shifted away from carbon and strontium isotope ratios expected for their diets and from their tooth enamel, still retain enough of their original biogenic composition to preserve the carbon isotopic differences seen between grazing (C_4-feeding) and browsing (C_3-feeding species). They conclude that bones with moderate crystallinity indices may have sequestered their biogenic signal soon after burial.

Human burials dating to the apogee of development of social complexity of Mississippian society at Mound 72 at Cahokia (Ambrose et al.) show that high status individuals had high protein diets with small amounts of

maize. Conversely, low status burials, apparently ritually sacrificed young females, many with skeletal indicators of nutritional stress, had low protein diets. Their relatively low collagen $\delta^{13}C$ values suggest they consumed only slightly more maize, but very high apatite $\delta^{13}C$ and apatite-collagen difference values suggest low status individuals at low protein diets, with maize as the primary staple. This study, conducted by Krueger, produced the largest diet-related difference in chemical composition between social groups ever reported. Without apatite isotopic analysis, the amount of maize in low status diets would have been severely underestimated.

The collagen carbon isotope record of eastern North America shows a rapid, widespread increase in consumption of maize after 1100 BP. Harrison and Katzenberg take advantage of the sensitivity of apatite carbon isotopes to the total diet rather than the protein source, to identify the initial low levels of consumption of maize following its introduction to southern Ontario before 1500 BP. This kind of study should be conducted using early Late Woodland populations in the Midwest USA, where small amounts of maize have been directly dated to ca. 2000 BP. The high apatite-collagen spacing values observed among Late Woodland farmers contrasts with the low values of marine foragers from San Nicholas Island, California.

The Moatfield Ossuary site, reported by van der Merwe et al., is a prime example of the practice of minimally invasive bioarchaeology of culturally sensitive prehistoric sites. This study may also illustrate the additional dimensions of diet revealed by collagen and apatite carbon isotopes. Analysis of associated fish bones provides useful baseline information for food web stable isotope end-members of late prehistoric agriculturalists in southern Ontario. High nitrogen isotope ratios for humans demonstrate heavy reliance on fish, but high collagen carbon isotope ratios also demonstrate heavy reliance on maize. The authors of this study calculate similar percentages of maize consumption from apatite and carbon isotope ratios, using results of a controlled diet study with pigs. However, if the diet-apatite relationship obtained in studies cited above is used, then maize consumption was somewhat higher.

Models of vertical interactions between communities of the Pacific coast and the Andean Altiplano, and horizontal interactions between coastal polities have strongly influenced archaeological research agendas in Ecuador, Peru, and northern Chile. Paula Tomczak has analyzed large samples of Chiribaya culture populations from sites in the Osmore Valley at different distances and altitudes from the coast, and documented substantial isotopic shifts over short distances. Nitrogen isotope values decrease and apatite-collagen difference values increase systematically from the coast to the interior, as expected where diets comprised different proportions of marine protein and terrestrial C_3 and C_4 resources. These data suggest that local communities obtained much of their dietary resources locally.

In Tierra del Fuego, Yesner et al. found a pattern of inter-individual isotopic differences in collagen and apatite nitrogen and carbon isotope composition similar to that in the Osmore Valley. This study is valuable because it provides an independent line of evidence for prehistoric diet which demonstrates that ethnographic and ethnohistoric data on post-contact subsistence provides an inaccurate picture of the diets of prehistoric populations.

Northern Borneo, with its hot, humid climate, is a hostile environment for bone collagen preservation, so the prospects for paleodietary analyses are presently limited to isotopic information obtainable from tooth enamel. Because of the high isotopic fidelity of this tissue, and because the carbon isotopic composition of the diet is relatively monotonous (virtually all C_3), Krigbaum was able to assess the canopy effect on foodweb carbon isotope ratios, and thus to reconstruct changes in environment from closed forest foraging to open forest and cleared field agriculture. This kind of study should be feasible in other tropical closed-forest ecosystems, where agricultural societies have flourished and declined, and forests have reclaimed ancient farmlands.

Conclusion

The studies in this volume represent a small subset of studies within the growing field of skeletal paleobiogeochemistry in bioarchaeology. If Hal Krueger had succumbed to conventional wisdom, and had he abandoned his efforts to extract reliable geochemical information from ancient bones and teeth, none of the papers in this volume would have used bone apatite carbonate for dietary and environmental reconstruction. We all owe Hal a nod and a wink for the added dimensions to past lifeways made possible by his efforts.

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