RADIOIMMUNOASSAY FOR COCAINE IN MUMMY HAIR TO DETERMINE ANTIQUITY AND DEMOGRAPHY OF COCA LEAF CHEWING PRACTICES IN SOUTHERN PERU AND NORTHERN CHILE

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Chewing of the leaves of the coca plant (Erythroxylon coca) is a uniquely Andean practice (Allen, 1988). In spite of clerical and governmental attempts to prohibit the tradition, it still persists primarily in the highlands, and is generally limited to native Indians of the region. Archaeological evidence and ethnohistorical writings indicate this custom has an ancient tradition (Plowman, 1984). Some information regarding methods and frequency of its use can be derived from ethnohistorical accounts near the time of the Conquest in Peru (Garcilaso, 1987 [1609]:511). While there are concerns about the nature and accuracy of their interpretations (Murra, 1986), a common synthesis of their content suggests that royal efforts to restrict coca use began about A.D. 1230 in the reign of Inca Roca, but the horticulture and use of the coca plant did not become a state monopoly until about two centuries later. At that time the plant acquired divine status and played a central role in religious rituals. Under the strains of imperial expansion just prior to the Spanish Conquest, royalty found it necessary to share the privilege first with the nobility and then with progressively lower social classes until, at the time of the Conquest, all but the lowest classes enjoyed virtually unrestricted access to coca growth and use (Mortimer, 1974:65; Phillips and Wynne, 1980:9).

Stylized lime containers in Ecuadorian tombs suggest coca leaf chewing was practiced there as early as 3,000 B.C. and reached the northern coast of Peru about a millennium later. Ceramic figurines demonstrating the bulging cheek characteristic of the coca leaf chewer can be found in central Peruvian lowland areas and Moche tombs about A.D. 300 (Lumbreras, 1974). However, tobacco was also chewed and its effect often was enhanced by the simultaneous use of lime. There may, therefore, be some risk in diagnosing coca leaf chewing on the bases of such indirect evidence as figurines. In the drier regions of the Andes where survival of biological material for centuries is possible, many tombs contain coca leaves. Coca leaf-filled textile bags (chuspas) are common in the tombs of various cultures on the coast of southern Peru and northern Chile after about AD 500 but are rare before that. In addition, both subadult and adult mummies from these later groups have been found with a coca quid in situ (Figure 2).

Evidence suggests that the coca leaf chewing practice has changed little and today is carried out in a manner very similar to that in prehistoric times. Extraction and/or absorption of the leaf alkaloids are enhanced by addition of an alkaline substance (lime or plant ashes called yupta, liipient, or yuptka in Peru) in one of two ways (Lumbreras, 1947; Plowman, 1984; Schultes, 1987). In one method the coca leaves are folded into a wad and placed between the teeth and inside of the cheek. If this method is used, the alkaline substance could be applied directly, but more commonly a moistened stick was used for the transfer. This use can be seen in a Moche effigy vessel circa 100 A.D. that represents powdered lime being removed by a spatula for such purposes (Figure 3). In another method dried coca leaves are ground into a fine powder and mixed with the ashes. The subsequent mixture was then held between the teeth and the cheek. The absorption of the cocaine occurs both through the oral mucosa and gastrointestinal tract (Paly et al., 1980). After about an hour in the oral cavity, the quid is expelled, although occasionally it may be swallowed. Chewing of 20 to 60 grams of leaf daily is common in modern populations (Zapata Ortiz, 1952) and provides a daily cocaine dose of 200 to 300 mg. (Phillips and Wynne, 1980) with resulting plasma cocaine levels up to mean values of about 250 ng/ml. These levels can cause weak physiological responses, however, they are far less than the amount measured in street users of concentrated cocaine and in individuals after use of cocaine as a topical anesthesia (Van Dyke et al., 1976).

It is clear coca leaf chewing antedated the Spanish arrival. What is not clear is its antiquity, geographic origin, diffusion routes, chronology, demography, or purpose. Most historians agree with the several chroniclers including Garcilaso de la Vega (Garcilaso 1987 [1609]) that the Inca people indulged in this practice during pre-Hispanic times but they frequently disagree on the details (Murra, 1986). What is needed is a sensitive and specific test for detecting drugs in individuals, since a large number of mummified pre-Columbian remains have now been recovered. Because hair analysis for drugs of abuse has been reported in recent users we decided to apply this procedure to samples from mummified
human remains in a pilot study. This demonstrated that cocaine and its primary metabolite benzoylecgonine (BZE) are compounds sufficiently stable to be demonstrable in ancient mummy hair (Cartmell, et al., 1991).

Hair analysis for detection of heavy metals has been used for many years. However, hair as the matrix for drug detection is a rather recent innovation. When first reported (Goldblum et al., 1954) the methods for drug detection in hair at that time, were cumbersome and not readily applicable to multiple analyses. A national concern for drug abuse in the 1970s stimulated the development of new technologies. Specific in this regard was the development of a very sensitive radioimmunoassay (RIA) procedure. Hair analysis, using RIA, was first reported for opiates in 1979 (Baumgartner et al., 1979) and was followed shortly by other drugs including cocaine (Valente et al., 1981).

The half-life of cocaine in the human body is measurable in minutes. However, some of its metabolic products (most notably BZE) demonstrate considerable stability (Goodman and Gillman, 1985). Although the exact mechanism is not fully explained, BZE and cocaine are absorbed by the hair follicle and subsequently incorporated into the keratinous hair shaft. This hair shaft therefore serves as an ideal source for testing. Hair may accumulate evidence of coca use over period of months to years. Since hair grows at a rate of 1 to 1.5 cm. per month, the shaft becomes a virtual timed record of cocaine exposure.

The anatomy and physiology of fingernails and toenails are somewhat similar to those of hair follicles. Nails have been shown to be useful materials for detection of some drugs (Suzuki et al., 1984). We theorized that nail tissue might serve as an alternate sample for BZE analysis when nails were present in mumified bodies without hair. Fingernails grow at an average rate of only about .09 mm. a day and toenails grow at a rate of one-third to one-fourth that of fingernails. Therefore a one centimeter long fingernail segment would represent approximately four months' growth.

POPULATIONS SAMPLED IN THIS STUDY

Seven populations with distinctly different cultural adaptations were sampled in the course of this study (Table 1). As illustrated in Table 1, five of the populations were recovered from archaeological sites located within the Azapa Valley and one from the Camarones Valley south of Arica, Chile (Figure 1). The remaining samples were derived from three Chiribaya sites excavated within the Osmore River drainage. Two of the three Osmore sites also show evidence of Tiwanaku occupations. The Azapa Valley and Camarones cultural sequence is summarized briefly in the following paragraphs to illustrate the environmental and cultural contexts of the peoples who served as the basis for this research.

The Atacama Desert on the western slope of the Andes is the world's most arid desert. Stretching from about 17° to 27° south latitude, its interior is habitable below 3,300 m only within certain stream-containing valleys and along the
coast. While the lower Osmore Valley lies just north of the Atacama Desert, its climate is only slightly less arid than that of the Azapa and Camarones Valley within the Atacama itself.

The Chinchorro culture represents the earliest (ca. 7000 B.C.) evidence of human occupation in the coastal area of northern Chile. Its members formed a tradition of preceramic fishermen and hunter-gatherers whose technological advances were associated with a simple maritime subsistence. Highland contact appears to have been minimal. Various origins have been proposed including a tropical one.

The Quianí people probably represent the descendants of the Chinchorro. While they were still preceramic they developed a more complex fishing-tool technology and appear to have begun some agricultural experimentation and basketry. They represent a transitional group between their marine-hunting predecessors and the subsequent agriculturists.

About 1000 B.C. the Alto Ramirez people settled along the lower econiches. Believed to be immigrants from the Lake Titicaca area, they brought with them their highland traditions of camelid herding and agricultural practices. By A.D. 400 they were succeeded by the people of the Cabuza phase. These were agroganaderos (pastoralists and agriculturists) and represent Tiwanaku-related people.

Just prior to the collapse of the Tiwanaku empire the Chiribaya became identifiable in the Azapa Valley near Arica in northern Chile. Their ceramics acquired a richer palette, and the herding of camelids and their overall agricultural technology improved along with an increase in density and expansion of the population.

The San Miguel group appear to be Azapa Valley successors to the Chirabaya population there, differentiated on the basis of their ceramics and somewhat more technologically developed agricultural methods. The group designated Cam-9 is a small group whose beach burial site is located at the north of the Camarones Valley. Their grave goods clearly define a maritime subsistence. In spite of the presence of Inca-type clothing and pottery in their graves, they appear to be a group of local origin.

MATERIAL AND METHODS

Tissues were acquired by anatomic dissections of archaeologically excavated, spontaneously mummified human remains from coastal and valley sites in the region of the Atacama Desert. Age and sex were estimated using methods commonly employed by physical anthropologists (Ubelaker, 1989). As indicated in Table 1, 163 samples originated in the Human Biology Section files at the Archeological Research Institute at the University of Tarapaca in Arica, Chile. The remaining 82 were derived from Osmore Valley samples. Selection was made solely on the basis of availability of hair samples in the files or from the excavated mummies.
At least 30 mg of hair were removed from each individual studied. If scalp was attached the hair was transected as close as possible to the skin and orientation maintained within the plastic storage bag. After removal of gross debris the hair sample was rinsed once with 100 ml of sterile, distilled water. Following centrifugation the rinse water was saved for subsequent analysis to check for the presence of BZE. The hair sample was wrapped in a 4-x-4 inch sterile, loose gauze with stapled ends. This was placed in a plastic bag together with 100 ml of water containing .1 ml of a commercial detergent shampoo (Prell, Proctor & Gamble, Cincinnati, OH 45202); the hair was then thoroughly washed. After 10 more rinses the hair was air dried for 48 hours. The individual hair shafts were then cut into lengths of 1-3 mm. For the two individuals on whom we performed segmental analysis, a long braid was cut into 13-mm-long segments (about one month's growth), and each segment was prepared as an independent sample for analysis.

Paired samples of hair and nails from the same individual were obtained from the Osmore Valley Chiribaya excavations. The nails were grossly cleaned with a stiff brush and then washed with soap and rinsed as noted above. The nails were dried for 48 hours and then macerated using a mortar and pestle. The subsequent nail matrix powder was subjected to extraction as described below.

The extraction procedure has been described by Valente et al. (1981). The hair or nail sample was suspended in a test tube containing 2 ml of .1 normal hydrochloric acid and extracted overnight in a water bath at 45°C. The mixture was then suspended in .1 ml of one normal sodium hydroxide and .9 ml of phosphate buffer at pH 7.2 and centrifuged 10 minutes at 3,000 rpm.

An aliquot of 25 ml of the extract was analyzed by radioimmunoassay for the cocaine metabolite in urine following the manufacturer's recommendation (Diagnostic Products Corporation, 1990). Standard solutions of BZE provided in the kit include 0, 100, 300, 900, 2,700, and 5,400 ng of BZE/ml. In addition we also prepared 10 and 30 ng/ml standards. These were all analyzed at the same time as the hair-extract samples. An Abbott Auto Logic Gamma counter was used to obtain the raw counts per minute, and the data were entered into a Nuclear Medical Labs 6000 CT computer that prepared a graph plot of standard concentrations against the percent of each standard found. This curve was employed to predict the concentrations of BZE in the individual hair samples.

A hair and nail sample for a negative control was taken from a laboratory employee, and for a positive control a hair sample was acquired from known cocaine users. No positive nail control was obtained. However, all nail samples were performed in the same "run" with positive hair controls. A cutoff value of 5 ng/ml was employed to differentiate a "negative" and a "positive" result since this conservative value is commonly used in clinical studies.

The first rinse water from 13 positive specimens with high values was analyzed to assess external contamination of the hair. A second aliquot was fortified with deuterated BZE and deuterated cocaine, extracted to isolate the BZE, and chemically derivatized. A control sample was also run to verify BZE
levels. These samples were analyzed by a Finnigan gas chromatograph mass spectrometer (GCMS) in a selection mode. Further instrumental and procedural details have been presented elsewhere (Springfield et al., 1991).

RESULTS

Of 254 individual hair samples tested by RIA, 114 showed positive reactions (45 percent). Of these 113 samples reacting positively for BZE by the RIA method, 61 (54 percent) were also subjected to analysis for BZE by GCMS, to ensure quality control. Sixty of these 61 (98 percent) were positive by the GCMS method (the unconfirmed sample developed a cloudy solution, preventing analysis). Twenty samples that were negative (nonreactive) by RIA were all also negative by GCMS analysis. The first rinse water of 13 highly reacting positive hair samples was tested using RIA; all rinse-water tests (100 percent) were nonreactive for BZE.

While GCMS remains the legal standard, RIA is finding increased acceptance because of its simplicity and economy coupled with its excellent performance. Specificity tests reported by the manufacturer demonstrate no reactivity of the antibody with 19 drugs of abuse other than cocaine or its metabolic product BZE, even at concentrations up to 10,000 ng/ml (Diagnostic Products Corporation, 1990). The detection limit of the procedure is 3 ng/ml. Both false positive and false negative results are rare. The latter are usually the result of the conservative cutoff value selected for legal applications. For BZE solutions at 100 and 5,400 ng/ml the intra-assay coefficients of variation for precision tests were 9 percent and 4 percent, and for inter-assay were 12.6 percent and 9.7 percent. Recovery results for specimens «spiked» with 2,500-10,000 ng/ml ranged from 103 to 123 percent.

Data on the test results for hair samples from archaeologically-recovered prehistoric human remains are itemized in Tables 1-3. Two positive samples were selected for segmental analysis. A 24-year-old Inca-period female showed an episodic pattern with use one month prior to death (Table 2). A 50-year-old female from the Azapa Valley Chiribaya group had a pattern of continuous use for 14 months preceding death (Table 2).

A total of 22 nail samples from which we had corresponding hair were analyzed by the above method. No distinction was made between toenails and fingernails. Identical results were found in 12 of 22 hair-nail pairs from the same individual; all of the remaining 10 revealed positive reactions in hair and negative reactions in the nails. In no case were positive BZE reactions in nail tissue found if the hair sample from the same individual was negative (Table 3). This correlation indicates that nails sometimes take up the drug over time intervals different from hair, and/or at levels which can be detected by RIA but not as frequently as hair and in much lower concentrations. In the absence of hair, nail can be used as the matrix but there will be a loss of sensitivity.
DISCUSSION

Chewing the leaves of the coca plant (Erythroxylon coca) causes measurable amounts of cocaine and its major metabolite BZE to be incorporated into the growing hair shaft. This has been proved in both recent (Henderson et al., 1992) and ancient (Cartmell et al., 1991) coca chewers. Although archaeological and ethnohistorical accounts of coca leaf use are numerous, this radioimmunoassay is the most direct method for assessment of such use.

The earliest group tested includes members of the Chinchorro culture (Table 1). While a north Peruvian variant of the coca plant was successfully cultivated in coastal areas during pre-ceramic times, this was not true in the coastal area of the Atacama desert. Acquisition of coca leaves by our studied groups, therefore, would have been possible only through trade. The Chinchorros were primarily-marine oriented and with little archaeological evidence of ongoing trade with the highlands or even northern coastal Peru. As expected this group showed no evidence of coca leaf use. The transitional Quiani group was very small (only three samples). These, like their predecessors the Chinchorros, were primarily marine-oriented but did some limited gathering. These samples are likewise negative.

Certain members of the early phase Alto Ramirez (Pisagua) people have been radiocarbon dated to about 1000 B.C. Test results indicate one positive out of nine from that earlier group and an additional positive out of three tested samples from the later group (about A.D. 350). This demonstrates coca leaf use in this coastal area beginning some 3000 years ago. The tests on the Tiwanaku-related Cabuza people reveal use by over half of those members tested. This cultural group, similar to those of the Alto Ramirez, were highland migrants, suggesting they transported the practice from the highlands to the more arid coastal regions.

The frequency of coca-leaf chewing among the Inca-period group is of special interest. Funerary artifacts in their tombs provide no reason to believe this was an elite subgroup. Indeed, their worn, frequently patched and repaired clothing suggests they represent the lowest socioeconomic status. Nor do they fit the agricultural image of the imperial, Cuzco-related highland Incas. Their beach-site location, abundant marine-oriented grave goods, and chemical dietary-reconstruction values (Aufderheide and Allison, 1992) all clearly identify them as sea hunters (and, probably to a limited extent, sea traders). Radiocarbon dates also indicate the tested individuals lived during the last century of the Inca empire. Consistent with the model described by Santoro et. al. (1987) the most probable structure of this group was that of a local population that continued to function as a specialized subgroup of marine resource harvesters, trading their products with highland dwellers. Admittedly more group members need to be tested to reach a desirable degree of validity, but finding even nine positive results out of 13 individuals tested suggests that no significant restrictions for coca use applied to this group. It is emphasized, however, that these results cannot be used as a
model for Inca practices at this period, because this small, remote group does not represent members of the imperial Incas.

The two Chiribaya populations reflect widespread exposure to cocaine, 51 percent of the population had positive test reactions (Table 4). This group is also large enough to evaluate subgroup use. There is a high frequency of positive reactions in the 0 to 2 years age group as compared with other subadults (Figure 4). There are two possible routes of exposure in these infants. Cocaine and BZE cross the placenta. Newborn hair has been suggested as the specimen of choice to monitor maternal use in the last trimester since this is the period of fetal hair growth (Graham et al., 1989); therefore this could reflect maternal use. The second possible route of exposure is oral-through the breast milk or by medicinal administration, as perhaps a tea. Separation of these two possibilities rests on future availability of newborn mummy hair for testing.

Table 1.

<table>
<thead>
<tr>
<th>Culture</th>
<th>Pos. Tested</th>
<th>From</th>
<th>To</th>
<th>Subsistence Strategies</th>
<th>From</th>
<th>To</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chinchorro</td>
<td>0 23</td>
<td>7000 B.C.</td>
<td>2000 B.C.</td>
<td>primarily coastal marine resources</td>
<td>3000 B.C.</td>
<td>2000 B.C.</td>
</tr>
<tr>
<td>Quiani</td>
<td>0 3</td>
<td>2000 B.C.</td>
<td>1500 B.C.</td>
<td>coastal marine, valley mouth gathering</td>
<td>1500 B.C.</td>
<td>1250 B.C.</td>
</tr>
<tr>
<td>Alto Ramirez</td>
<td>2 12</td>
<td>1000 B.C.</td>
<td>A.D. 300</td>
<td>migrants from highlands, early agriculture</td>
<td>350 B.C.</td>
<td>250 B.C.</td>
</tr>
<tr>
<td>Cahuza</td>
<td>10 16</td>
<td>A.D. 400</td>
<td>A.D. 1000</td>
<td>migrants from highlands, early agriculture</td>
<td>A.D. 400</td>
<td>A.D. 1000</td>
</tr>
<tr>
<td>Chiribaya</td>
<td>37 82</td>
<td>A.D. 1000</td>
<td>A.D. 1250</td>
<td>primarily agriculturists</td>
<td>A.D. 1000</td>
<td>A.D. 1250</td>
</tr>
<tr>
<td>Chiribaya</td>
<td>54 97</td>
<td>A.D. 1000</td>
<td>A.D. 1250</td>
<td>primarily agriculturists</td>
<td>A.D. 1000</td>
<td>A.D. 1250</td>
</tr>
<tr>
<td>San Miguel</td>
<td>2 8</td>
<td>A.D. 1050</td>
<td>A.D. 1300</td>
<td>primarily agriculturists</td>
<td>A.D. 1200</td>
<td>A.D. 1350</td>
</tr>
<tr>
<td>Cam-9</td>
<td>9 13</td>
<td>A.D. 1400</td>
<td>A.D. 1500</td>
<td>primarily marine resources</td>
<td>A.D. 1400</td>
<td>A.D. 1500</td>
</tr>
<tr>
<td>Total</td>
<td>114 254</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Number of positive = number of individuals with positive reaction.

a Azapa Valley
b Osmore Valley
c Camarones Valley
Table 2.
Benzoylcegonine (BZE) Test Reactions in Successive 13-mm Segment Hair Samples

<table>
<thead>
<tr>
<th>Hair Segment Number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 y/o «Inca»</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 y/o Maitas</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: + = positive reaction; - = negative reaction.

Table 3.
Comparison of BZE Reactions in Pairs of Hair and Nail Samples From the Same Individual.

<table>
<thead>
<tr>
<th>Hair +, Nail +</th>
<th>4</th>
<th>Agree</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hair 0, Nail 0</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Hair +, Nail -</td>
<td>10</td>
<td>Don't Agree</td>
</tr>
<tr>
<td>Hair 0, Nail +</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Total Agreement: 0.545
Kappa statistic: 0.22535
z 1.67142
p > .05

Table 4.
Age and Sex Distribution of Positive Benzoylcegonine (BZE) Reactions in Hair Samples of Azapa and Osmore Chiribaya Population only.

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Total Number of «Positive» Individuals</th>
<th>Total Number of Individuals Tested</th>
<th>% «Positive» Individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2 years</td>
<td>18</td>
<td>33</td>
<td>54.4</td>
</tr>
<tr>
<td>3-14 years</td>
<td>11</td>
<td>33</td>
<td>33.3</td>
</tr>
<tr>
<td>15-34 years</td>
<td>24</td>
<td>46</td>
<td>52.2</td>
</tr>
<tr>
<td>35+ years</td>
<td>38</td>
<td>67</td>
<td>52.1</td>
</tr>
<tr>
<td>Female adults</td>
<td>24</td>
<td>57</td>
<td>42.0</td>
</tr>
<tr>
<td>Male Adults</td>
<td>26</td>
<td>50</td>
<td>52.0</td>
</tr>
</tbody>
</table>

a Percent of total number of tested individuals yielding positive BZE reaction.
b Three Azapa Chiribaya adults listed in Table 1 are not included here because it was not possible to estimate their gender.
ACKNOWLEDGEMENTS

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