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## **Incorporation of Nandrolone in Horse Hair – Importance of Sweat**

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### **Introduction**

Interest in long-term detection of drugs using hair analysis had become greater during the last years. Hair seems to be a suitable specimen not only in forensic cases but also in control of stallions selected for breeding.

Substances can be incorporated into hair by different ways, via blood into the root or by sweat and sebum into the hair shaft. It is known that the incorporation rate of a substance depends on several conditions, its chemical structure for example and also hair colour or cosmetic treatment could be important [1].

Target concentrations of substances which are incorporated due to their lipophilicity into hair and not bound to melanine are rather low, e. g. steroids. The objective of this study was to examine most important routes and rates of incorporation of 19-norsteroids into horse hair especially after transdermal application.

### **Application study**

A commercially available transdermal formula was applied containing a mixture of the nortestosterone precursors 4-estrene-3 $\beta$ ,17 $\beta$ -diol and 4-estrene-3,17-dione.

Transformation of these so called prohormones should lead to nandrolone in the organism (Fig. 1). Nandrolone is an anabolic steroid and not endogenously produced in geldings and mares but in stallions [2].

Previous studies showed an increased bioavailability and delayed elimination period compared with oral administration of these substances in humans [3].

Four geldings were treated by topical application with a total amount of 2 mg of prohormones per kg body weight. The transdermal product was applied at the abdomen. Sweat, tail hair,

coat hair, plasma and urine samples were collected immediately before application, 17 hours and 30 days after treatment. Further sample collections are in progress.

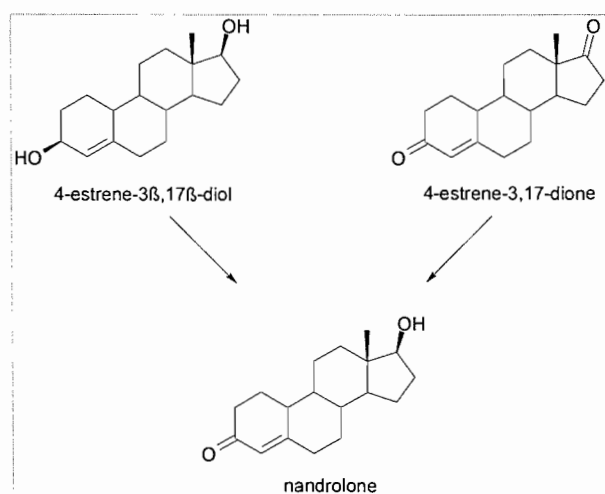


Fig. 1: Transformation of precursor substances to nandrolone

To get sweat samples the horses had to undergo physical exercise on a treadmill under controlled conditions. Samples were collected at neck and breast (between front legs) to prevent contaminations from application site and also to detect possible differences of the metabolic patterns depending on body region. Coat hair samples were taken from neck by shaving. Tail and coat hairs were always collected after exercise.

## Experimental

Sweat and plasma:

Sample preparation was carried out by solid phase extraction (XAD-2) and liquid-liquid extraction with n-pentane at pH 9 to isolate unconjugated steroids (norandrosterone-d4 was added as internal standard) [3]. Dried residues were derivatised using MSTFA / ammonium iodide / propanethiol and then samples were analysed by GC-HRMS (AutoSpec, Micromass) [3], target ions used for quantification are: 418.2723 (nandrolone); 416.2567 (4-estrene-3,17-dione); 420.2880 (4-estrene-3 $\beta$ ,17 $\beta$ -diol).

Hair:

Hair samples were washed with methanol / water (1:1) for decontamination. After cutting into segments each sample was pulverised. Samples (100 mg) were spiked with testosterone-d3 as internal standard, treated with 2.5 ml of methanol and incubated in ultrasonic bath (5 hours, 50°C). Solutions were evaporated, dissolved in buffer (pH 9) extracted with n-pentane and further purification was carried out by HPLC [3]. Collected LC-fractions were evaporated to

dryness and treated with MSTFA / ammonium iodide / propanethiol to form bis-TMS derivatives. Analyses were performed by GC-MS/MS (AutoSpec, Micromass), following fragmentations were detected for quantification: 418.2→194.1 (nandrolone); 416.3→194.1 (4-estrene-3,17-dione); 330.2→240.2 (4-estrene-3 $\beta$ ,17 $\beta$ -diol).

## Results

Sweat:

Metabolites and administered steroids were only found in the unconjugated fraction. 17 hours after treatment nandrolone is already detectable, although the amount of 4-estrene-3 $\beta$ ,17 $\beta$ -diol and 4-estrene-3,17-dione is predominant (Table 1). After 30 days the amount of nandrolone in sweat is comparable to calculated concentrations at day 1, but the values of 4-estrene-3 $\beta$ ,17 $\beta$ -diol and 4-estrene-3,17-dione are decreased (Table 2). Differences of total amount and metabolic patterns in samples taken from neck compared with breast could be determined, especially at the first day after treatment.

Table 1: Concentrations of 19-norsteroids in sweat, one day after application

		Horse 1	Horse 2	Horse 3	Horse 4
nandrolone [ng/ml]	neck	29	77	307	5
	breast	18	49	41	34
4-estrene-3,17-dione [ng/ml]	neck	244	1231	1137	59
	breast	264	345	260	1400
4-estrene-3 $\beta$ ,17 $\beta$ -diol [ng/ml]	neck	47	250	1362	6
	breast	37	176	79	68

Table 2: Concentrations of 19-norsteroids in sweat, 30 days after treatment

		Horse 1	Horse 2	Horse 3	Horse 4
nandrolone [ng/ml]	neck	41	77	116	23
	breast	20	82	120	32
4-estrene-3,17-dione [ng/ml]	neck	39	68	25	39
	breast	39	18	26	76
4-estrene-3 $\beta$ ,17 $\beta$ -diol [ng/ml]	neck	5	17	25	8
	breast	2	17	33	2

Plasma:

Caused by their nonpolar and lipophilic properties unconjugated 19-norsteroids are more readily incorporated into hair from blood, in contrast to conjugated metabolites.

Free nandrolone could only be measured in plasma samples taken 17 hours after application. Concentrations ranged from 2 to 5 ng / ml.

Coat hair:

Coat hair only grow during a short time period in spring and autumn, there is no permanent growth. This study took place in winter time and no growing of hair was observed. That is why possibly detected steroids could only be incorporated via sweat or sebum into the hair, not via blood.

Investigation was carried out in the complete length of hair (approximately 3 cm). Nandrolone could be found in all samples, calculated concentrations were higher in samples taken after 30 days than at day 1 (Fig. 2). The administered substance 4-estrene-3,17-dione could also be identified, but no 4-estrene-3 $\beta$ ,17 $\beta$ -diol was detected.

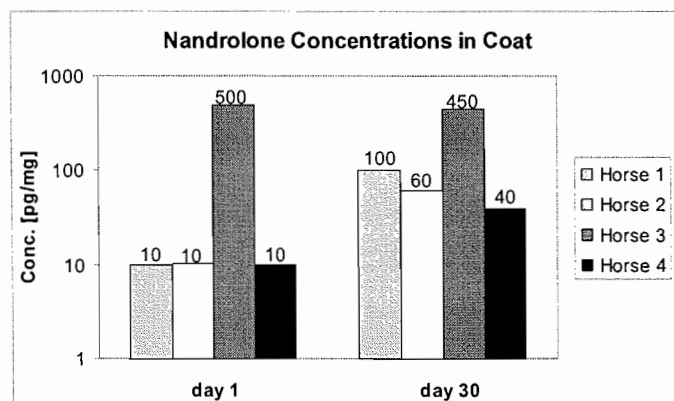


Fig. 2: Concentrations of nandrolone in coat hair one day and 30 days after treatment

Tail hair:

Mane and tail hair of horses have a permanent growing rate of approximately 2 - 3 cm per month. Analyses of collected tail hair samples were carried out in segments of 2 cm of length. Samples taken at day 1 were investigated from roots up to 4 cm of hair length, samples taken after 30 days ranged up to 8 cm.

High amounts of nandrolone were determined in all samples and hair segments grown before treatment also contained nandrolone. This clearly can be shown by concentrations measured in hair samples taken 17 hours after administration: nandrolone was detectable in segments up to 4 cm of length which were already grown one and two months before treatment, respectively (Fig. 3).

This distribution of steroids over a wide length of hair indicates the importance of its incorporation into hair by sweat or sebum.

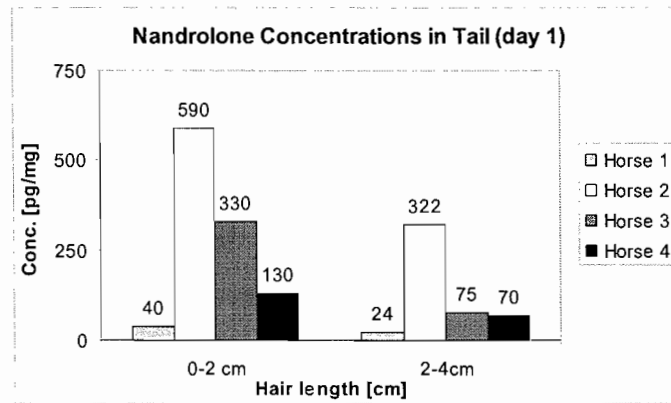


Fig. 3: Concentrations of nandrolone in tail hair samples after one day (length of segments 0 - 2 cm and 2 - 4 cm)

According to results in coat hair samples 4-estrene-3,17-dione could also be found in all segments.

### Conclusion

- Steroids seem to be mainly incorporated via sweat or / and sebum into coat and tail hair.

Indications for this assumption:

Concentrations of 19-norsteroids found in sweat were much higher than in plasma samples.

Detection of nandrolone was already possible in hair samples taken 17 hours after topical application of the precursor substances 4-estrene-3 $\beta$ ,17 $\beta$ -diol and 4-estrene-3,17-dione.

- An unexpected wide spreading of nandrolone over the hair strand was observed. In cases of suspicious hair samples this should be taken into account for interpretation of results and estimation of treatment period.

### Literature

- [1] P. Kintz, in: R. K. Müller, D. Thieme (Eds.), Progress in Hair Analysis (1), Sport und Buch Strauß, Cologne (2000), 79-89
- [2] E. Houghton et al., Equine Vet J. 18(6): 491-3 (1986)
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