The biochemistry and microbiology of buried human bone, in relation to dietary reconstruction

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Summary

This paper presents a survey of the background to the biochemical and microbiological study of human bone, reviews previous and current applications of this work, and discusses the implications for the reconstruction of past human diets. Information has been drawn from a range of published sources, not all of them easily accessible to the archaeological community. It is intended that this paper should provide a summary and reference point for students and researchers with an interest in palaeodiétary studies, but without a specialist biochemical background or time to undertake a literature search themselves.

Introduction: bone chemistry and diet

Attempts have been made to reconstruct the diets of prehistoric communities from a study of their skeletal tissue using two approaches: the study of stable isotope ratios (mostly either carbon or nitrogen) in the organic matrix and the analysis of trace elements in the inorganic bone mineral.

The isotopic method is based on two observations: (i) bone collagen isotope ratios (13C/12C and 15N/14N) reflect the corresponding isotope ratios in the animal's diet (DeNiro and Epstein 1978; 1981); and (ii) different foods have characteristically different isotope ratios depending on the base of the food web (Van der Merwe 1982; Schoeninger and DeNiro 1984). Stable carbon and nitrogen isotope ratios have therefore been used to distinguish between a reliance on terrestrial and marine food sources in the diet of prehistoric humans. Carbon isotope ratios have also been used to distinguish between the consumption of C3 and C4 plants (defined below; see also DeNiro and Epstein 1978; Van der Merwe 1982). Nitrogen isotope ratios can also reflect the differential utilisation of nitrogen-fixing organisms (legumes and N2-fixing cyanobacteria) and non-nitrogen fixing plants (DeNiro and Epstein 1981).

The inorganic fraction of bone contains a ring of trace elements, some with presumed dietary significance. The first element to be so identified was strontium (Sr), which is still the most important element in palaeo-diétary reconstruction. Other potential indicators are zinc (Zn), magnesium (Mg) and barium (Ba), although the behaviour of these elements in the body is not well understood. There are also other elements which may be deposited in bone, such as lead (Pb) and copper (Cu). The analysis of which may provide information about the general health of the individual.

This paper seeks to summarise the biology and chemistry which underlie palaeodietary studies, and to draw together and critically review recent developments in the field.

Background: the structure of bone (Figure 19)

Bone is a structural tissue in a living 70 kg adult human, the skeleton will weigh approximately 10 kg (Snyder et al. 1976). In
life, it performs a number of functions: (i) it provides support for soft tissue; (ii) it protects delicate organs such as the brain; (iii) it allows movement by providing points of attachment for muscular tissue; (iv) it acts as a reservoir for the storage of essential elements, and, to some extent, acts as a repository for unwanted elements (Armelagos et al. 1989, 230).

It is a highly specialized composite material, which exhibits as principal characteristics rigidity and strength, while retaining a degree of elasticity. These properties derive from bone's structure: a complex mineral substance (containing chiefly calcium, phosphate, hydroxyl, citrate and carbonate ions) deposited within a soft organic matrix, composed largely of the protein collagen. Although the composition is variable, the average composition of dry compact bone is 70% (by weight) insoluble organic matter, 20% organic matter and 10% water (i.e. that lost below 105°C).

Figure 19. The microscopic structure of bone (based on Anthony and Thibodeau 1984).
The organic matrix

Approximately 90% (by weight) of the organic fraction is made up of a fibrous structural protein of the collagen family. Collagen is widely distributed throughout the connective tissue of the body, and are largely responsible for the strength and elasticity of such tissues.

Proteins are polypeptides consisting of interconnected chains of amino acids, linked by peptide bonds. Amino acids have the general formula:

\[
\text{H} \quad \text{R} \quad \text{NH}_2 \quad \text{COOH}
\]

where R is one of a number of organic radicals— the simplest being H, giving glycine (Gly), the simplest member of the amino acid family. The peptide bond is formed when the amine (NH₂) radical of one amino acid links to the acid radical of the next, with the elimination of a water molecule:

\[
\text{H} \quad \text{R} \quad \text{NH}_2 \quad \text{COOH} + \text{H} \quad \text{R} \quad \text{NH}_2 \quad \text{COOH} \rightarrow \text{H} \quad \text{R} \quad \text{NH} - \text{CO} - \text{NH} \quad \text{R} \quad \text{H}
\]

This elimination reaction can continue until a protein chain has been formed containing many thousands of amino acid residues. The protein is characterised by the sequence of amino acids, labelling from the nitrogen-containing radical end of the chain. There are 23 "natural" amino acids found in protein, but collagens typically only contain about 17 of these. The dominant sequence of collagens is the repeated unit: glycine (Gly) — proline (Pro) — hydroxyproline (Hypro) resulting in an average composition of 33-38% Gly, 6-13% Pro and 9-17% Hypro in most kinds of collagen, with the other amino acids together making up less than half of the total residues. Hypro is an unusual constituent in that, of all the proteins, it only appears to be a significant component of collagen.

Collagen is made up of a rope-like structure consisting of three polypeptide chains, twisted together in a right-hand helix (polypeptides are chains of amino acids linked by peptide bonds). Each individual chain is twisted to the left, one turn per three residues (thus aligning the glycine molecules at every third residue), with ten turns of each chain per turn of the triple helix. The relatively small size of the glycine molecule makes for a tightly twisted chain, and the strength of the triple helix derives from hydrogen bonding between the amide nitrogen of glycine in one chain and a non-glycine carbonyl oxygen in the adjacent chain. There are seven or eight common sequences of amino acid chains in collagens, most simply described as types 'a' to 'g' (McGillivary and Goldstein 1983, 164) and collagens in different tissues are made up of different combinations. The most common collagen (referred to as Type I) makes up approximately 90% of body collagen, and occurs in bone, tendon, cornea, soft tissue, and scar tissue. It is made up of two chains of type 'a' plus one of type 'g', and is therefore described as a.g. The majority of the rest are made up of three identical chains, such as Type III (d) occurring in blood vessel walls.

Bone collagen fibrils have an average molecular weight of 300,000 Daltons (a Dalton is the mass of one atomic unit, with a length of 260 nm and a diameter of 1.4–2.0 nm. In collagen fibrils, the fibrils are aligned head to tail, with a gap of 40 nm between fibrils and a stagger of 65 nm (one quarter of the length of the molecule) between adjacent rows. This gives rise to the characteristic 65 nm banding that is visible in electron micrographs of collagen. Mature collagen is insoluble in water because of the cross-linking between adjacent polypeptides in the helix. Solubility increases however, as the protein is denatured (i.e. as the overall molecular weight is reduced), as when bones are boiled for soup.

The other organic components of bone (approximately 10 weight percent) are often grouped together as non-collagenous proteins (ncp). Carbohydrates include proteoglycans (predominantly glycosaminoglycans), consisting of protein combined with acidic carbohydrates. Common in dentine (a major constituent of teeth), but not in bone, are phosphoproteins, with an unusual amino acid composition of 50% serine and 40% aspartic...
acid, and a total phosphorus content of 26 weight percent. Other proteins include osteocalcin, in which the glutamic acid side chain has been carboxylated. These so-called 'gla' proteins are the major component of the apatite fraction in bone. The lipid component in dense and detached bone makes up only about 0.1 weight percent of the tissue in bone, by contrast; three-quarters of this is triglyceride, the rest predominantly cholesterol (Williams and Elliott 1989, 366).

The mineral phase

The principal components of the inorganic phase are calcium and phosphate ions. It is poorly mineralized (i.e., having much amorphous material and small crystals), but is normally described as calcium hydroxyapatite \((\text{Ca}_x\text{PO}_4\text{OH})_3\). Apatites have the general formula \(\text{Ca}_x\text{(PO}_4\text{)}_yX_z\), where \(X\) is commonly either \(\text{OH}\) or \(F\). Fluorapatite is a relatively common mineral, but hydroxyapatite is rare outside the animal kingdom. Apatites are ionic crystals (apart from the covalent phosphate ion), with the phosphate ions forming hexagonal close-packed sheets, with sheets stacked in the 'ala' system (i.e., the third layer ions are directly over those in the first layer). This kind of structure has two types of spaces (intersitial vacancies) within it, labelled A and B. The A position is octahedral in structure; running all through the structure in hydroxyapatite, two of three of these channels are occupied by Ca\(^{2+}\) and the other by \(\text{OH}\). The same channels are lined with the remaining Ca\(^{2+}\) ions, leaving in a perfect crystal, the B position (the octahedral sites) unfilled. This structure is therefore very porous, so that dead bone easily takes up ions from the groundwater.

Pure hydroxyapatite is difficult to synthesise, and in biomimatisation a number of substitutions are common, which distort the hexagonal structure. Any ions may be involved, providing they are of closely similar size and, less importantly, charge. Thus, AsO\(_4^{3-}\) will substitute freely for PO\(_4^{3-}\), HPO\(_4^{2-}\) and CO\(_3^{2-}\) will substitute in a limited way, Sr\(^{2+}\), Ba\(^{2+}\) and Pb\(^{2+}\) will substitute freely for Ca\(^{2+}\), Na\(^{+}\), H\(_2\)O (and ion vacancies) in a limited way, and K\(^{+}\) and Mg\(^{2+}\) in a restricted fashion. Hydroxyl ions are freely substituted by halide ions (fluoride, F\(^-\), chloride, Cl\(^-\), bromide, Br\(^-\), and iodide, I\(^-\)), but only in a limited way by H\(_2\)O and vacant sites. Double substitutions can also occur—for example, two hydroxyl ions can be replaced by CO\(_3^{2-}\) or O\(_2\)\(^-\).

Synthetic hydroxyapatite crystals can be made with sites in the millimetre region, but commonly they only have dimensions of a few microns, particularly if they are non-stoichiometric (i.e., the ratio of ions does not conform to the formula for the mineral), as is common in biosynthesis. In bone, the typical crystal has a needle shape, of length 15-75 \(\text{nm}\) and diameter 5 \(\text{nm}\) (Sillen 1989, 213). This has a number of important consequences in life: it has been calculated that 1 gram of mineral has a surface area in excess of 100 \(\text{m}^2\) (McLean and Urist 1968, 61), so a 70 kg human will have a total surface bone crystal area of approximately 0.5 \(\text{m}^2\). This implies that this vast mineral surface is bathed by only a few litres of body fluid, and thus that most metabolic processes must be the result of surface phenomena.

The calcium minerals in bone are poorly crystalline in situ, as well as being small in size: in mature rat femur, the crystallinity has been estimated as 65% (McLean and Urist 1968, 65). Other phases may be present, including whitlockite \((\text{Ca}_3\text{PO}_4\text{F})_2\), monemite \((\text{Ca}(\text{HPO}_4\text{)}_2\text{H}_2\text{O})\), \(\alpha\)-talc calcium phosphate \((\text{Ca}_3\text{(HPO}_4\text{)}_2\text{PO}_4\text{)}\cdot\text{H}_2\text{O}\) and amorphous calcium phosphate, \(\beta\)-talc, which may contain the structural unit \(\text{Ca}_3\text{(PO}_4\text{)}_2\) (Williams and Elliott 1989, 328). In addition, substitution may occur, especially of the hydroxy in hydroxyapatite by fluoride ions, to yield fluorapatite, which is the most suble of the apatites. Bone also contains significant quantities of carbonate (2.5% by weight) and citrate against Ca\(_3\text{(PO}_4\text{)}_2\)\(\cdot\)CO\(_3\), or more likely, as surface-adsorbed species on the apatite minerals (especially in the case of citrate, which is too large to be substituted).

Bone metabolism

At the highest level, two major types of bone are recognised in most vertebrate groups: (a) cancellous, trabecular or spongy bone and (b) compact or cortical bone. Cancellous bone is characterised by a porous structure, consisting of a network of trabeculae (struts). The distribution of cortical and cancellous bone throughout the skeleton is governed largely by biomechanical considerations. In general, cancellous bone is found beneath cortical bone, surrounding the blood-rich marrow
at the epiphysial ends of long bones. Cancellous bone is supplied with blood by a network of arteries entering from all sides and branching into the bone marrow. For the external, cortical bone, four major types have been recognised: woven bone, lamellar bone, haversian bone and fibrolamellar (plexfiform or laminar) bone (Currey 1984). In adult humans, haversian bone predominates, in which blood vessels and nerves, housed in vascular canals, are surrounded by a cement sheath and concentric layers of bone (the lamellae). Except in certain locations, living bone is covered by connective tissue—externally, by the periosteum, on internal spaces (e.g. bone marrow channels, cancellous trabeculae and haversian canals) by the endosteum. Growth involves deposition on the outer (periosteal) surfaces, as well as at the epiphyses (ends of bone), and resorption takes place from the internal (endosteal) surfaces.

When a living tissue, bone contains a number of specialised cells carrying out a variety of functions. Mineralisation takes place through the activity of osteoclasts, which occur on the surface of growing or developing bone, in a continuous layer in active bone. Bone production proceeds by the deposition of bone mineral onto collagen fibrils. Mineralisation of hydroxyapatite from the extracellular fluid probably proceeds via an amorphous calcium phosphate precursor, with the Ca/P molar ratio rising from 1.5 to 1.67 in the fully mineralised hydroxyapatite. As the osteoclast becomes surrounded by calcified tissue, it becomes an osteocyte, which probably regulates the release of bone mineral into the bloodstream. The structural organisation of human bone (after the first year of life) results in new bone being laid down in lamellae, arranged in a cylindrical pattern around the haversian canal system, in units called osteons, which are usually orientated along the long axis of the bone. The collagen fibrils of each lamella run spirally along the axis of the canal. In a human femur, these osteon bundles are approximately 0.25 mm in diameter. The outer surface of compact bone is surrounded by a circumferential layer of osteons under the periosteum and the endosteum.

Osteoclasts are giant multinuclear cells which usually occur on the surface of the bone, associated with osteoclastic bone resorption. They usually lie in lacunae, which are thought to result from erosion by the osteoclasts themselves. Resorption is the result of removal of both the mineral fraction (by acidic action, or the result of a chelating agent) and the organic matrix (as a result of the action of a collagenase enzyme, which breaks down and renders soluble the collagen). In a normal adult, approximately 3-5% of the skeleton is remodelled at any one time, with turnover being more rapid in cancellous bone (Armelagos et al. 1988, 235). The cycle of resorption and rem minimisation can take from three months in a year, according to skeletal component, age and health.

In vivo, bone is bathed by the blood plasma and the intercellular fluid which, combined, make up 20% of the body weight. This serves to transport oxygen and carbon dioxide around the cells, and also to exchange minerals between the bone and the fluid. The intercellular fluid contains, in a 70 kg individual, approximately 530-700 mg of calcium ions, compared with 2.2 kg in the skeleton. Adult body plasma contains approximately 10 mg per 100 ml calcium (half as ions, half as protein complexes) and 3 mg per 100 ml total phosphorus (mostly in the form of HPO4 ions). The recommended adult daily intake of calcium is 0.8 g, although as little as 20% of the dietary content may be utilized—the rest being eliminated in the faeces. The daily requirement of phosphorus is approximately 1 g (McLean and Urist 1968, Ch. XI).

Bone is therefore a reservoir for both calcium and phosphorus, allowing mobilisation via osteoclastic resorption when needed. Various other chemical elements, many with no known physiological function, reside in the skeleton, although sodium and magnesium may be accessed when needed by other tissues. Nearly half of all body sodium is stored in skeletal mineral, but potassium is not a bone-seeking element. Other ingested elements, especially strontium and lead, are stored in the skeleton. Fluoride ions have a high affinity for bone mineral, converting hydroxyapatite to fluorapatite.

Biochemical background
Carbon dioxide fixation pathways in plants (Figure 20)
Photosynthetic plants trap carbon dioxide and assimilate it by one of the following mechanisms, or occasionally by a combination of two:
1. Calvin-Benson cycle (C₃ photosynthesis)

The first step is the carboxylation (i.e. the addition of CO₂ to an existing organic molecule) of the 5-carbon sugar ribulose 1,5-bisphosphate (RuBP). This forms an unstable 3-carbon compound, which hydrolyzes spontaneously to form two molecules of 3-phosphoglyceric acid (3-PGA). The 3-PGA thus produced is further phosphorylated by the enzyme phosphoglycerate kinase to yield 1,3-diphosphoglyceric acid (1,3-dPG). The 1,3-dPG is phosphorylated by the enzyme triokinase to give 3-phosphoglycereraldehyde (3-PGAL). A fraction of this PGAL is further converted by triokinase to 1,3-bisphosphoglycerate (1,3-BPG) and NADP⁺ to give 3-phosphoglycerate (3-PG). The 3-PG is then converted by aldolase to dihydroxyacetone phosphate (DHAP). Fructose-1,6-bisphosphate (F⁺1,6-BP) is synthesized from the remaining PGAL, and the synthesized DHAP, and glucose is produced from this by a sequence of enzymatically-catalysed reactions. Algae autotrophic bacteria, and most terrestrial and aquatic higher plants assimilate CO₂ using this mechanism.

2. Hatch and Slack cycle (C₄ photosynthesis)

In this mechanism, there is a preliminary carboxylation in the mesophyll cells of the leaf. Carbon dioxide is trapped during the day and converted into the three-carbon organic acid phosphoenol pyruvic acid (PEP), giving a four-carbon acid, malic acid. This is transferred to the cells surrounding the vascular bundle (the bundle sheath cells), where it is decarboxylated to release CO₂. This is then assimilated by the Calvin-Benson cycle described above. The C₄ cycle is found mainly in tropical grasses and is an adaptation to exploit higher light levels and temperatures and to counter limited water availability.

3. Crassulacean acid metabolism (CAM)

A third pathway, mainly found in succulents, and important only in certain tropical ecosystems, involves the synthesis of malic acid by carboxylation at night, and the subsequent daytime breakdown of the malic acid to liberate CO₂ for photosynthesis. In darkness, stored carbohydrates are broken down by glycolysis to PEP, which is carboxylated to malic acid, which is stored in the vacuole. In the daytime, the malic acid is decarboxylated to yield pyruvic acid and CO₂, which is then used in the normal Calvin-Benson cycle.

Carbon isotopic fractionation

Variations in the isotopic composition of natural carbon are due to two fractionation mechanisms:

(i) a kinetic effect, resulting in the depletion of the heavier ¹³C isotope during the assimilation of CO₂ by photosynthetic plants;

(ii) an enrichment of ¹³C in marine bicarbonates, and eventually in limestone, when compared with atmospheric CO₂.

The main features of the observed variations in the ¹³C/¹²C ratio in plant material can therefore be explained by systematic differences between marine plants, C₃ terrestrial plants, C₄ tropical plants, and CAM plants (Van der Merwe 1962). Since the ratio of ¹³C/¹²C is small, it is normal to discuss variations in this ratio using the δ notation, where δC is defined as follows:

\[
\delta^{13}C = \left( \frac{\text{measured/standard}}{\text{standard}} - 1 \right) \times 1000 \text{ per mil}
\]

where the reference standard is the ratio of ¹³C/¹²C in a particular carbonate rock (Pee Dee Belemnite, PDB). A similar expression is used to define F viper, with the ratio ¹⁵N/¹⁴N replacing the carbon ratio. For nitrogen the reference standard used is taken to be the ratio of ¹⁵N/¹⁴N in atmospheric nitrogen. Using this notation, increasingly positive δ values imply an enrichment of the heavier isotope, and vice versa.

Dietary implications

Because of the discrimination against the heavier isotopes of carbon in terrestrial plants, the δC values for these plants are more negative than those found in atmospheric carbon dioxide. Thus δC in atmospheric carbon dioxide is -6 to -7 per mil, whereas in terrestrial C₃ plants it is between -22 and -34 per mil (Tauber 1981; DeNiro and Epstein 1978). Animals feeding on these plants should show similar values, although observations by Van der Merwe (1989) have shown a difference of ~5 per ml in animals feeding on C₃ plants, and ~6 per ml for C₄ plants.
consumption. This difference (fractionation) probably results from isotopic variation in the dietary components in the same organism, protein and carbohydrates are found to have the same isotopic value, but lipids are isotopically lighter. Normally, lipids are not used in the manufacture of bodily protein (in this case, collagen), but care must be taken to ensure that the correct fractions are being compared (Chisholm 1989).

In a marine environment, the absorption of \( \text{CO}_2 \) and the subsequent production of hydrogen carbonates and eventually carbonates is governed by kinetic factors which enrich the carbonate in the heavier isotope, giving marine carbonates a 8\(^{18}\)O value of about 0 per mil (i.e., more positive than atmospheric \( \text{CO}_2 \)). When marine organisms assimilate these carbonates (and hydrogen carbonates) by photosynthesis, there is discrimination against the heavier isotope, giving a 8\(^{18}\)O value of around -10 to -18 per mil in marine plants, again becoming less negative as the carbon is passed up the food chain.

There should therefore be a simple distinction between the 8\(^{18}\)O values of animals whose food chain is based on either C\(_3\) or C\(_4\) terrestrial plants or on marine plants, and it is this distinction which has been exploited in the carbon isotopic characterisation of the diets of ancient communities. Chisholm (1985) has suggested the use of collagen 8\(^{18}\)O end-point values of -20 to -7 and -13 per mil respectively for C\(_3\), C\(_4\), and marine communities, allowing the approximate calculation of the relative importance of each food source in cases where one variable may be eliminated, or the contribution calculated from other information.

Further refinement in this analysis may be possible, since a number of amino acids are considered to be essential, i.e., they are required for protein formation, but cannot be synthesized by the body. These essential amino acids are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (McGillivary and Goldstein 1983, 318). These must be obtained directly from dietary intake of protein, and therefore comparison of the isotopic composition of one or more of these amino acids with that of the 'non-essential' components may yield further detail (Silen et al. 1989).

Nitrogen isotope systematics (Figure 21)

Based on their nitrogen assimilation mechanism, living organisms can be divided into three categories:

(i) Atmospheric N\(_2\)-fixing organisms (legumes and cyanobacteria)

Since atmospheric nitrogen is the defined standard for the isotopic ratio, and little fractionation occurs during nitrogen fixation, 8\(^{15}\)N is approximately 0 for these organisms. This should be true irrespective of whether the plants are terrestrial or marine—published 8\(^{15}\)N values for terrestrial nitrogen-fixing plants show a mean of +1 to +2 per mil, whilst that for marine nitrogen-fixing plants is between 0 and +3 per mil (Schoener and DeNiro 1984). N\(_2\)-fixing cyanobacteria and the zooplankton which feed upon them have lower 8\(^{15}\)N values than the phytoplankton, which do not directly fix nitrogen, and the corresponding zooplankton which feed upon them (Wada 1980). Fish feeding on coral reefs, areas noted for large concentrations of cyanobacteria, have 8\(^{15}\)N values much lower than fish of equivalent trophic position in the open ocean (Schoener and DeNiro 1984).

(ii) Terrestrial organisms (other than those subsumed in (i))

The second group contains the majority of terrestrial plants and consequently the animals which feed directly or indirectly on them. The major sources of nitrogen for terrestrial plants are inorganic nitrogen and ammonium ions. Nitrogen uptake by plants involves very little fractionation, and most non-nitrogen fixing plants have 8\(^{15}\)N values between 0 and +6 per mil (DeNiro and Epstein 1978).

(iii) Marine organisms

The third group contains all the marine organisms, excluding cyanobacteria. In deep oceans, denitrification (reduction of nitrates and nitrites to nitrogen) occurs, with relatively large fractionation. The remaining nitrates are thus enriched in 15N, and therefore the nitrate utilized by plankton at the base of the marine food chain is richer in the heavier isotope than that used by terrestrial plants. This enrichment is carried up the food chain, causing marine phytoplankton, zooplankton and fish to have 8\(^{15}\)N values more positive than those of terrestrial organisms (Sweet et al. 1978).
Figure 21. The nitrogen cycle.
Dietary implications

This suggests that the stable nitrogen isotopic ratio in bone collagen can be used to distinguish between three potential food sources: nitrogen-fixing plants, terrestrial plants (non-nitrogen-fixing) and marine plants (non-nitrogen-fixing). The biochemical mechanisms of nitrogen assimilation are, however, much less well understood than those relating to carbon assimilation, although the position is potentially simpler in view of the fact that nitrogen only occurs in dietary protein. In a study of both prehistoric and historic human bones, Schoeninger et al. (1983) showed that historic Eskimo populations with a primarily marine food intake had collagen δ¹⁵N values ranging from +7 to +20 per mille, whilst those in historic European and Mesopotamian agriculturalists ranged from +6 to +12 per mille.

Diet and trace elements in bone

In addition to the major elements required to support life (calcium, magnesium, phosphorus, sodium, potassium and chlorine), fifteen trace elements are now regarded as essential: chromium, cobalt, copper, fluorine, iodine, iron, manganese, molybdenum, selenium, zinc, arsenic, lithium, nickel, silicon and vanadium (Arimemogos et al. 1989; 240). These elements often play a part in enzymatic catalysis, and deficiencies can result in various abnormalities, although excesses can also be deleterious. Other elements absorbed from the diet can be regarded as extraneous (e.g. strontium), or disadvantageous (e.g. lead and cadmium), and are either excreted or deposited in the skeleton.

More than 99% of the strontium in vertebrate animal tissue is found in the mineral component of the bone (Schroeder et al. 1972). In animals, strontium is discriminated against (by renal excretion) in favor of calcium for the synthesis of bone tissue (Comar et al. 1977). Price et al. (1985) reported that the ratio of strontium to calcium in the bone of a herbivore is approximately five times lower than that ratio in plants consumed by the animal. Carnivores further discriminate against strontium, so that their bones exhibit a lower ratio still. Omnivores exhibit strontium ratios intermediate between those of herbivores and carnivores, in proportion to the relative importance of plants and meat in their diet; this should be the case with human strontium levels. The situation is somewhat different in the marine environment, where higher strontium is found in organisms at the base of the food chain in oceanic waters (Rosethil 1963). Palaeodietary studies of coastal populations from Alaska have suggested that strontium concentrations can be used to distinguish between the importance of marine and terrestrial organisms in subsistence (Connor and Slaughter 1984).

Zinc is also potentially a useful palaeodietary indicator. Hatch and Geidel (1985) suggest that high zinc levels in bone arise from a larger dietary contribution from animal sources (since blood and flesh are rich in zinc). Gilbert (1977), however, reported that both strontium and zinc can reach extremely high levels in certain nuts and shellfish, as can other elements such as vanadium, copper and manganese. In addition, humans subsisting wholly or largely on a marine diet might ultimately be expected to have higher zinc levels than these subsisting on a terrestrial diet, through the concentrating effect of sea-water as noted above. Magnesium, being another alkaline earth metal, has also been suggested as a possible dietary indicator (Lambert et al. 1979). Lambert et al. (1982) suggested that the elements most closely connected with diet are strontium, zinc and magnesium, and they noted that similar correlated levels of all three were found in both the ribs and femora of the same individual.

Applications of palaeodietary reconstruction

There is an extremely large and rapidly-growing body of literature on palaeodietary reconstruction, best summarised in Price (1989). Trace element studies have been carried out on European populations (Antoine et al. 1988a; Renz 1987; Tauber 1981). North America (Hatch and Geidel 1985; Lambert et al. 1979; 1982; Schoeninger 1979; and others), and in the Middle East (Schoeninger 1981; 1982). Stable isotope analysis of bone collagen has been widely used since its introduction by Vogel and Van der Merwe (1977) to reconstruct the diets of prehistoric humans and animals (e.g. Ambrose and DeNiro 1986; Antoine et al. 1988a; 1988b; Burleigh and Bridwell 1978; Chisholm et al. 1982; 1983; Sealey and Van der
Bone diagenesis

It has become increasingly clear in all this work, and has occasionally been forced to be pointed out (e.g. by Hatvock et al. 1989), that buried bone may be subject to considerable alteration and many authors have doubted that the dietary signal is retained in these cases. Considerable effort has been expended on the detection and elimination of diagenetic effects. Several strategies have been recommended, but none has been proposed as uniformly applicable.

The simplest case appears to be that of collagen. It has been known for many years that collagen gradually disappears from buried bone—in fact, the gradual removal of protein (monitored by the decrease in nitrogen content) has been used as a method for the relative dating of bone (Lynne and Jeffries 1962). It has generally been accepted that the isotopic ratios $\delta^{13}C$ and $\delta^{15}N$ of bone collagen are free of diagenetic effects (Chisholm 1989). Ambrose (1991) has demonstrated that the C:N ratio in bone 'collagen' remains at modern levels (2.9–3.5, expressed as an atomic ratio) providing a significant quantity (greater than 2% of the total bone weight) of 'collagen' remains in the bone. He uses the term 'collagen' to refer to the whole of the glycosylated residue from bone, which may be derived from other proteins in addition to collagen. It is possible that the 2% cut-off for the stability of the C:N ratio implies that at this stage only non-collagenous proteins is left. This work suggests that, providing structurally-intact collagen is obtainable from the bone, the isotopic ratios are likely to be unchanged from the same ratios in vivo, as required for palaeodietary research.

The situation with the trace elements in bone is considerably more complicated, and the dietary significance of measurements on excavated bone is still the subject of debate. Several authors have studied variation in trace element composition as a function of skeletal component (e.g. ribs versus femora, Lambert et al. 1982), and variations in trace element distribution in bone cross-section (Lambert et al. 1984). These latter authors found iron, aluminium, potassium, manganese and magnesium to be concentrated along the outer margins of the bone, indicating that these were post-depositional contaminants, but concluded that the uniform distribution of zinc, strontium, lead, sodium and calcium confirmed that these elements are not enriched post mortem. Analysis of associated soil, however, showed increased levels of calcium and magnesium around the bone, indicative of leaching.

Lambert and others have asserted that careful cleaning and removal of the outer layer of bone yields material which can be safely analysed and interpreted from a dietary viewpoint. Measurement of the stable isotope ratios of strontium ($^{87}Sr/^{86}Sr$) in modern and prehistoric marine and terrestrial animals has, however, yielded evidence for the post mortem contamination of buried bone by strontium from ground water (Nelson et al. 1986). They studied the strontium content in the bener of modern seals and reindeer, and found the seal bone to be richer in strontium (about 1000 ppm, compared with less than 200), in accordance with the model of the transmission of strontium through trophic levels outlined above. In excavated bone, however, there was no difference in strontium content between seal and reindeer—both had the order of 900-1000 ppm. The strontium isotopic ratio was also different in modern terrestrial and marine mammals, but uniform in excavated bone. This is interpreted as being the result of the interaction of the buried bone with a common source of strontium, probably ground water.

Several studies have concentrated on the mineralogy of excavated bone. In view of the poor crystallinity and large surface area of living bone, it is not surprising mineralogical changes take place post mortem, which will affect the trace element composition (Pollard et al. 1991). Hancock et al. (1989) and others have stated that deviations from the figure of 2 for the Ca/P weight ratio in fired archaeological bone signifies diagenesis, and Sillen (1989) suggests that an increase in Ca/P may result from recrystallisation of the hydroxyapatite to other calcium phosphates of differing solubilities, and the deposition of calcite crystals into the bone. It is not clear what effect these processes will have on the dietary indicators—strontium, zinc, magnesium, and so on, but it is likely that recrystallisation will reduce the trace element concentrations in hydroxyapatite (since recrystallisation is a standard purification technique in chemical synthesis), although external contamination might subsequently
increase them, depending on the composition and pH of the groundwater. Turner et al. (1986) have shown that bone cement levels and hydroxyapatite crystals are both increased with time for animal skeletons exposed on the surface for ten years.

It has become common to analyse soil from the burial context in an effort to assess the potential exchange between soil and bone. Pate and Hutton (1988) have emphasised the need to take soil chemistry into account in this process, considering the availability of exchangeable ions rather than the total soil composition. This requires an understanding of the physics and chemistry of soil, including factors such as the soil pH and temperature, the presence or absence of organic matter, as well as the geochemistry of the ionic species present and local groundwater movement.

The microbiology of buried bone

Clearly, the diageneis of excavated bone is still an area requiring considerable research effort. A particularly important aspect which has so far received very little attention is the effect of microbial and fungal attack on buried bone. Very few chemical studies have taken into account the implications of such microbiological decay: the results of such attack are often recognised, but cannot be included in the assessment of diageneis because of the lack of detailed microbiological information (e.g. DeNiro 1983; Price et al. 1985; Pate and Hutton 1988). Saprophytic soil fungi (including Macr and Faustium species) have been reported as being associated with microscopic focal destruction (tunneling) in archaeological bone (Marchafava et al. 1974; Hackett 1981; Pieperbrink 1986; Grupe and Piepenbrink 1988), but no model has been put forward for the mechanism involved.

Tunneling is probably the result of a process similar to osteoclastic resorption, and the bone mineral is probably dissolved by organic acids produced by microorganisms as a by-product of metabolism. If the micro-organisms are utilising the bone protein as their only source of both organic nitrogen and organic carbon, as seems likely, then those microorganisms capable of producing collagenase enzymes (i.e. enzymes which cleave the collagen field) are most likely to produce tunneling, since this will provide a means of attacking the collagen and breaking it down for utilisation. The number of bacteria and fungi known to be capable of producing collagenase is slowly increasing, but detailed work on bacterial attack is only just beginning (Child and Pollard 1991). Recent work (Child, in preparation) has isolated several micro-organisms from soil, human insects and non-human human tissue which are capable of producing collagenase at 10°C (taken to be a representative soil temperature in temperate regions). One of these (Pseudomonas fluorescens from soil) which produces a very active collagenase, inoculated onto sterile pig carpal has caused considerable degradation within nine weeks.

The implications of this and related work for those investigating extracted archaeological protein could be significant. The work on bacterial attack was initiated as a result of an unanticipated scatter in the determination of age at death using the recrystallisation of aspartic acid in dental collagen (Gillard et al. 1991). Blind tests on modern individuals gave good results, but identical measurements on excavations of unknown age from a church crypt (Christchurch, Spitalsfields, London; time elapsed since death approximately 200 years) occasionally gave deviant ages, even in multiple determinations from the same individual. Since deviation was observed in both directions, microbiological activity was suspected, even in this relatively uncomplicated burial environment. Amino acid sequencing of the extracted collagen showed no apparent change, and consequently microbiological racemose activity was considered; this work is progressing in parallel with the detection of collagenase production.

It is known that microbiological attack on protein can take one or more forms, including cleavage into large fragments, gradual reduction of chain length (both resulting in increased solubility), removal and modification of specific amino acids and peptide sequences, and racemisation. It seems likely that work of this nature will be necessary to study many aspects of archaeological protein chemistry, including radionuclei dating and stable isotope palaeodietary reconstruction. Little work has yet been attempted on the trace element implications of microbiological colonisation, although the possibility of introduction of contaminants has been considered in connexion with the spread of fungal hyphae through the bone.
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References


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