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The potential use of hair in doping control in general and in the detection of stanozolol in particular

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Introduction

The use of hair as biological specimen for Drugs-Of-Abuse (DOA) testing has been well established. Numerous scientific articles regarding this subject are still being published. A major advantage of using hair specimen for analysis is the long detection window in hair. As one objective in doping analysis is to increase the length of a detection window of particular doping agents, hair analysis may be of interest. The greatest improvements regarding detection windows in doping analysis up to now have been achieved by introducing more sophisticated analytical techniques [1]. This report describes the potential to use hair as biological specimen in doping control. Several years ago Dr M. Möller presented at the Workshop a short lecture regarding this topic.

The Netherlands Institute for Drugs and Doping Research (NIDDR) is interested in the evaluation of non-invasive techniques in drug testing. The potentials for use of saliva as a non-invasive technique in the field of doping analysis has reported in the Proceedings of 1996 [2]. A complete report on the detection of stanozolol in hair has been published in the *Journal of Analytical Toxicology* [3]. A review regarding drug testing in hair is available in the thesis of Höld 1996 [4].

Anatomy and physiology of hair

Hairs can be found almost all over the body and are embedded into the skin in hair follicles (Figure 1). Sitting in the hair follicle is the hair shaft (A). The deepest point of the follicle is called the hair bulb (B). Hair grows from the hair bulb, as the bulb contains matrix cells, which, as they divide and become larger, pushes the hair shaft upward. The matrix cells are supplied with necessary nutrients by arterial blood capillaries (C). Venous blood capillaries (D) transport waste products from the bulb. Hair follicles are surrounded by sweat (E), sebaceous and apocrine (F) glands. The sweat glands have ducts near the hair follicles.

Hair does not grow continuously, but grows in cycles. The growth rate is depending on many factors. There are a wide range of reported growth rates. The average growth rate is approximately 1 to 1.5 cm per month.

Hair contains proteins (65-95%), water (15-35%), lipids (1-9%) and minerals (0.25-0.95% on a dry weight basis) [4]. One of the most important hair proteins is keratin. It is composed of several amino acids, of which, cysteine is the major one. Hair color is derived from melanin, which is a polymer derived from tyrosine.

Mechanisms of substance transfer from blood into and on hair

The possible routes that may lead to a drug and/or metabolite incorporated into and on hair are:

1) passive transcellular diffusion from blood into the matrix hair cells;
2) transfer from blood to the outer skin surface via sweat and sebum and deposition on the outside of the hair;
3) transfer from the external environment to hair.

Passive transcellular diffusion is the transport of substances across the capillary wall to the matrix cells in the hair bulb. During this process, the rate of incorporation in the hair is mainly determined by the melanin affinity and the lipophilicity of a substance and the pH gradient between blood (pH 7.4) and the hair matrix (acidic) [5]. In general it can be stated

that basic substances are incorporated more readily than neutral or acidic substances. Transfer of a substance onto hair via sweat and sebum probably leads to deposition of the substance on the hair. The substances are not tightly bound this way. External contamination of hair from drugs that may be smoked is also a potential way to deposit drugs to hair. These less tightly bound drugs however, can be removed by washing during the sample preparation, although the efficiency of the washing procedures is still controversially.

Collection and analysis of hair

The collection of hair specimens is non-invasive and can be easily performed. Hair on the crown of the head is the most suitable place to collect. It is relatively uniform and consistent in its growth patterns and phases. Also for cosmetic reasons it is an acceptable area to collect hair.

Washing procedures to remove the unbound drugs on the exterior surface of hair, enzymatic or chemical hydrolysis processes to digest hair and conventional extraction methods are the principal steps. The detection of compounds in hair specimens can be achieved with similar analytical techniques as in other biological specimens such as urine and blood.

Detection of doping agents in hair

The doping agents which have been detected in human hair (Table 1) are typical examples of DOA. The reason for this is, that especially in DOA-testing hair specimen collection and analysis have proven its significance. The concentrations observed in hair are dependant on the ability of a compound to be incorporated and the dose [6]. For example, buprenorphine and clenbuterol are administered at relatively low dosages and, therefore, it is impossible to observe high concentrations.

Table 1: Selection of doping agents and respective concentrations reported to be found in human hair	
doping agents and/or metabolite	concentration range (ng/mg hair)
amphetamines	
- amphetamine	up to 12
- 3,4-methylenedioxyamphetamine	up to 10
- 3,4-methylenedioxymethamphetamine	up to 60
(nor)buprenorphine	0.02 - 0.59
cannabinoids	
- tetrahydrocannabinol	0.06 - 7.6
- 11-nortetrahydrocannabinol-9-carboxylic acid	0.06 - 3.9
clenbuterol	0.025 - 0.160
cocaine	
- benzoylecgonine	0.1 - 5.8
- cocaine	0.3 - 89
fentanyl	0.013 - 0.048
opiates	
- codeine	0.45 - 39.6
- 6-monoacetylmorphine	0.09 - 48.2
- morphine	0.3 - 45

For those compounds for which a long detection window would be very important, the anabolic steroids are of special interest. These compounds are used during training periods in such a way, that testing for the use of anabolic steroids after sport events is almost useless. Out-of-Competition testing is therefore necessary, but this type of testing is not always easy to organize and is considered to affects the privacy of an athlete. Hair specimen collection

could be a solution, at least if these kind of compounds are incorporated in hair. However, no examples of the detection of anabolic steroids and/or its metabolites in human have been reported.

In animal studies the incorporation of the anabolic steroid stanozolol in hair has been examined [3]. It was observed that after the administration of 20 mg/kg body weight/day for 3 days, stanozolol was incorporated preferentially into pigmented hair at mean concentrations in the range of 0.3 ng/mg hair. Although dosage extrapolations have to be very carefully applied, the examined dosage of stanozolol was compared to humans high. It also should be noted that stanozolol is a special steroid as its contains an amine function in its molecular structure and that in contrast to other steroids it is relatively basic. However, the fact that at least certain anabolic steroids can be incorporated in hair has been demonstrated in the respective animal study.

Potential role for hair in doping analysis

If the average growth rate is considered to be 1 to 1.5 cm per month, than in a hair specimen of 10 cm the window of detection is in the range of 6 to 10 months. In such a sense hair specimen analysis could be very useful in doping analysis.

Looking at reported concentrations found of doping agents in human hair, one must conclude that in general very sensitive analytical detection techniques are required. Even if for example 50 mg of hair is collected, limits of identification in the low ng range probably are necessary.

Conclusions

1. Hair as a biological specimen is of interest because of its long detection window.

2. Analysis of drugs in hair requires very sensitive techniques.

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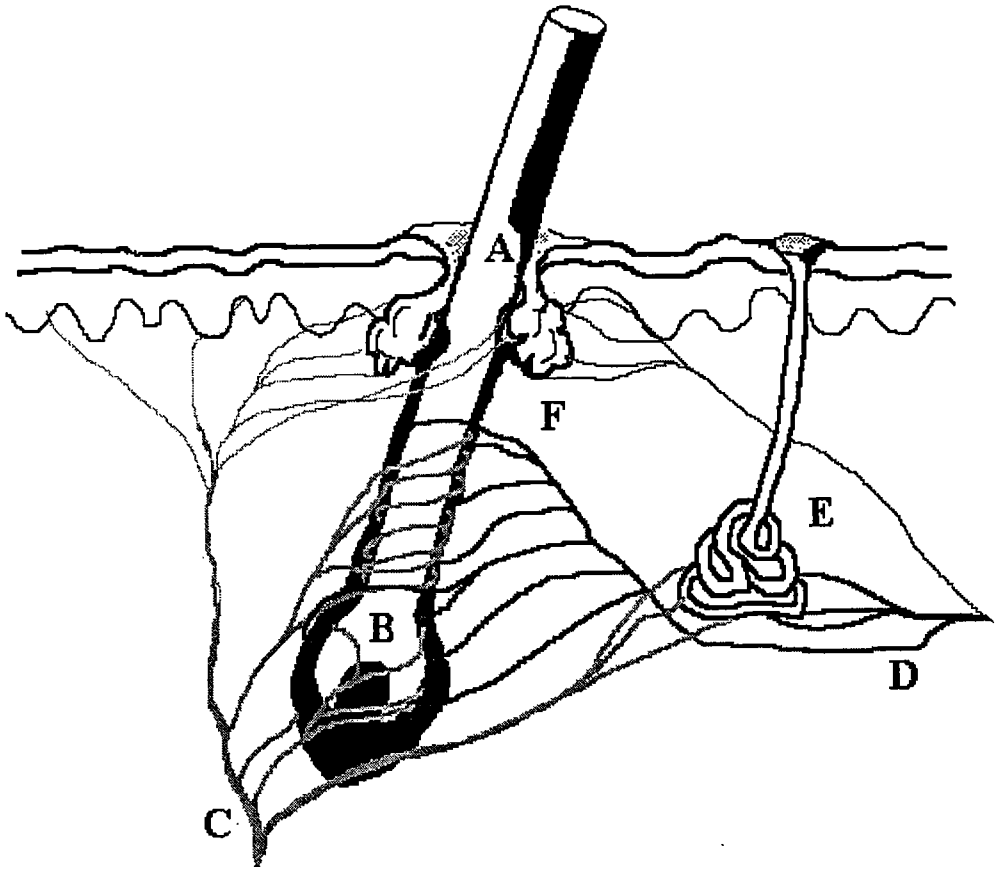

Figure 1: Schematic representation of the hair follicle embedded in the skin, showing the hair shaft (A), hair bulb (B), arterial blood capillaries (C), venous blood capillaries (D), sweat glands (E), sebaceous and apocrine glands (F).