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What Were they Drinking? A Preliminary Study of Alcohol Metabolites in Andean Mummy Hair

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Abstract

Consumption of alcoholic beverages has occurred for millennia. Sugar is common in most natural fruit juices and our ancestors likely discovered the fermentation process accidentally. Many indigenous populations developed their own fermented drink, which varied depending on local ingredients. In the Andean region, a cloudy beer called Chicha was the favored fermented beverage. Very little direct evidence is known concerning the pre-Hispanic pattern of use. When alcohol was introduced in this society in a widespread manner is also unknown. A direct alcohol marker would enhance our understanding of ancient use as it has with other drugs. Fatty acid ethyl esters (FAEE's) have been identified as metabolic products of ethyl alcohol in modern hair and recently tests have become available for their detection. This preliminary study was undertaken to determine if FAEE's could be found in mummy hair to serve as markers of alcohol exposure.

Alcohol consumption both intentional and unintentional has occurred for millennia. The incidental exposure to alcohol would have occurred to all our ancestors foraging on fermented fruit. It has even been suggested that consumption of high caloric alcohol was an adaptive advantage to our ancestors the last 3-5 million years and this is supported by the health benefits of low-level alcohol intake in modern humans. Dudley (2002) since sugars in many common natural fruit juices will ferment to an alcoholic beverage our ancestors surely discovered the fermentation process accidentally. The end result is a very useful drink free of harmful microorganisms and also a psychoactive compound. Millikan LE (1999) that combination assured the survival of alcohol to future generations.

Many indigenous populations developed their own fermented drink, which varied depending on local ingredients. Valee (1998) In the Andean region of pre-contact South America, a cloudy beer called *chicha* was the favored and most likely the only fermented beverage. *Chicha* has a low to moderate alcohol content and is generally made from maize although it could also be fermented from a variety of other plants. Incas; Lords of Gold and Glory 1992 The process of making *chicha* varies widely even today but involves three basic steps and requires a period of only one to three days. (3) Either mixing the pulverized maize with saliva or malting starts the process. Saliva contains the enzyme diastase that converts starch to sugar. "Malting" is the second method that allows the maize to germinate, which begins the conversion of starch to sugar. Either method will allow for more complete fermentation and higher alcohol content than simply allowing the maize to ferment naturally. Cooking the brew and then separating the desired liquid from the by-products then completes the process. (Cutler et al., 1947).

There is little doubt that *chicha* played an important and varied role in ancient Andean society including ceremonial, economic, and nutritional. Moore (1989) Missionaries in post-contact Peru were appalled that during religious ceremonies the mummified bodies of Inca nobility were given cups of the corn beer to toast each other. Not only was the brew made for families but recently a large brewery was discovered on a mountain top in Peru that could produce enough drink for hundreds of people. However exactly when chicha began to be produced and the prevalence within the cultures in this region is unknown. Stable isotope ratio studies of bone apatite and collagen demonstrate that maize did not become a staple in the diet of pre-Colombian Americans until about 3000 years ago yet the cultivation of this plant and its ancestors began 7000 years ago. The grain in the plant predecessors of maize was not useful as a food item but the stalks were high in sugar. Some researchers suggest

that the domestication of the plant before it had a suitable dietary grain demonstrates that the stalk was probably used as a sugar source for beer making 7000 years ago. Smalley, et al., (2003) however this concept remains controversial. Direct markers of alcohol exposure could help resolve the issue of timing of wide spread introduction of an alcoholic drink and also the patterns of use in these ancient populations.
Detection of alcohol consumption is an area of research that has focused mainly on acute or current exposures. It is well known that immediately following ingestion, ethanol can be measured in any body fluid as well as expired breath air. The subsequent specific ratio levels can be correlated to amount consumed. However, using ethanol itself or it's by product aldehyde can only indicate recent exposures due to their quick metabolism and lack of potential to accumulate appreciable amounts for long periods of time. Detecting chronic or long-term exposure to alcohol is a more difficult task.

One of the most promising recent tests for chronic alcohol exposure concerns a metabolic product of ethyl alcohol. Fatty acid ethyl esters (FAEEs) are products of the non-oxidative metabolism of ethanol that are formed by the conjugation of ethanol to endogenous fatty acids. They were identified some 50 years ago as ethyl alcohol metabolites but only recently have they been applied clinically. Laposata (1998) FAEEs appear in the blood shortly after ethanol is absorbed and begin to decrease 2 hours after the last drink. They persist in blood for more than twenty-four hours after significant alcohol consumption. These esters generally reflect the amount of alcohol exposure and social drinkers have lower levels than alcoholics. Although elevated levels are attributed to alcohol some individuals have very low to trace endogenous levels which probably originate from sources other than alcohol consumption (Soderberg et al., 2001). Using hair analysis as a tool for the retrospective detection of illicit and/or therapeutic drug consumption is an emerging field with much potential. It has been used successfully for the analysis of various compounds such as cocaine and nicotine in both ancient and modern hair. (Cartmell et al., 1991). As a result of their hydrophobic nature, FAEEs have the potential to accumulate significantly into the hair and remain for the life of the hair or until it is cut. Consequently, FAEEs may be suitable long-term markers for identifying and quantifying alcohol use.. FAEEs have been reported in hair of adult alcoholics whereas hair taken from children and teetotalers was negative. Auwarter et al., (2001) For social drinker's levels of FAEEs were much lower than alcoholics. Neonatal hair analysis also has recently been used as a marker for maternal alcohol consumption. Klein et al., (2002) We were encouraged to expand our studies of hair from mummies by previous success in demonstrating cocaine (from coca -leaf-chewing practices) and nicotine in hair from Andean mummies. This study is undertaken to determine if FAEEs are stable enough to be found in mummy hair in measurable quantities

Anthropological features of the studied group

The studied hair samples came from mummies found in the Atacama Desert of northern Chile and southern Peru. This desert lies on the western slope of the Andes and continues to the Pacific Ocean. Stretching from 17° to 27° south latitude, its interior is habitable only within certain stream-containing valleys and along the coast. Being one of the most arid regions of the world makes survival difficult but these conditions allow preservation of many biological materials such as hair, tissue and textiles. The studied hair samples were taken from mummies of the Maitas Chiribaya culture. This culture became identifiable just prior to the collapse of the Tiwanaku in the Azapa Valley near Arica in northern Chile. They were primarily agriculturists and are dated AD 1000 to AD 1250

Materials and methods

Hair samples were obtained from the Human Biology Section files at the Archaeological Research Institute of the University of Tarapaca in Arica, Chile. These are archaeologically excavated spontaneously mummified human remains from coastal and valley sites in that region of the Atacama Desert. Age and sex were estimated using methods commonly employed by physical anthropologists. Ubelaker (1989) The samples tested were taken from five males and 2 females with age ranges from 15 to 50+.
The esters were extracted from the hair samples overnight with a mixture of n-heptane and dimethyl sulfoxide. The extract was then evaporated and the residue was reextracted with the use of headspace solid-phase microextraction. The final extract was analyzed by gas chromatography-mass spectrometry. Details of this type of analysis have been published by one of the authors. Klein et.al. (2000)

FATTY ACID ETHYL ESTERS (FAEE'S) IN ANDEAN MUMMY HAIR

AGE	SEX	RESULTS NG/MG HAIR
15	M	BDL
55+	M	BDL
17	M	BDL
20	M	0.010
50+	M	0.057
47	F	0.598
50+	F	BDL

Table 1

Results

Of the seven samples tested 3 or 43% had quantifiable levels of FAEE"S. The remainder were below detectable levels (BDL) (Tab. 1) The positive levels ranged from 0.010 to 0.598 ng/mg. In modern human hair a reasonable FAEE cut-off to distinguish between social/moderate and heavy drinking/alcoholism in hair is 0.4ng/mg. Wurst (2004) The esters were present in both sexes. These results indicate that FAEE"S are highly stable in this arid environment and may play a role in the study of alcohol consumption in ancient populations. This also adds to the growing body of evidence that FAEE"S may be suitable as a long-term biomarker of alcohol exposure. This is only a preliminary/pilot report but the results seem to justify larger cross-cultural studies to further define the introduction and pattern of alcohol use in ancient populations.

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