THE PUEBLO GRANDE PROJECT, VOLUME 6:

THE BIOETHNOGRAPHY OF A CLASSIC PERIOD HOHOKAM POPULATION

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ELEMENTAL VARIATION IN SUBADULT REMAINS FROM PUEBLO GRANDE

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Anthropologists and clinicians alike have become involved in the study of trace elements in human bone. Bone is a dynamic tissue that provides the body support, leverage, protection, and storage (Shipman, Walker, and Bichell 1985), and it is this last function in which trace-element analysis is most interested.

Bone is composed of an inorganic and organic fraction. The organic fraction consists largely of collagen; the inorganic fraction is made up of hydroxyapatite crystals and numerous minor and trace minerals. Nutrients, including trace elements, are transported via extracellular fluid surrounding blood vessels in the haemispheric canals into compartments of interstitial fluids in the canaliculi and lacunae (Martin and Burr 1989). Free ion exchange occurs between the fluids and the hydration shell surrounding the hydroxyapatite crystals. Trace elements thereby pass into the bone matrix.

Many of these elements assist in normal bone physiology. For example, copper, manganese, and zinc (Zn) have been related to proper bone formation (Underwood 1977), silicon to skeletal maturation (Carlisle 1986; Cutress 1983), and vanadium, strontium (Sr), and fluorine to dental health (Hadjimarkos 1973; Navia, Aponte-Merced, and Punyasingh 1988; Nielsen 1986). The normal physiology of other tissues as well as electrolyte balance are maintained by the transport of minerals into and out of bone tissue.

Bone, in its capacity as a mineral reservoir, has provided anthropologists with a means for measuring ancient dietary and disease patterns. The trace-element composition of bone reflects those elements incorporated into the matrix at the time of mineralization during growth and lifelong remodeling, as well as those that diffuse into the tissue after mineralization is complete (Curzon 1983). Although physiology, internal crystal chemistry, and environmental factors all may influence elemental concentrations, Schroeder (1965) found that diet played the largest role in the trace-element uptake in humans. In this manner, bone provides a composite record of lifetime metabolic interactions. Therefore, "the same features that make [bone] resistant to degradation, make it an excellent repository of past biological activity" (Armelagos et al. 1989).
APPLICABILITY TO ARCHAEOLOGICAL RECONSTRUCTIONS

Trace-element analysis provides an invaluable supplement to traditional anthropological methods and has furnished anthropologists with a powerful tool for the reconstruction of past lifeways. Research on age and sex variation, disease reconstruction, and dietary analysis all have benefited.

Variations in trace-element concentrations along the dimension of age have been documented by Lambert, Szpunar, and Buikstra (1979) in a comparison of Middle and Late Woodland populations. Factors contributing to these differences were attributed to the normal physiological changes associated with aging, dietary differences, and postdepositional effects.

Unquestionably, the most-frequent application of trace-element analysis on archaeological bone has been for studies of prehistoric diet. For example, certain elements have proved useful in the assessment of trophic levels of ancient populations (Price 1985). As with the analysis of the adult remains from Pueblo Grande (Chapter 6, this volume), Sr (Price, Swick, and Chase 1986) and Zn (Lambert et al. 1983) have been used for determining the relative contributions of plant versus animal resources.

Dietary information derived from trace-element concentrations also has been applied to questions of social status. Traditional methods such as burial location, grave goods, and osteological evidence of increased stature and decreased numbers of Harris growth arrest lines have been used to establish status (Hatch and Geidel 1985; Schoeninger 1979). Application of trace-element analysis provided an independent confirmation that individuals inferred to be of high status from skeletal and mortuary evidence from a variety of New World (Brown 1973; Schoeninger 1979) and Old World (Runia 1987) sites demonstrated the elevated Zn and depressed Sr levels associated with increased meat consumption.

Even the inheritance of status has been examined from a trace-element perspective. Brown and Blakley (1985) found no differences between high- and low-status individuals from the protohistoric King site. This was interpreted to indicate the bestowal of earned (achieved) status. Ascribed (inherited) status, it was argued, would have resulted in the accumulated lifelong effects of differential food resources that in turn would have appeared in the bone trace-element record.

Whether reconstructing diet per se or related aspects of status and inheritance, all of the previously mentioned investigations have expanded traditional archaeological studies. Most important, they have avoided such biases as food-processing practices and the indirect evidence provided by floral and faunal remains.

Accomplishments notwithstanding, critical areas of trace-element investigation remain relatively unexplored. For example, age studies such as those by Lambert, Szpunar, and Buikstra (1979) have focused on changes across the adult age range. Children have been by and large avoided because subadult remains are believed to be excessively complex due to the effects of growth and development, as well as increased susceptibility to diagenesis (postdepositional effects).

There have been some exceptions to this pattern of avoidance. For example, Sillen and Smith (1984) investigated subadult skeletons from the Eastern Mediterranean coast and found reduced Sr-to-Ca ratios in infants prior to age 3 typical of high milk consumption. The reduced ratios were followed by a subsequent increase, after age 3, corresponding to the ingestion of higher Sr in weaning foods. The timing of this shift was consistent with ethnographic accounts of weaning practices in the area.
RATIONALE FOR THE PUEBLO GRANDE INVESTIGATION

The purpose of this phase of the Pueblo Grande research was to conduct an exploratory trace-element analysis of the subadult remains. This was justified by the paucity of such data currently in the literature as well as by the issue of reliability, particularly when preservation is poor. Assessment of the results was facilitated by selecting two areas of investigation for which predictive hypotheses could be generated. First, analysis of infant mortality and morbidity (Chapters 2, 3, and 5, this volume) suggested that weaning occurred near the third year. This age is consistent with that estimated for other archaeological populations, as well as a wide range of ethnographic data from living populations. Based on Sillen and Smith’s (1984) research on Mediterranean populations, we would predict a lower Sr-to-Ca ratio among infants 3 years and younger followed by a rise after age 3 at Pueblo Grande.

In addition to the time of weaning, our analysis of iron-deficiency anemia (Chapter 5, this volume) provides an opportunity to construct a second dietary hypothesis. Ever since Carlson, Armelagos, and Van Gerven (1974) first proposed the iron-deficiency/cribra orbitalia hypothesis, the phenomena of weaning stress and weaning diarrhea have been central to the argument.

Specifically, it was argued that near age 3, frequencies of both cribra orbitalia and porotic hyperostosis become elevated due to the increased effects of anemia, exacerbated by the stresses of weaning. These stresses include a loss of access to mother’s iron (Fe) stores (available in utero as well as in mother’s milk), the shift from liquid to solid food, and with this shift, increased exposure to intestinal bacteria.

Given the evidence for Fe deficiency at Pueblo Grande, a second weaning hypothesis could be tested. We predicted that infants 3 years and younger should have higher Fe levels than their older counterparts. This pattern, if observed, would provide important support for the iron-deficiency hypothesis and also bear on the issue of using subadult remains in trace-element research. If well-known phenomena such as Fe deficiency and the Sr-to-Ca ratio appear interpretable at Pueblo Grande, where preservation is poor, a more-thorough investigation of subadult remains in general would be warranted.

Because the reliability of subadult remains has been the subject of considerable debate, a detailed description of the procedures of sample preparation and analysis applied to the remains (including the adults presented in Chapter 6, this volume) was provided.

MATERIALS AND METHODS

Sample Preparation

Five-gram samples of cortical bone were extracted from the femoral midshafts of 42 individuals ranging in age from birth through 15 years. The femoral midshaft was selected because it is highly compact and resistant to diagenetic (postdepositional) contamination (Lambert et al. 1985; Sheridan 1992). Age estimation was made to the nearest year using standard developmental criteria as discussed in Chapter 2, this volume. The 5-g quantity was chosen to ensure homogeneity (Klepinger, Kuhn, and Williams 1986).

Samples were prepared at the University of Colorado in the Department of Anthropology’s Trace-Element Laboratory. The lab is equipped with a Class 100 Clean Hood, which was used during all sample preparation (International Atomic Energy Agency Advisory Group 1984). Trace-element analysis-grade chemicals, ultrapure triple-distilled, deionized water (specific resistance=18MW at 25°C), and acid-cleaned glass and polyethylene labware were employed (Wolf 1986). All samples were handled with Teflon tweezers, spatulas, and gloved hands. The samples were prepared following a combination of established analytical procedures:
Prior to bone sampling, all polyethylene test tubes, balls, blades, specimen cups, weighing dishes, and tubing were filled with or soaked in 20 percent Tracepur nitric acid (HNO₃)/triple-distilled, deionized water) at room temperature for at least 30 days to leach adhering elements. The acid solution was then discarded and the containers were rinsed three times with copious amounts of triple-distilled water. All containers were dried in the laminar-flow Clean Hood.

Five-gram samples (Brown and Blakey 1985; Klepinger, Kuhn, and Williams 1986) of femora were extracted. It should be noted that no intact specimens were used in this research. Only broken femurs with exposed midshaft portions that could be trimmed were used.

The outer surfaces of the bone samples were cleaned by abrasive grinding with a Dremel power tool (IAEA Advisory Group 1984; Olmez, personal communication 1990). Removal of this outer layer of cortical bone has been recommended to remove surface contamination (Lambert et al. 1983).

Each sample was placed in an acid-cleaned polyethylene specimen cup and soaked for 24 hours in triple-distilled, deionized water. The water was then decanted and the specimen and cup thoroughly rinsed with triple distilled, deionized water. Cups with specimens were refilled with ultrapure water, and ultrasonically cleaned (Klepinger, Kuhn, and Williams 1986; Price 1988; Runia 1987; Stedt 1979). Three 30-minute sonications were performed for each sample.

After cleaning, samples were dried for approximately 48 hours at 85°C to a constant weight (Giordano et al. 1984; Iyengar, personal communication 1980; Mahanti and Barnes 1983; Price 1988, 1989).

Each sample was then ashed in a muffle furnace at 800°C for approximately eight hours (Brown and Blakey 1985; Burton and Price 1990; Giordano et al. 1984; Klepinger, Kuhn, and Williams 1986; Lambert et al. 1984b; Price 1988; Shiraishi, Kawamura, and Tanaka 1987). Samples were ground to a fine powder (Brown and Blakey 1985; Kawamura, Tanaka, and Shiraishi 1986; Price 1988).

Samples were then weighed to 0.1±0.002 g sample and heated to 110°C for 1.5 hours. Each test tube was covered with an acid-cleaned polyethylene ball to minimize the escape of trace elements in the gases produced during heating, yet loose enough to avoid explosion of the tube. Samples were vortexed repeatedly during heating.

Samples were allowed to cool, then brought to a final volume of 47 ml with ultrapure triple-distilled, deionized water and vortexed (Brown and Blakey 1985; Burton and Price 1990).

All samples were analyzed using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) within a week of digestion. They were analyzed on an ARL 3560 ICP-AES at Trace Minerals International, a Boulder, Colorado-based clinical chemistry firm specializing in elemental analysis. Machine operation was the responsibility of the senior author.

RESULTS AND DISCUSSION

Diagenesis

Even when skeletal remains are extremely well preserved, the possibility of chemical enrichment, leaching, or both, after interment (diagenesis) must be considered. In the case of poorly preserved
remains such as those from Pueblo Grande, some degree of diagenetic alteration is a virtual certainty. The question becomes whether such material is sufficiently well preserved to warrant trace-element analysis.

A principal method for assessing diagenesis is to compare calcium-to-phosphorus (Ca to P) ratios between values from archaeological remains and modern values. Because Ca and P are the principal minerals in bone, it has been argued that a significant shift in this ratio may indicate postdepositional alteration. According to published estimates, the Ca-to-P ratio in modern bone is 2.16 (Hancock, Grypoas, and Alpert 1987; Kyle 1986; White and Hannus 1983). The Ca content of bone ash by weight is approximately 37 percent (Tanaka, Kawamura, and Nomura 1981), and P about 15 to 17 percent (Price 1989).

Figure 7.1 illustrates the Ca-to-P ratio of the Pueblo Grande subadults. The average Ca-to-P ratio was 2.38, which was consistent across the subadult age range, and most likely reflected some degree of chemical alteration during interment. The Pueblo Grande adults averaged 2.14 (see Chapter 6, this volume). Bone Ca levels averaged 404,747.03 ± 35594.56 ppm compared to a soil average of 161,040.24 ± 29481.60 ppm. Bone P averaged 165,899.79 ± 23595.23 ppm compared to a soil average of 18,331.12 ± 3582.34 ppm. As a consequence, comparison of relative element differences between pre- and postweaning individuals should not have been adversely affected. Although diagenesis was inevitable in the calcium carbonate-rich soil of Pueblo Grande, it is unlikely that the remains were differentially affected across the subadult age range (Price 1985).

**Strontium/Calcium Ratio**

The Sr-to-Ca ratios (Sr to 1000 Ca) for the pre- and postweaning subadults from Pueblo Grande are presented in Figure 7.2. The average value for 23 individuals aged 0 through 3 years was 0.522 ± 0.168 ppm with a coefficient of variation (CV) of 0.32. The postweaning average was 0.386 ± 0.108 ppm with a CV of 0.28. The associated probability of significance was greater than 99 percent. Although highly significant, this difference was the reverse of that anticipated by the weaning hypothesis. Sillen and Kavanagh (1982) and Sillen and Smith (1984) stated that whereas Sr is absorbed at a very high rate in infants relative to adults, milk is a poor source of the element. This is because of discrimination against Sr, first by the mother's intestinal tract and again in the mammary gland. Consequently, infants have very low levels of available Sr prior to weaning (Sillen and Kavanagh 1982; Sillen and Smith 1984).

At the same time, milk is an excellent source of Ca. Consequently, the ratio of dietary Sr to Ca for a nursing infant is extremely low. With weaning, the infant is exposed to higher levels of Sr in the form of adult food, particularly plant resources. Given the relatively higher levels of Sr among the adult females at Pueblo Grande (Chapter 6, this volume) and the tendency for infants to eat what their mothers eat, an increase in Sr relative to Ca at weaning would be expected.

One possible explanation for the elevated Sr-to-Ca ratio among the youngest infants at Pueblo Grande is dietary. If the level of available Ca is low, discrimination against Sr decreases (Sr is substituted) and relative Sr levels rise. However, as illustrated in Figure 7.3, Ca levels for both pre- and postweaning infants at Pueblo Grande were well within the normal range. Mean Ca for the midshaft femur of a modern cadaver sample analyzed by the senior author (using the same preparation protocol and instrumentation) was 399861.74 ± 17778.74 ppm (Sheridan 1992). The nursing infants from Pueblo Grande were within 1.5 percent of that value, and the older children within 5 percent. Bone Ca levels also were higher than soil Ca, which averaged 161,041.24 ± 2948.60 ppm with a CV of 18.31 percent. It is therefore unlikely that the bone underwent sufficient diagenetic
Figure 7.1. Calcium/phosphorus ratio for subadults from Pueblo Grande.

Figure 7.2. Strontium/calcium (Sr/1000 Ca) ratio for pre- and postweaning subadults from Pueblo Grande.
enrichment to mask a dietary deficiency.

Sr, on the other hand, may have been high. As illustrated in Figure 7.4, the mean for the younger age group was $200.55 \pm 84.77$ ppm and $185.71 \pm 53.83$ ppm for the older, compared to an average of 149 ppm for Black Mesa (Martin et al. 1991). Although Sillen and Kavanagh (1982) did not report Sr values directly, they reported a range of Sr-to-Ca ratios for a sample of modern British children aged 0 to 3 years of 0.22 to 0.27 ppm. The maximum value in this range was 48 percent below the average for the comparably aged individuals at Pueblo Grande and 31 percent below the older children. Assuming typical Ca values, this difference implies proportionately lower Sr concentrations in the modern sample than at Pueblo Grande.

There appear to be two possible explanations for the high Sr-to-Ca ratios at Pueblo Grande. The first is diagenetic Sr enrichment from the soil. A second is the complicating factors of growth and development. Soil Sr values at Pueblo Grande averaged $743.24 \pm 127.53$ ppm with a CV of 17.16 percent. This value is 270.6 percent higher than the preweaning average and introduced the distinct possibility of diagenetic enrichment. In support of the second explanation Sillen and Kavanagh (1982:73) suggested that "except for certain special studies...the bones of children should generally be excluded from Sr analyses to infer prehistoric diet."

The present results may provide small and very tentative support for the suggestion of Sillen and Kavanagh (1982). The adult Sr average at Pueblo Grande of $193.08 \pm 81.99$ ppm (Chapter 6, this volume) fell well within the observed range of 87.0 to 380.6 ppm reported for prehistoric popu-
Figure 7.4. Strontium levels for pre- and postweaning infants from Pueblo Grande.

populations, and the sex and diachronic patterning of the Sr relative to Zn appears consistent with a biocultural interpretation. It is the subadult pattern that appears anomalous from the standpoint of Sr values.

Given the poor quality of preservation at Pueblo Grande, diagenetic alteration is a certainty. From the standpoint of Sr, all segments of the population were no doubt altered. The fact that the relative adult values remained interpretable while the subadult values did not may be a reflection, even after death and diagenesis, of the more-complex biology of the subadult skeleton.

**Iron**

Although levels of bone Fe, like Sr, no doubt were affected by diagenesis across all age segments of the Pueblo Grande population, the relative difference in Fe concentration between pre- and postweaning infants and children was consistent with our iron-deficiency hypothesis. As illustrated in Figure 7.5, the average iron level of infants aged 0 to 3 years was 163 percent higher than among children aged 3 to 15 years (774.56 ± 469.59 ppm and 294.17 ± 186.42 ppm respectively). This difference had an associated probability of significance greater than 99.99 percent.

The values also were consistent with those reported for other prehistoric populations. Martin et al. (1991) reported a range for prehistoric populations of 404 to 578 ppm. It is interesting that the postweaning mean for Pueblo Grande fell 27 percent below the lower limit of the prehistoric range.

Fe must be considered an element with a high potential for diagenesis (Lambert,
Figure 7.5. Iron levels for pre- and postweaning infants at Pueblo Grande.

Szpunar, and Buikstra 1979; Lambert et al. 1983, 1985). This was particularly true in the high Fe soils at the site (mean Fe=23245.26±3499.64 ppm). Nevertheless, the age patterning within the Pueblo Grande population as well as the low average level at the site compared to other populations was consistent with the skeletal evidence for high rates of iron-deficiency anemia. Indeed, the adult Fe mean of 134.97 ppm (Chapter 6, this volume) suggested a further decline in Fe during the adult years. This adult decline was indicated skeletally in the pattern of life-long diploic thickening (Chapter 5, this volume).

Finally, the relative elevation of Fe observed among the preweaning infants was consistent with the biology of maternal milk as an Fe resource. Weber, Vaughan, and Stini (1982) have argued that "human milk can provide sufficient Fe for infants during their first year of life." However, the concentration of Fe in milk decreases over time (Weber, Vaughan, and Stini 1982). Figure 7.6 illustrates a polynomial regression of Fe on age for the Pueblo Grande subadult sample. The dramatic reduction in Fe following the first year is intriguing in light of Weber, Vaughan, and Stini's (1982) observation. Although some differential enrichment of the very young may have contributed to this postinfantile reduction, diagenesis is unlikely to have been the entire cause. This interpretation is supported both by the high degree of comparability in preservation as well as by the similarity in Ca-to-P ratios across the age range.
CONCLUSIONS

The present results illustrate a theme that runs throughout this volume. The analysis of ancient patterns of health and disease is an exercise in the art of deduction. As with the evidence from paleodemography or childhood stress, there are no witnesses to the weaning practices, Sr levels, or Fe content of preweanling children. Our case is circumstantial and depends upon the internal consistency of our evidence. In the case of subadult Fe, the evidence is consistent with the iron-deficiency hypothesis. The infants and children at Pueblo Grande appear to have suffered from a syndrome known as weanling diarrhea.

The evidence for the onset of weaning based on Sr-to-Ca ratios is less clear. The age patterning is inconsistent with both the modern biological and archaeological evidence. This suggests that the utility of subadult Sr values should be considered suspect, at least until more is understood about diagenetic effects and biology of Sr relative to growth and development. One thing is clear, however. Data such as these from a wide range of ancient populations, interred under a variety of conditions, are essential to our eventual understanding of prehistoric human diet and bone chemistry.