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OVERVIEW OF HAIR ANALYSIS: A REPORT OF HAIR ANALYSIS FROM DAKHLEH OASIS, EGYPT

Larry W. Cartmell*, Cheryl Weems**

*Valley View Regional Hospital, 430 N Monta Vista, OK 74820, U.S.A. E-mail: <u>cartmell@chickasaw.com.</u> **930 East 17th Street, Ada, OK 74820, USA. E-mail: <u>cweems@compworldnet.com.</u>

During growth and keratinization certain chemicals, including drugs, are "trapped" in hair. These chemicals can subsequently be released and recovered for analysis. Hair analysis can provide long term information, from months to years, concerning drug exposure in an individual. Both modern and ancient hair samples have proven useful in analyses. This paper will examine scientific issues such as the incorporation of drugs into the hair shaft, external contamination, etc. in addition to a report on analysis of 18 hair samples from Dakhleh Oasis, Egypt.

Key words: Mummies, forensic, toxicology.

Durante el crecimiento y la keratinización, ciertos químicos, incluyendo drogas, son "atrapados" en el pelo. Subsecuentemente, estos químicos pueden ser liberados y recuperados para su análisis. El análisis del pelo puede proveer información de largo plazo, de meses a años, respecto a la exposición de un individuo a drogas. Tanto muestras modernas y antiguas han sido útiles en el análisis. Este trabajo examinará temas científicos como la incorporación de drogas en las hebras del pelo, contaminación externa, etc., y reportará información sobre un análisis de 18 muestras de pelo del Oasis de Dakhleh en Egipto.

Palabras claves: Momias, forense, toxicología.

Hair analysis for drugs of abuse has received much attention in the medical and forensic literature. But in spite of it's potential usefulness in ancient remains, very little has been written in the anthropology literature. There are only a hand-full of published reports and only one population based study (<u>Cartmell et al. 1991: 260-268</u>). This report will deal briefly with an overview of hair analysis for drugs of abuse and then report on a study of 13 hair samples from Dakhleh Oasis.

Hair analysis has had its ups and downs in the past. Hair analysis for elemental poisoning has been accepted for almost 150 years. This is probably the most famous use of this type of analysis. Other methods of hair analysis have met with less success. In the early 1970's trace metal concentration in human hair attracted considerable publicity when researchers tried to prove a relationship between such elements with intelligence and mental disease. Unfortunately clinical studies were released before basic research had been completed. By 1980 this practice was dropped.

Probably the most popular use of hair analysis was its application to the evaluation of nutritional deficiencies. Many people from food faddist to physicians were convinced health status could be improved by studying the concentration of trace metals in hair. After much experience with this type of analysis, most practitioners became disenchanted with this technology. A 1985 article by Barret in JAMA sounded the death knell among legitimate practitioners for nutritional analysis of hair. Even though in disrepute nutritional hair analysis can still be obtained. In 1997 I submitted a hair sample from an

adult male Maitas Chiribaya mummy approximately 1000 years old from the Azapa Valley of Northern Chile. The completed report was followed by a number of suggestions for nutritional supplements which they sell by the way including stress tablets and 6 a day fiber supplement. So if you subscribe to this type of analysis you can conclude stress and fiber depletion are not modern phenomena.

Chemical analysis of hair for drugs is passing the test that others have failed. A growing body of literature now substantiates the legitimacy of drug testing. The first report of drugs in hair was in 1954 by a dermatologist studying the deposition of drugs in hair as the cause of barbiturate induced dermatitis (<u>Goldbloom et al. 1954: 121-128</u>). It was 18 years later before another article appeared in the literature along with a few scattered other reports. These early techniques, however, were cumbersome and were not well suited for most laboratories. The national concern about substance abuse in the late 1960's and early 70's however stimulated the development of new technologies. Very specific in this regard was the development of sensitive radio immunoassay procedures which were primarily designed for urine analysis. The first article using these techniques for hair analysis appeared in 1979 by Dr. Baumgartner who was the pioneer in the development in hair analysis for drugs of abuse. The first article specifically regarding cocaine was again published by Dr. Baumgartner in 1982 (<u>Baumgartner 1982: 790-792</u>).

In the late 80's and 90's there have been a plethora of articles primarily in the forensic literature regarding hair analysis. At the current time, not only have numerous drugs of abuse been report but at last count, over 60 therapeutic drugs. The first study of drugs in ancient hair was in 1990 and reported cocaine in Chilean mummy hair (<u>Cartmell et al.</u> 1991).

How do Drugs Enter the Hair?

The pathways of drug entry into hair are still not fully understood. The most common theory of drug incorporation in the hair matrix is that it takes place at the root level. As chemicals circulate in the blood stream, they are incorporated in the growing hair matrix. Scalp hair grows between 1 -1.5 cm a month and the hair shaft becomes a virtual timed record of drug use.

Hair follicles are skin addnexa which arise in the dermis. The growing portion of the hair bulb is surrounded by numerous arterial capillaries. Usually associated with most hair follicles are sebaceous glands which secrete a waxy substance know as sebum which covers the hair and has a protective role. In addition to the sebaceous glands ecorine sweat glands usually are found in association with hair follicles. Although sweat is not directly secreted on the hair follicle as sebum is, the proximity and liquidity of the sweat ensures that it comes in contact with the hair.

The primary function of the hair bulb is production of the hair shaft. Hair consists of an outer cuticle and an inner medullary portion. The medullary portion of hair consists of keratin chains wound into strands known as microfibrils. The microfibrils are organized into larger bundles of microfibrils that make up the medullary portion of the hair shaft. Surrounding this is a layer of epithelial cells which overlap like shingles to serve as a protective barrier. The cuticle is usually intact at the hair closest to the scalp. However, as the hair grows and is exposed to normal wear and tear this cortex degenerates and becomes frayed. In tips of extremely long hair the cuticle can be completely absent.

This anatomy and physiology allows several separate ways for drugs to enter the hair. Drugs present in the blood stream can enter the hair follicle when it is nourished at the root. Fusion of drugs from arterial blood capillaries to matrix cells in the base of the follicle is considered a primary means of drug deposition on hair. As these cells pass up the hair shaft, they gradually die and form the keratin hair fiber with drug embedded in the matrix (Cone 1996: 438-443). Some authors have postulated that the concentration of drugs in hair is proportional to their concentration in blood (Baumgarner et al. 1987:53)

Numerous water soluble drugs have been detected in sweat. This review primarily concerns cocaine. Cocaine and lesser amounts of the metabolites ecgonine and benzoylecgonine have been recorded in sweat up to 48 hours after ingestion (<u>Cone et al.</u> <u>1993: 298-305</u>). It is easy to understand that this excretion of cocaine in sweat could be transferred to hair. In one study, cocaine was transferred from a cocaine dosed subject to drug free hair in 30 minutes when held tightly in the hand (<u>Hebderson 1993: 19-20</u>).

Sebaceous glands are present on the entire hair growing surface of the body with the exception of the dorsum of the foot. They are most concentrated on the scalp. The sebaceous glands secrete sebum which is a waxy lipid material. There has not been a great deal of investigation of the role of sebum in drug incorporation of hair. However, drugs have been demonstrated in hair and theoretically can be transferees to hair follicles (Faergamann et al. 1993: 305-309).

The skin itself could also serve as a transporter of drugs either into the body or exiting from the body. In a small study, which has not been reported, we checked for cocaine in skin. We found cocaine in mummy skin samples from individuals who had tested positive in hair. However in our small sample we found if the hair is negative, the skin sample is also negative. Theoretically, the drug could be present in sebum or sweat not inherent within the skin.

External Contamination

The real Achilles heel of hair testing for drugs is external contamination. That is the possible source of entry of drugs into hair from the environment in a person who did not use the drug. In spite of a great deal of publicity about external contamination in modern hair in court cases, very little is actually known. Smoke and solid forms of drugs in the environment can contaminate hair with both cocaine and nicotine and give false positive results.

External contamination is also of concern in ancient hair samples (Table 1). The external contamination could come from several sources. Contamination could be pre-mortem from environmental contaminants. It is known for example that nicotine was both smoked and also used in solution as a tobacco paste. Either one of these methods could have allowed nicotine to be transferred from the hair when the person was not actually a tobacco user. A second source of contamination could be during the funerary process. Of the populations I have studied very little information about what solutions if any were used in the Egyptian's elaborate processes but this could potentially be a source of contamination.

Pre Mortem	Post Mortem
 Smoke External application (medicinal or ceremonial, etc.) 	 –Funeral process –Period of burial –Leaching from grave goods –Post excavation and curation –Tobacco use by staff –Storage issues

Table 1. Possible sources of false positives (contamination) in ancient hair samples

A third source of external contamination for ancient hair samples could be during the period of burial. It is known, for example, in the ancient Andean populations cloth bags of varying sizes containing coca leaves have been found in mummy bundles. In addition,

some mummies have been found with a coca "quid" in situ. Theoretically either of these sources could leach into the hair during post-mortem autolysis or subsequent centuries of burial. I have examined both quids and leaves from Andean mummies over 500 years old and found the drug content to remain high so the potential exists. In a separate study of mummy hair from Chiribaya in southern Peru it was learned that positive mummy hair had no correlation with coca in tombs (Aufderheide 1992).

A fourth way that ancient hair could have been contaminated is post-excavation. Obviously some mummies have been excavated for many years. The curation practices were somewhat haphazard in the early days and even today there are no universal standards for maintenance of smoke free environment, etc. Black and white film of early excavations shows many of the principals smoking tobacco. George Risner who spent more than forty years excavating on the Gisa plateau almost continually smoked his pipe. Could this type of contamination account for the finding of vegetal material in the abdomen of Rameses which had been open after excavation for 100 years?

Is there a way to check for external contamination prior to testing? This issue has not been completely settled. However, there is one method that seems to be gaining acceptance. The first routine step in drug hair testing is to wash the sample in an attempt to reduce external contamination and exogenous debris. This first rinse water of modern hair samples is frequently used as a possible check for external contamination. In a similar manner, we tested the rinse water of 50 ancient hair samples which were positive for cocaine. All rinse water from these ancient samples were negative indicating no contamination.

What is the magnitude of external drug contamination in ancient hair? The current thinking is that it is probably minimal. None of the studies mentioned previously demonstrated any contamination. In great part this is due to the arid conditions that preserve most mummies. This dryness makes it very difficult for molecules to move around. External drug contamination remains a theoretical possibility in archaeological samples and we should remain diligent. However, with proper handling after excavation and use of routine decontamination procedures, this should not be a great obstacle in archeological samples.

The technical aspects of hair drug testing will not be detailed in this paper. These are readily found in the literature. Basically the drug is extracted from the hair and the subsequent liquid is tested as any other body fluid. The criteria for a positive result should include analysis by two fundamentally different techniques. The two most commonly used are sensitive radioimmunoassay and a highly specific chromatographic test such as GC/MS.

As an example of the use of this technique, I will discuss a recent study of cocaine and nicotine in ancient hair samples from Egypt. Tobacco producing plants are of the genus Nicotinia which are of the Nightshade family. This is primarily a plant of the new world and use of tobacco is generally accepted to be an American innovation. However, Nicotinia species have been recorded in other continents including Africa. In addition, other members of the Nightshade family and other plants containing nicotine are found in Egypt. In contrast to nicotine, no native cocaine containing plants have been recorded outside the Americas. Ancient use of tobacco or cocaine has not been recorded by Egyptologists. Recently, however, Egyptian mummy tissue including hair, soft tissue and bone have been reported by Balabanova et al. to contain cocaine, hashish (THC), and nicotine (Balabanova et al. 1992) (Table 2). Their research has also been popularized by a recent one hour program on the Discovery Channel entitled "Curse of the Cocaine Mummy". The study consisted of nine adults from a period 1070 B.C. to 395 A.D. All mummies were reported positive for cocaine and hashish and eight were positive for nicotine.

Age	Sex	Cocaine	Nicotine Ng/Mg
12	M	0	1.7
25	Μ	0	2.1
21	F	0	2.2
25	F	0	1.7
10	U	0	0.7
6	Μ	0	0
45	F	0	2.2
55	Μ	0	1.9
55	М	0	2.1
Adult	I	0	1.9
Adult	I	0	0
Adult	1	0	1.8
42	Μ	0	0
2	1	0	0
23	F	0	1.9
55	M	0	2
55	М	0	1.9

Table 2. Dakhleh oasis drug hair study

The ancient Kellis site is located within the Egyptian Oasis of Dakhleh in the western Sahara. The site has been inhabited since Paleolithic times but there is very little information concerning this Oasis in the ancient Egyptian texts. This oasis is approximately 800 kilometers south, southwest of Cairo on the same latitude as Luxor. It is approximately 425 kilometers northwest of Kharga Oasis. In antiquity it served as the trading center of the caravan route through the desert from upper Nile to Libya. In 1993, I was invited to be a part of a group to study these mummies that had been previously excavated.

Hair samples were taken from 18 mummies from the Dakhleh Oasis which had under gone extensive autopsies and some of which had radiocarbon dating. These were subjected to cocaine and nicotine analysis by previously reported techniques.

Hair samples were taken from a variety of ages and sexes. No samples were taken from individuals under six years of age. These results indicate that all individuals were negative for cocaine and 10 of the 13 individuals had nicotine levels consistent with dietary or minimal tobacco exposure.

These results indicate that all individuals were negative for cocaine and 14 of the 18 individuals (78%) were positive for nicotine. The positive levels clustered in a very narrow range from 0.7 to 2.1 ng/mg of hair. A cut off value of 2 ng/mg is used in modern testing to discriminate tobacco use from passive exposure and dietary sources of nicotine. In a study of ancient hair samples for Andean mummies this also was determined to be a valid cut-off value. All of the Dakhleh values are consistent with dietary origins. In contrast to the low levels found in Egyptian mummy hair the tobacco used in the South American population had values exceeding 20 Ng/mg hair.

This small preliminary study of Dakhleh Oasis Egyptian mummies indicates the source of nicotine to be dietary in origin. A larger study is planned which will include gender and age differences. Perhaps ethnobotanists might also investigate the source of nicotine in this population.

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