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Effect of Mollusc Eating on Human Bone Strontium Levels

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Empirical aspects of the movement of strontium through the food chain suggest that the level of bone strontium can be used as an indicator of the percentage of meat in human diet. In general, skeletal remains from agricultural peoples are expected to have high bone strontium levels relative to hunter-gatherers from the same geographical region because plants contain relatively higher amounts of strontium when compared with animal products. The results of the study described in this paper, however, indicate that the inclusion of molluscs as a component of the diet may produce the opposite of the expected strontium values. Burials from an Archaic (*c.* 2500 BC) hunting-gathering population excavated from Lu°25, an archaeological site in northern Alabama, USA, exhibit a mean bone strontium level ($\bar{X}$=465 p.p.m. atomic absorption; $\bar{X}$=475 p.p.m. neutron activation) that is higher than the mean level from an agricultural Mississippian (*c.* AD 1400) population ($\bar{X}$=295 p.p.m. atomic absorption; $\bar{X}$=255 neutron activation) that was buried at the same site. The samples were analysed by two techniques (atomic absorption spectrometry and neutron activation analysis) and the results compared favourably; therefore, the results can be accepted as valid rather than being due to technique error. We propose that the ingestion of molluscs, whose meat is known to contain large amounts of strontium, has produced this reversal from expected results.

Keywords: DIETARY RECONSTRUCTION, BONE STRONTIUM LEVELS, MOLLUSC EATING, AMERINDIAN, ARCHAIC, MISSISSIPPIAN, ATOMIC ABSORPTION SPECTROMETRY, NEUTRON ACTIVATION ANALYSIS.

Introduction

The amount of strontium in human bone can be a sensitive indicator of the relative composition of the diet of prehistoric human populations (Brown, 1973; Schoeninger, 1979*a*, *b*). There are, however, certain aspects of the fractionation of strontium through the food chain which must be considered before the level of strontium in human bone

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can be used as a specific indicator either of dietary composition or of a population's position in the food chain. The assumption that agriculturalists can be separated from hunter-gatherers by the relatively greater amounts of strontium in the former's skeletal material may be overly simplistic. An example is provided through measurement of the level of stable strontium in the bones of a hunter-gatherer population and an agricultural population that, although separated in time by 3000 or more years, lived and then buried their dead in the same archaeological site. Because the diet of the hunter-gatherers included molluscs, whose flesh contains relatively high levels of strontium (Schroeder *et al.*, 1972), we expected the levels of bone strontium within this population to be higher than would be predicted based on their position in the food chain. As expected, the level of strontium in their bones was significantly higher (1·5 to 1·8 times) than that of the agriculturalists, a marked reversal of the levels predicted by the most simplistic food chain model.

The argument for the use of strontium as an indicator of diet begins with environmental strontium (Toots & Voorhies, 1965). Although the sedimentary cycle of strontium appears to be stable, the distribution of strontium throughout the physical environment is uneven (Odum, 1951; Odum, 1971). The amount of strontium in living organisms is determined by the amount of strontium present in the soil and by biotic factors. In general, the ratio of strontium to calcium in plants is the same as that in the soil, although some plants discriminate against strontium and others concentrate it (Bowen & Dymond, 1955; Vose & Koontz, 1955; Comar *et al.*, 1957). Because the distribution of strontium is uneven throughout the physical environment, the same species of plant may contain different amounts of strontium when grown on different soils.

The level of strontium in the bones of terrestrial vertebrates reflects the level of strontium in their diet minus the large and constant percentage that is eliminated through excretion, lactation or placental transfer. Unlike calcium, strontium does not appear to be under homeostatic control in vertebrates; a constant percentage of dietary strontium crosses the gut and enters the blood stream (Comar & Wasserman, 1964; Berg, 1972). There seems to be no initial discrimination against strontium by bone mineral (hydroxyapatite) and no differential removal of strontium relative to calcium from mature bone mineral (Neuman *et al.*, 1963; McLean & Urist, 1968; Marchall *et al.*, 1973; Reeve & Hesp, 1976); therefore, the amount of strontium in bone is a reflection of the amount of strontium available in the blood (Underwood, 1977). As a result, the amount of strontium in the bones of terrestrial vertebrates is ultimately determined by the level of strontium in the diet. Since 99% of the strontium stored in the body is sequestered in bone mineral while the soft tissues contain very low amounts of strontium (Schroeder *et al.*, 1972), a diet composed largely of meat should contain a relatively low level of strontium. As a consequence, herbivores will have a larger amount of strontium in their diet and ultimately in their bone mineral than will carnivores who subsist on animal flesh which contains very little strontium.

The above generalization is true only when both the herbivores and carnivores are located within the same geographical region and are part of the same biotic community. Because of the uneven distribution of strontium throughout the physical environment, the bone strontium levels of skeletal populations from different geographical regions cannot be directly compared. In addition, comparison of bone strontium levels in a modern skeletal series with those of a prehistoric series may not be meaningful for two reasons: (1) the geographic location in which the two populations lived may be vastly different, and (2) the modern transportation industry provides a diet which comes from the four corners of the continent if not the world. An additional exception to these generalizations about plants and animals is provided by aquatic molluscs. Ophel (1963), who traced ^{90}Sr in the community metabolism of a lake, demonstrated that this isotope

was concentrated 730-fold in the meat of freshwater molluscs (see also Kulebakina, 1975).

Anthropologists have used the level of strontium in bone either as an indicator of a population's dependence on domesticated grasses or to show differential consumption of vegetable foods and animal protein between social strata within agricultural societies (Brown, 1973; Gilbert, 1975; Schoeninger, 1979*a*, *b*). These analyses depended on the assumption that, in general, agricultural populations would have ingested and retained significantly higher amounts of strontium from their plant diet than would omnivorous hunter-gatherers, who, in turn would have incorporated greater amounts of dietary strontium than would hunters almost wholly dependent on animal products (e.g. Netsilik and Copper Eskimo; Balikci, 1968; Damas, 1972). We recognize the problems in assuming that hunter-gatherers necessarily must ingest greater amounts of animal products than must agriculturalists. However, given the amount of animal bone deposited in Archaic period sites, the assumption probably is sound for this portion of North America. Such archaeological evidence, used in conjunction with trace element analysis, is the best indicator of prehistoric diets since, as discussed in Lee & DeVore (1968, pp. 92–94), the diet of extant hunter-gatherers distributed in marginal areas may not be indicative of the diet of prehistoric hunter-gatherers living in areas where game was abundant. On the other hand, even if the general assumption is accepted, the linear carnivore to herbivore scale may not adequately classify the diet of the population if molluscs had been incorporated in the diet.

In order to investigate the effect of molluscs in the diet on the level of strontium in bone mineral, this element was measured in a small sample of skeletal remains from two populations, one of which regularly consumed molluscs, the other of which did not. To minimize unwanted environmental variation in the sources of strontium, both samples were drawn from burials interred in the same archaeological site. This site, Lu°25, was a large shell mound situated on the north end of Seven Mile Island, Lauderdale County, Alabama (Webb & DeJarnette, 1942, 1948). The prehistoric utilization of this site spanned a period from before 3000 BC to after AD 1400. There were, however, two major occupations within this time span: one in the Late Archaic period (*c.* 2500 BC); the other in the Mississippian period (*c.* AD 1400). Almost all of the 1025 burials excavated from this site prior to its inundation by the closing of the Pickwick Dam can be assigned to one or the other of these periods. Ribs were taken from three late Archaic burials and three Mississippian burials. Only burials with clear archaeological context

Table 1. Measures and sample statistics for Sr in bones of Archaic and Mississippian burials at Lu°25

	Burial No. (Snow)	Sex	Age	Strontium (p.p.m.) Atomic absorption	Strontium (p.p.m.) Neutron activation
Mississippian	178	M	56–75	324	236
	401	F	20–22	297	277
	609	M	22–26	263	250
				$\bar{X} = 295$	$\bar{X} = 255$
				$s = 31$	$s = 21$
Archaic	574	F	25–30	462	415
	530	M	45–50	478	471
	086	M	21–35	454	538
				$\bar{X} = 465$	$\bar{X} = 475$
				$s = 12$	$s = 61$

were considered, and the individuals were matched as closely as possible for age and sex (Table 1). In this way differences in diet became the major source of variation between the two samples.

Late Archaic populations of the Middle Tennessee Valley, like other "Riverine Archaic" groups in the eastern United States, were predominantly gatherers and hunters of wild foods, although horticulture did play a minor role in their subsistence pursuits. The majority of their diet came from wild plants, including small seeds, nuts, and animals including deer, small mammals, turkey, migratory waterfowl, fish, turtle and molluscs (Asch, Ford & Asch, 1972; Ford, 1974, 1977; Marquardt & Watson n.d.; Dye, 1977). Although molluscs were a minor component in the diet as a whole, they were a seasonally important source of food.

In the Middle Tennessee Valley the Late Archaic populations exploited 56 species of shallow water molluscs and 22 species of fresh water snails (Morrison, 1942). If, as has been estimated, 25% of the shell mounds were composed of shell, then Lu°25 contains approximately 10,000 m^3 of snail and molluscan remains. Despite this mass of shell, deer and other animals contributed far more protein and calories to the Late Archaic diet than did molluscs (Morse, 1967; Parmalee & Klippel, 1974; Dye, 1977). Molluscs, however, provided an important source of vitamin C, niacin, iron and potassium (Watt & Merrill, 1963; Parmalee & Klippel, 1974), and as Morse (1967) suggests, they probably served as an important short-term staple when other sources of food failed.

In the 3000 years that elapsed between the Late Archaic and Mississippian periods, the major change in diet was the growth of an agricultural regime which displaced the breadth of the Late Archaic subsistence system. Corn, beans and squash became the dominant vegetable foods, although nuts and wild plants retained an important place in the diet (Griffin, 1967; Ford, 1974, 1977). The exploitation of animal species retained essentially the same order of importance between the two periods, and deer held their pre-eminent position in the diet (Smith, 1975, 1978). Molluscs, however, seem not to have been utilized by Mississippian populations in the Tennessee Valley, although they continued to be locally abundant. In brief, between these two periods, the proportion of plant to animal foods shifted even more in favour of plants, and molluscs were eliminated from the subsistence system. The former change should increase the level of strontium in bone mineral, the latter should decrease the level of strontium markedly.

Beyond the conceptual and empirical problems noted thus far, one of the major limitations in the analysis of strontium in bone is the lack of any recognized standards with a bone-like matrix, such as those available from the National Bureau of Standards for trace elements in other kinds of biological samples (e.g. NBS Bovine Liver SRM1577). In the absence of such standards, the unknown samples should be analysed by two independent methods and the results compared (Morrison, 1976). Such comparison provides a check on random errors that may result from the interaction of a specific method with the matrix of the sample. The check is necessary because when the matrix of the standard differs from the sample matrix the two react differently to any given analytical technique (see Szpunar, 1977, pp. 59–67). The samples from Lu°25 were analysed by both atomic absorption spectrometry and neutron activation analysis. The second technique was considered doubly important in light of Helsby's (1974) demonstration that strontium values were depressed in the analysis of teeth by atomic absorption spectrometry, even when precautions were taken against ionization and interference.

The sample for analysis by atomic absorption spectrometry was a weighed portion of bone (*c.* 500 mg after drying to a constant weight) which was disolved in 3 ml of concentrated HCl. An aliquot (0·2 ml) of the completely dissolved bone was diluted

20 : 1 in 3·8 ml of 10% trichloracetic acid which contained 1% La and 0·5% K. The prepared sample was analysed by a Varian Techtron with a nitrous oxide acetylene flame. The wavelength was set at 460·8 nm, the lamp current at 10 mA, and the slit width at 0·5 Å. The percent absorbance was compared to that of prepared standards.

The sample for neutron activation analysis was bone that had been dried to a constant weight, ground in an agate mortar, and then ashed at 600°C. Approximately 50 mg of each sample was heat-sealed in suprasil quartz tubing, which had been sterilized by boiling for several hours in aqua regia. The samples, a blank, and two standards were irradiated in the reactor pool for 30 hours. Three weeks after removal from the pool each sample was counted for two hours. The radionuclide that was used to measure strontium concentration was strontium—85 ($T_{\frac{1}{2}}=62·5$ days) which emits gamma rays of 514 keV.

The results (Table 1) indicate that there was a significant difference in the amount of strontium in the Mississippian and Archaic skeletons as measured by both atomic absorption and neutron activation (AA, $t=7·28$, df$=4$, $0·01>P>0·001$; NAA, $t=4·81$, df$=4$, $0·01>P>0·001$). The similarity of results produced by the two techniques (Mississippian NAA *v.* AAS, $t=1·52$, df$=4$, $0·4>P>0·2$; Archaic NAA *v.* AAS, $t=0·24$, df$=4$, $0·9>P>0·5$), indicates that the observed levels are valid and not the result of technique error.

Diagenesis does not appear to be a likely explanation for the observed differences for several reasons. First, Parker & Toots (1980) found no difference in the strontium content of enamel, dentine and bone of fossil *Subhyracodon*. If post-mortem chemical changes had occurred, the denser enamel should have had a different composition than the other two materials since it would be less subject to chemical alteration. Second, Wyckoff & Doberenz (1968) compared strontium content in animal bone from the sites of early humans in the western United States, pleistocene animals from California and Arizona, Tertiary animals from Arizona, and even older fossils from all over the world. Where possible the bone strontium levels in these animals were compared to the bone strontium levels in their modern dietary analogues. There was no significant difference in bone strontium levels between the time periods, although there were significant differences in these levels in animals from different geographical areas and between animals ingesting different diets. Third, Nelson (1967) investigated the possibilities of solution and redeposition of strontium in prehistoric clam shells from shell mounds on the Clinch and Tennessee rivers. These shell mounds are temporally and geographically close to the mound from which we took samples. Nelson concluded that no diagenesis had occurred because a heterogeneous distribution of strontium among annual layers was preserved. If measurable solution and redeposition had occurred, this heterogeneity would have been obliterated. In reference to the human skeletons, if diagenesis had occurred and the element followed concentration gradients, the movement of strontium should have been out of the bone. Nelson reports levels of strontium in midden clam shells that are lower than the levels we found in bone, therefore, if solution and redeposition had occurred, the expected result would be a lowering of the amount of strontium in the Archaic skeletons toward equilibrium with the clam shell.

Eliminating technique error and diagenesis as probable explanations, the difference in bone strontium levels is probably due to a dietary shift from the earlier to the later time period. This explanation is in agreement with the remainder of the archaeological record. The change in the level of strontium, however, is not in the direction that would have been predicted: the agriculturalists have much less strontium in their bones than the hunter-gatherers. We believe that the inclusion of molluscs in the diet of the hunting-gathering population is the reason for this reversal.

These results suggest that trace element analysis of human bone, especially the analysis

for strontium, if it is to yield measures of dietary composition, must be carried out in conjunction with some prior knowledge of the range of plants and animals consumed by the population. If organisms such as molluscs that concentrate strontium are included in the diet of one group but not another, then, although these groups might occupy broadly similar positions in the food chain, the strontium analysis would assign them to different trophic levels. In the case discussed here, the Archaic hunter-gatherers would be classified as far more "herbivorous" than the Mississippian agricultural population. In addition, until standards are developed for the trace element composition of human bone, two different techniques of trace element analysis should be employed—one serving as the standard and check for the other.

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