TRACE ELEMENT ANALYSIS OF HAIR FROM A NUBIAN POPULATION

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Twenty eight hair samples of adults excavated from two Christian era Sudanese cemeteries were analyzed for trace element content using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES). Quantitative levels of eight elements related to nutrition and disease were determined for this study group. Significant iron levels were found which agree with earlier studies on Nubian bone and hair. This particular study demonstrates the utility of trace element hair analysis as a tool in studies of ancient diet and disease. [Ascent, 4(1):57-64]

INTRODUCTION

Trace element analysis of hair has become of considerable interest to anthropologists as well as researchers in the nutritional, forensic and medical sciences. The approach was first used on biopsy material in 1945 when indications of toxic materials such as lead (Pb), arsenic (As), and mercury (Hg) poisoning began to appear at autopsy. Hair analysis continues to be used by health specialists, chiropractors and physicians for determining diet, disease and environmental toxicities (TMI, 1990; Katz and Chatt, 1988).

Most recently, trace element techniques have turned to the analysis of antique and ancient hair. For example Napoleon's hair was analyzed for evidence of arsenic, and the remains of Zachary Taylor were likewise investigated in an attempt to determine the manner of his death (Grupe and Herrmann, 1988). Dietary reconstructions of prehistoric populations have also been attempted with varying degrees of success (Benfer et al., 1978; Sandford, 1984; Lubec et al., 1987).

Basic Hair Structure

In order to understand how trace elements are incorporated during hair fiber synthesis, a basic knowledge of hair structure and formation is needed. Hair is the end product of numerous chemical and biological functions controlled by the body's regulatory systems. All hair originates from follicles in the skin; the follicle is actually a depression in the epidermis lined with epidermal cells. Thousands of these hair follicles cover the surface of the skin, although most remain inactive. Those on the scalp and face (in men) produce large amounts of hair. In both regions, hair follicles are clustered into dense groups of two to five.

The color of hair also plays a role in density. Blond hair is the most dense, followed by red, then brown and finally black. The low density of black hair is due to its course texture.

Hair formation goes through 3 stages:

1) Anagen stage - Hair cells form and collect large amounts of amino acids.

2) Catagen stage - Hair cells are in a period of transition between active and resting stages.

3) Telogen stage - Newly formed hairs are in a resting stage.

Mature hair is composed of three distinct structures. The outermost layer is composed of three to nineteen overlapping circular bands known as the cuticle. The cuticle allows for hair shaft flexibility and may also "open up" like the petals on a flower allowing water, toxins and other environmental elements to enter the outer cortex or cortical fibers of the hair shaft (Figure 1). The center of the hair consists
of a sponge-like canal system known as the medulla. Trace elements accumulating within body tissues from both dietary and environmental origin are concentrated in the cuticle layer, the cortical fibers and the medulla portions of the hair.

In preadolescent children, the medulla is poorly developed and consequently, trace element concentrations vary widely from individual to individual. As a result, trace element techniques are best applied to mature hair.

The bulk of the hair shaft is made of cortical fibers which are interwoven progressively tighter as they near the center of the shaft. These fibers give the hair its strength and elasticity. Most of the hair's pigment (melanin) is located in the cortical fibers. Waste materials and excretions of the hair are transported through the air passages of the medulla.

All hair growth begins in the papilla area where the cells are constantly pushed upward and outward by new cell growth. The cells closest to the scalp are the youngest mature hair cells, those furthest from the scalp are the oldest. Over time, the protective cuticular layer becomes worn, thus allowing the cortical fibers to be altered. This permits environmental contaminants to enter the hair shaft; a factor of concern to both clinical and anthropological investigations of hair trace element concentrations.

Hair Analysis of Ancient Populations

Trace element analysis of ancient hair presents several challenges, including limited amounts of material and the distinct possibility of contamination of the cortical fibers. However, when proper controls are employed, such analysis has proven a useful tool for studying numerous biological and environmental factors affecting prehistoric populations (Sandford, et al., 1983). Examples of such studies include an investigation of the structural stability of hair from Egyptian mummies, and an overview of nutritional factors affecting an ancient Peruvian population (Lubec et al., 1987; Beñer et al., 1978).

The purpose of this research was to expand the investigation of ancient remains through an analysis of scalp hair from a Medieval Sudanese Nubian population. Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP-AES) was used to determine elemental levels in the hair. This analysis was part of a multisite investigation of ancient patterns of diet and disease using trace element distributions. Other tissues examined
include bone (Kolbrenner and Sheridan, this issue) and teeth (Chiu and Sheridan, this issue).

MATERIALS AND METHODS

Study Group

The individuals used in this study were a Christian population excavated from two cemeteries, dating to AD 550-750 and AD 750-1500. The earlier cemetery represents a Medieval population characterized by extensive religious and political unification throughout the Nubian corridor. The later cemetery includes the Feudal period descendents of the Medieval population. Unlike their forebears, these people occupied the area during a time of regional isolation and political autonomy much like the Middle Ages of Europe.

The cemeteries were located in an area of the Lower Nubian desert known as the Batn el Hajar, near the town of Kulubnarti in modern-day Sudan. The arid climate of the Nubian desert permitted remarkable preservation of bone as well as soft tissues including hair (Figure 2).

For this investigation, hair samples from adults (age 15 to 50+) of both sexes were analyzed. Because body hair tends to absorb elements from sweat, samples were collected exclusively from the scalp. When possible, multiple scalp areas were sampled.

Contamination Control

Contamination introduced before and during burial can have a significant influence on the accuracy of trace element concentrations found in hair. Prior to death, factors such as the individual’s age, sex, exposure to environmental toxins, and the location of the hair on the scalp affect ambient levels. After burial, diagenesis (the process of post-depositional alteration) can greatly affect trace element levels. Rates of diagenetic change differ among bodily tissues. In hair, numerous changes take place over time including reduction of elasticity and tensile strength due to dehydration (Lubec et al., 1987) and

Figure 2. An adolescent female from the Feudal cemetery. Note the intact corn rows demonstrating the remarkable degree of preservation found among this Nubian population.
contamination introduced from the surrounding soil.

Given the possibilities for the introduction of contaminants before and after burial, the importance of adequate removal of exogenous minerals prior to analysis becomes paramount. Proper clean-room handling procedures during all phases of analysis are also a necessity.

**Sampling Procedures**

There is no standard method for the collection and preparation of ancient hair samples such as the Nubians (Sandford, 1984; Sandford et al., 1983; Meglan, 1991; Valkovic, 1988). Therefore, the following sample preparation procedure was adapted from numerous studies (Katz and Chatt, 1988; TMI, 1990; Valkovic, 1988; Sandford et al., 1983; Meglan, personal communication).

Sample preparation was conducted at the University of Colorado-Boulder Department of Anthropology's trace element laboratory. In order to reduce airborne contamination, all samples were prepared in a Class 100 work station (Miziuke, 1983). All implements and storage vessels were cleaned with a 20% nitric acid (HNO₃) solution prepared from Tracepur® chemical and ultrapure distilled, deionized (18 megohm) water. The Tracepur® acids and ultrapure water were also used for digestion.

The following steps were used for sample preparation:

1) Hair samples were collected from various regions of the head to provide a homogenous mixture. Element concentrations vary along the length of a hair strand, so all samples were collected no more than seven centimeters from the scalp. Acid-cleaned plastic scissors were used to cut the hair; teflon coated tweezers were used to handle the specimens. Samples were stored in acid-cleaned polyethylene bags.

2) Hair samples were washed in a solution of hydrating non-ionic surfactant and ultrapure water (1 drop per 100 ml) and rinsed three times with ultrapure water.

3) Cleaned samples were dried in a Class 100 workstation at room temperature for 48 hours.

4) Five hundred milligram samples (Meglan, personal communication; TMI, 1990) were placed in acid-washed polypropylene test tubes and wet-ashed (digested) in 2ml of Tracepur® HNO₃ at 110°C for 90 minutes. One ml of 30% hydrogen peroxide was then added over the course of an additional 20 minutes. Samples were brought to a final volume of 5 ml with ultrapure water.

The samples were analyzed on an Inductively Coupled Plasma-Atomic Emission Spectrometer (ARL model 3560) by the second author. NIES hair standard was used as the certified reference material to monitor accuracy and consistency with the machine (NIES, 1985).

The most common elements found in hair using ICP-AES are: calcium (Ca), chromium (Cr), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), phosphorus (P), potassium (K), selenium (Se), silicon (Si), sodium (Na), zinc (Zn), molybdenum (Mo), aluminum (Al), cadmium (Cd), lead (Pb), mercury (Hg), and nickel (Ni) (TMI, 1990; Grupe and Herrmann, 1988). For this study, eight elements were analyzed: Ca, P, Zn, Mg, Mn, Fe, barium (Ba), and strontium (Sr). Although strontium and barium are not among the most common elements found in hair, they are of known dietary importance and thus considered important to this investigation.

Following data collection, summary statistics were calculated for the combined sample (Table 1) and comparisons were then made for each element by age, sex and cemetery using analysis of variance. In order to insure adequate sample sizes by age, age categories were created as follows: young adult, 15-25 yrs; adult, 26-35 yrs; older adult, 36-50+ yrs.

**RESULTS**

Summary statistics for the 8 elements investigated are presented in Table 1. All
TRACE ELEMENTS IN NUBIAN HAIR

<table>
<thead>
<tr>
<th>ELEMENT</th>
<th>MEAN (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barium (Ba)</td>
<td>5.952 ± 3.384</td>
</tr>
<tr>
<td>Phosphorous (P)</td>
<td>372.419 ± 218.846</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>204.883 ± 147.766</td>
</tr>
<tr>
<td>Calcium (Ca)</td>
<td>1194.739 ± 464.133</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>111.171 ± 28.209</td>
</tr>
<tr>
<td>Manganese (Mn)</td>
<td>25.367 ± 15.667</td>
</tr>
<tr>
<td>Iron (Fe)</td>
<td>381.079 ± 241.908</td>
</tr>
<tr>
<td>Strontium (Sr)</td>
<td>8.04 ± 4.106</td>
</tr>
</tbody>
</table>

Table 1. Summary statistics for the eight elements utilized for this study.

Mean values fall within the modern human range. The large standard deviations are also consistent with the wide variation observed among modern samples. Given the correspondence between the Nubian hair data and modern human values, the second phase of the research was undertaken to determine whether differences in element concentration existed by age, sex and cemetery.

Analysis of variance was performed for each element. As previously discussed, the independent variables were age at death, sex and cemetery (representing cultural period). Of the 8 elements examined, only Fe produced values significantly different (p ≤ 0.05) by any of the independent variables (Table 2). As illustrated in Figures 3 and 4, for the combined cemetery sample, Fe levels were significantly (p<0.05) lower in females compared to males and for the combined sex sample the early, Medieval sample had significantly (p<0.05) lower Fe levels than the later Feudal-age sample.

**DISCUSSION**

While limited to a single element, the pattern of difference by sex and cultural period is extremely conducive to interpretation. Over 11 years of research on the Kulubnarti remains has revealed a pattern of extreme stress with a substantial nutritional component. Beginning with a demographic reconstruction, Van Gerven and coworkers (1981) found extremely high frequencies of mortality with a mean life expectancy at birth of only 15 years. Evidence that nutritional stress contributed to high mortality has also been abundant. Hummert (1983) found reduced long bone growth in the Kulubnarti children and Van Gerven et al. (1985) found evidence for juvenile osteoporosis. This pattern of reduced growth and bone loss suggests chronic nutritional stress compounded by frequent episodes of disease.

The most direct evidence for nutritional stress has come from the analysis of cribra orbitalia. The term "cribra orbitalia" refers to lesions on the superior surface of the eye orbits. It is produced by an expansion of the blood-producing diploic (marrow) space with corresponding thinning of the outer surface (table) of the skull. In its less severe states, cribra orbitalia exhibits relatively minor porous openings in the orbital roof. In its most advanced stages there is considerable expansion of "honeycomb-like" cribrous bone into the area of the orbit normally occupied by soft tissue.

Extensive research at Kulubnarti and elsewhere in the Nile valley (Carlson et al., 1974; Van Gerven et al., 1981; Mittler, 1990) has linked this skeletal condition to iron deficiency anemia. Most importantly, the diet of Nubian populations has remained essentially unchanged for the last
several thousand years. In the past and today the primary foods are milled cereal grains such as millet and wheat, which contain critically little bioavailable iron. May (1961) noted that 75 to 80% of the population of modern Egypt do not have adequate iron intake and that the associated anemia is most severe among children and females. For women the problem is particularly compounded by repeated pregnancies. The probability that iron deficiency anemia will be present is highest during pregnancy and increases with each additional pregnancy. According to May (1961) the incidence reaches 56% of all pregnant females in contemporary Egypt.

The results of the present investigation accord well with this body of information. Given the low life expectancy at birth experienced by the Kulubnarti population, birth rates are likely to have been near the human maximum (Greene, et al., 1986). We would predict, given the combined effects of low dietary iron and high reproductive demands, very much what we see -- significantly reduced iron levels among the Nubian females.

Our observed difference in iron levels by cemetery is also consistent with a dietary interpretation. While the shift from the earlier Medieval period of political and religious unification to the Feudal pattern of local village autonomy effectively ended Nubia's political power, the biological well-being of peasant villagers such as those at Kulubnarti clearly improved. Lacking the constant economic demands of tithes and taxes, infant mortality was reduced (Van Gerven et al., 1981), growth retardation became less severe (Moore, et al., 1986) and most notably, cribra orbitalia was less frequent (Van Gerven, 1981; Mittler and Van Gerven, 1988). Our observation of a significant increase in iron levels for the combined Feudal population once again accords well with the body of earlier research.

CONCLUSIONS

Hair analysis among the Nubians has considerable value to the study of trace elements using ICP-AES in relation to
environmental, nutritional and disease stress among both extinct and extant populations. The present results expand our understanding of diet and disease in ancient Nubia and provide an important historical context for nutritional stress in the modern-day Nile valley. While the analysis of trace element variation in ancient remains is very much in its infancy, these results give reason to be optimistic for the future of such investigation.

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