Reprint from

RECENT ADVANCES IN DOPING ANALYSIS (3)

M. Donike
H. Geyer
A. Gotzmann
U. Mareck-Engelke
(Editors)

Sport und Buch Strauß, Köln, 1996

H. Geyer, W. Schänzer, U. Mareck-Engelke and M. Donike:
Factors Influencing the Steroid Profile
In: M. Donike, H. Geyer, A. Gotzmann, U. Mareck-Engelke (eds.) Recent advances in doping analysis (3). Sport und Buch Strauß, Köln, (1996) 95-113

H. Geyer, W. Schänzer, U. Mareck-Engelke and M. Donike

Factors Influencing the Steroid Profile

Institut für Biochemie, Deutsche Sporthochschule Köln, Germany

Abstract

The parameters of the urinary steroidprofile, analysed for dope control purposes, are very stable. These parameters can be influenced by the application of endogenous steroids, anabolic androgenic steroids, probenecid, diuretics, ethanol and trimethoprim. Also bacterial activities in the urine and side activities during the enzymatic hydrolysis may cause changes. All these factors lead to characteristic patterns of the steroidprofile.

Introduction

Steroidprofiling is a well known method of clinical endocrinology, used for detection enzyme deficiencies [1,2]. This method was introduced in the dope analysis by Donike et. al. [3,4] for the detection of the misuse of exogenous testosterone. Several studies have shown, that the steroid profile parameters, especially the steroid ratios used for dope control purposes are very stable. These ratios are not changed by training and severe physical endurance performance [5,6], by circadian rhythm [7], by the menstrual cycle [8] or by circannual rhythm [9]. In the following are presented factors which lead to obvious changes of these parameters and should be taken into account when steroidprofiles are interpreted.

Experimental

Isolation of steroids

2 ml of urine are adsorbed on Amberlite XAD-2 polystyrene resin. The XAD-2 column (pasteur pipette with a glass pearl,XAD-2 bed height: ca. 20 mm) is washed with 2 ml of twice distilled water, and conjugated and unconjugated steroids are eluted with 2 ml methanol. To the methanolic eluate 20 µl of the internal standard solution are added (Tab. 1). The methanolic eluate is evaporated to dryness, and the residue is enzymatically hydrolysed with 50 µl ß-glucuronidase from Escherichia coli K12 (Boehringer, 6800 Mannheim, Germany) in 1 ml 0.2M phosphate buffer, pH 7.0 for 1 hour at 50°C. After hydrolysis, the buffer solution is alkalised with 250 µl 5% potassium carbonate solution, and the steroids are extracted with

5 ml tert.butylmethyl ether. After centrifugation, the organic layer is transferred into a glass tube and evaporated to dryness under vacuum.

Derivatization for GC/MS

The dry residue is dissolved in 100 µl of MSTFA/NH_4I/ethanethiol,TMS (1000:2:6 v/w/v) and heated for 15 min at 60°C. 3 µl are injected directly into the injection port.

Tab. 1: Internal standard: concentrations of the working solution and resulting concentrations per ml of urine.

reference substances	working solution [µg/ml]	urine [ng/ml]
17α-methyltestosterone	50	500
[2,2,4,4-$^2H_4$]-etiocholanolone	50	500
[16,16,17-$^2H_3$)-testosterone	9	90
[16,16,17-$^2H_3$]-epitestosterone	1.5	15
[2,2,4,4-$^2H_4$]-11ß-hydroxy-androsterone	24	240

GC/MS quantitation

Quantitation of excreted steroids is performed with a GC/MS system [GC/MSD Hewlett-Packard (GC 5890/MS 5971A)], with the electron impact set at 70 eV, column: Hewlett-Packard, HP1, fused silica capillary column cross-linked methyl silicone (OV 1), 17 m, ID 0.20 mm, film thickness 0.11 µm. The carrier gas is helium (1 ml/min, split 1:10), and the temperature program is as follows: initial temperature 180°C, program rate 3°C/min to 229°C, 40°C/min up to final temperature 310°C.

Selected ion monitoring (SIM) is used with the ions presented in Table 2. Urine concentrations are calculated by the peak areas of the detected signals relative to the internal standard [2,2,4,4-$^2H_4$]-etiocholanolone m/z 438. In Figure 1 is presented an printout for a "normal" steroidprofile.

For calibration of the GC/MS instrument, a mixture of the reference substances (Tab. 3) and internal standards (Tab. 1) with the indicated concentrations per ml of urine is derivatized. To avoid wrong calibration factors, caused by a missing bological matrix in the standard we add a mixture of 1-(N,N-diisopropylamine)-alkanes (Dipa) [10]

Tab. 2: Retention times (RT), retention indices and ion traces of the endogenous steroids which are monitored for steroidprofiling in the Cologne laboratory. Also added are the internal standards (all steroids as per-trimethylsilyl derivatives).

substance	abbreviations	RT	index	m/z
5ß-androstane-3α,17α-diol		8.64	2419	241
5ß-androstane-3ß,17α-diol		8.99	2437	241
5α-androstane-3α,17α-diol		9.12	2433	241
5ß-androstandion		9.19	2437	432
androsterone	AND	10.36	2506	434
d_4-etiocholanolone	d4-ETIO	10.46	2511	438
etiocholanolone	ETIO	10.54	2515	434
5α-androstane-3α,17ß-diol	5αA3αD	10.68	2522	241
5ß-androstane-3α,17ß-diol	5ßA3αD	10.82	2529	241
dehydroepiandrosterone	DHEA	11.66	2572	432
epiandrosterone	EPIAND	11.78	2578	434
5α-androstandion		12.11	2595	432
5-androsten-3ß,17ß-diol		12.13	2596	434
5α-androstane-3ß,17ß-diol	5αA3ßD	12.15	2597	421
d_3-epitestosterone	d3-EPIT	12.15	2597	435
epitestosterone	EPIT	12.19	2599	432
dihydrotestosterone	DHT	12.41	2610	434
4-androstendion		12.85	2633	430
d_3-testosterone	d3-TEST	12.99	2640	435
testosterone	TEST	13.03	2642	432
11ß-hydroxy-androsterone	11OHAN	13.39	2660	522
11ß-hydroxy-etiocholanolone	11OHET	13.65	2673	522
methyltestosterone	MTEST	14.82	2733	446
pregnanediol*		15.37	2761	117
pregnanetriol**		15.98	2792	255

* 5ß-pregnane-3α,20α-diol

** 5ß-pregnane-3α,17α,20α-triol

Tab. 3: Calibration standard: concentrations of the working solution and resulting concentrations per ml of urine.

reference substances	working solution [µg/ml]	urine [ng/ml]
androsterone	200	2000
etiocholanolone	200	2000
testosterone	4	40
epitestosterone	4	40
11ß-hydroxy-androsterone	40	400
11ß-hydroxy-etiocholanolone	20	200
dehydroepiandrosterone	40	400
5α-androstane-3α,17ß-diol	8	80
5ß-androstane-3α,17ß-diol	18	180
pregnandiol*	100	1000
pregnantriol**	100	1000
tetrahydro-cortisol	100	1000
epiandrosterone	10	100
dihydrotestosterone	10	100
Dipa-C_{14} till Dipa-C_{23}	500 each	5000 each

* 5ß-pregnane-3α,20α-diol

** 5ß-pregnane-3α,17α,20α-triol

Results and Discussion

Application of endogenous steroids

After the application of **exogenous TEST** the most obvious changes of the steroidprofile are the increase of the TEST concentration and the ratio TEST/EPIT [3,4] and the decrease of the ratio AND/TEST (Fig. 2). A sample is sucpicious for TEST doping if the steroidprofile values of these parameters exceed the limits of population based reference ranges. Our criteria, based upon reference ranges of 5101 male and 1694 female athletes [11] are presented in Table 4.

Tab. 4: Parameters of the steroidprofile, which make a urine sample suspicious for an application of exogenous TEST.

Parameter	Men	Women
TEST/EPIT	> 6	> 6
AND/TEST	< 20	< 40
c TEST [ng/ml]*	> 130	> 60

*concentration corrected to a specific gravity of 1.020 [11,14]

The application of **exogenous DHT** leads to increases of the excretion of DHT and its 5α-metabolites 5αA3αD, 5αA3ßD, AND and EPIAND [12,13,14]. 5ß-steroids and EPIT are not influenced. Therefore steroidprofiles after the application of DHT are characterized by increased ratios of DHT/ETIO, DHT/EPI, 5αA3αD/5αA3ßD and AND/ETIO (Fig. 3). The criterias for a detection of DHT doping, based on reference ranges of 4631 male and 1341 female athletes [14] are presented in Table 5.

Tab. 5: Parameters of the steroidprofile, which make a urine sample suspicious for an application of exogenous DHT.

Parameter	Men	Women
c DHT [ng/ml]*	>21	> 18
AND/ETIO	> 2.9	< 2.1
5αA3αD/5ßA3αD	> 1.5	> 1.3
DHT/ETIO*	> 8.2	> 8.5
DHT/EPIT	> 0.73	> 2.3

*concentration corrected to a specific gravity of 1.020 g/cm^3 [11,14]

** DHT/ETIO value multiplied with 1000

If **exogenous 5α-androstandion** is administered, the same parameters as after a DHT administeration are changed, but in a different extent (Tab.6). 5α-androstandion itself is not excreted.

Tab. 6: Maximum values of excretion rates and ratios of a volunteer after administration of 20 mg 5α-androstandion and 25 mg DHT

Parameter	5α-androstandion	DHT
AND [µg/h]	2325	1075
5αA3αD [µg/h]	34.1	61.7
DHT [µg/h]	18.5	65.8
5αA3αD/5ßA3αD	2.8	6.4
AND/ETIO	22.6	8.5

The application of **exogenous AND** increases the excretion of AND and 5αA3αD. No TEST or EPIT is formed. This was shown with an excretion study with [2,2,4,4-$^2H_4$]-AND. Increased ratios AND/ETIO and 5αA3αD/5ßA3αD are the consequence of an AND application.

The use of **exogenous EPIT** increases the excretion of EPIT and of the 17α-diols, 5ß-androstane-3α,17α-diol and 5α-androstane-3α,17α-diol. These results were achieved by excretion studies with EPIT and deuterated EPIT ([2,2,4,6,6-$^2H_5$]-EPIT). Suitable parameters for the detection of an EPIT application maybe the EPIT concentration, the ratios TEST/EPIT and the ratio of the above mentioned 17α-diols to steroids which are not influenced by the EPI-metabolism like the 17ß-diols.

Application of anabolic androgenic steroids (AAS)

The application of AAS reduces the endogenous production androgenic steroids by a negative feedback mechanism leading to a decrease of their urinary excretion [5,15]. This is shown in Figure 4 for three volunteers who self-administered metandienone for 6 weeks in doses from 10mg/day to 40 mg/day, leading to substantial decrease in their urinary excretion rates.

A further effect of a longterm application of AAS is a suppression of the the 5α-reductase activity [5,16]. This effect remains also after cessation of the drug and leads to ratios of AND/ETIO which are far lower than 1 (Fig. 5).

Application of ethanol

The application of ethanol in high amounts may increase the ratio TEST/EPIT and decrease the ratio AND/TEST by increase of the excretion of TEST glucuronide and decrease of the excretion of AND glucuronide. These changes are connected with the presence of ethanol in urine (Fig. 6). These results have always taken into account if high TEST/EPIT ratios are found in dope control samples, especially in "out of competition" controls. To avoid false positive results, ethanol should be measured in urine, e.g. by Headspace/GC.

Application of probenecid and diuretics

The application of probenecid reduces the excretion of conjugated steroids [5,17]. This is shown in Figure 7 for AND and ETIO. Steroid ratios are not changed. This effect is due to a competitive inhibition of active transport mechanisms of organic acids in the proximal tubuli of the kidneys.

In contrary to probenecid, diuretics don't reduce the steroid excretion. These substances increase the urine flow and therefore also lead to lower steroid concentrations.

Application of trimethoprim- and sulfamethoxazol

Trimethoprim- and sulfamethoxazol-metabolites are eluted in a region, where many endogenous steroids are detected. They may disturb the chromatography of the endogenous steroids and lead to wrong evaluations of the steroidprofile. This is shown in Figure 8 for TEST and EPI. To separate the coeluting substances, we recommend a n-pentane extraction [18]

Bacterial activities in urine samples

The first indications for bacterial activities in urine sample are elevated pH values. The most often observed changes of the steroidprofile in such urines are the coming up of 5α-androstandion and 5ß-androstandion (Fig.9). The formation of these steroids is based on a bacterial deconjugation of AND- and ETIO-glucuronide and a following bacterial 3-hydroxy-steroid-dehydrogenase activity. Because of the bacterial deconjugation, high amounts of steroids, normally excreted as conjugates (as AND and ETIO) can be found in the free form.

Another effect of bacterial activities to the steroidprofile, only rarely observed, is the increase of the TEST concentration leading to elevated TEST/EPIT ratios (Fig. 10). We think, that the formation of TEST results from a bacterial hydrolysis of 5-androsten-3ß,17ß-diol sulphate and a following 3ß-hydroxy-Δ^5 steroid-dehydrogenase and steroid-Δ-isomerase

activity (Fig. 12). The bacterial formed testosterone is not conjugated and can be well separated from the TEST glucuronide by an ether extraction before the hydrolysis.

Incomplete hydrolysis

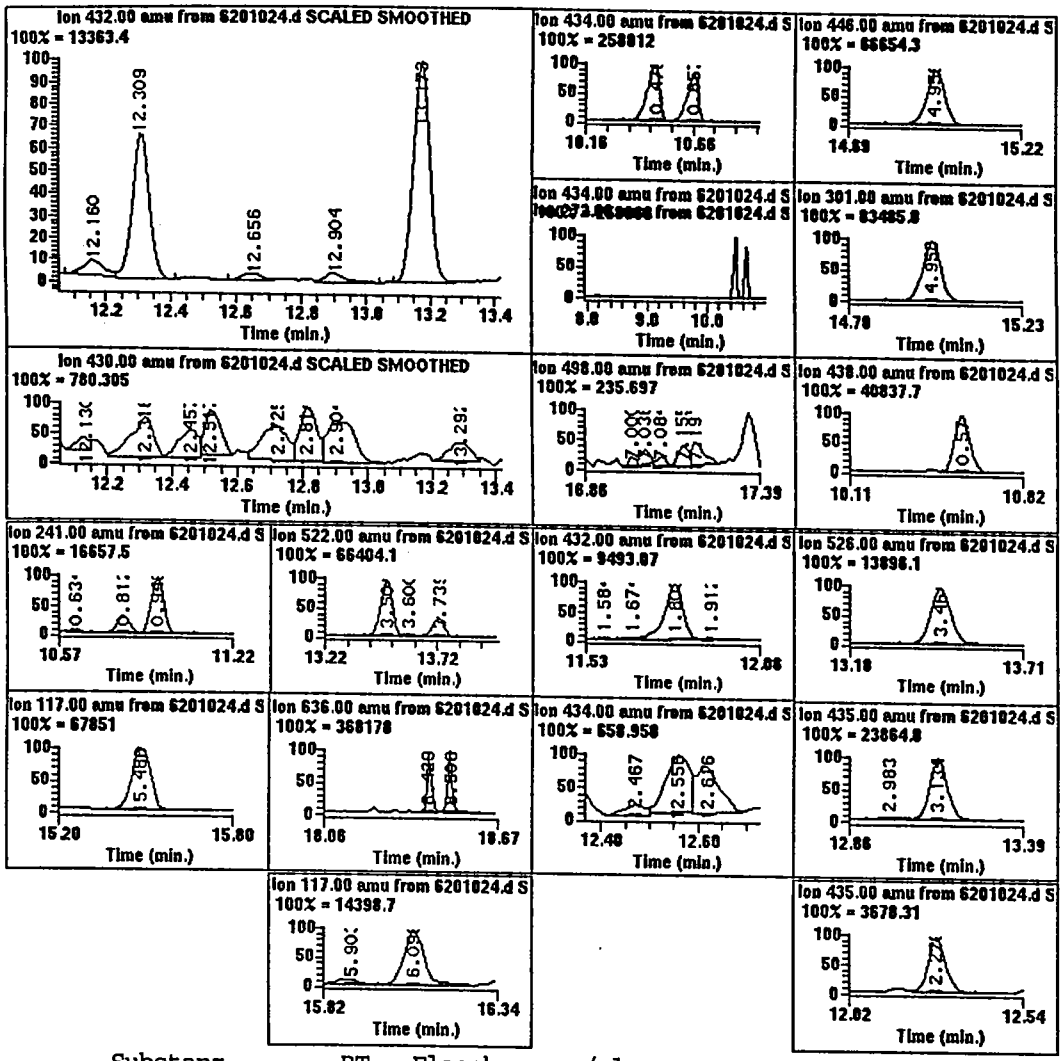
The time required for complete hydrolysis with ß-glucuronidase from E. coli varies between the different steroid conjugates [5]. TEST and EPI glucuronide are hydrolysed completely within some minutes. AND and ETIO glucuronide are hydrolysed slower, probably depending on the higher concentrations in urine. The hydrolysis of ETIO glucuronide is completed faster than the hydrolysis of AND glucuronide (Fig. 11). After incomplete hydrolysis, these different kinetics lead to characteristic patterns of the steroidprofile. The concentrations of AND and ETIO are low, whereas the concentrations of TEST and EPIT are normal. The corresponding ratios e.g. AND/TEST and AND/EPIT are extremly low. Additionally the ratio AND/ETIO maybe far below 1. Such patterns can be observed after the **application of amineptine**, which leads to an inhibition of the hydrolysis. In the case of amineptine the hydrolysis can be completed by longer incubation time or higher amounts of the enzyme preparation or the use of ß-glucuronidase/arylsulfatase from Helix Pomatia.

Only about 30 %-50 % of 11OHAN and 11OHET are hydrolysed with ß-glucuronidase from E.coli at 50° C. This is not changed by a prolonged incubation time at this temperature. The hydrolysis at 37°C for 15 hours or the use of ß-glucuronidase/arylsulfatase from Helix Pomatia leads to much higher values [5].

Side activities connected with the enzymatic hydrolysis

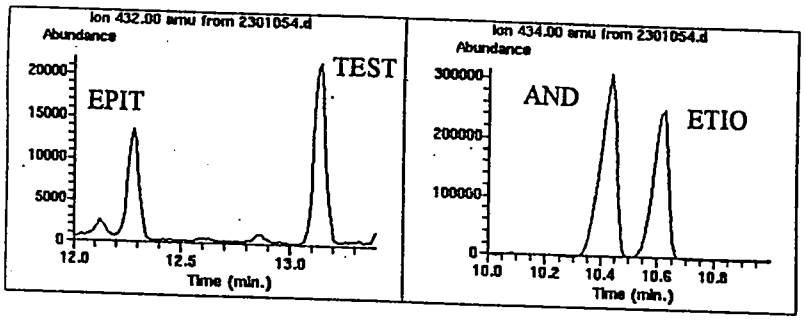
The use of ß-glucuronidase/arylsulfatase from Helix Pomatia for the enzymatic hydrolysis may lead to an increased ratios of TEST/EPIT and formation of 4-androstendion [5,19]. Reasons for these changes are side activities (3ß-hydroxy-Δ^5 steroid-dehydrogenase and steroid-Δ-isomerase) in the Helix Pomatia preparation which convert 5-androsten.3ß,17ß-diol to TEST and DHEA to 4-androstendion (Fig.12).

Similar effects can be observed at the hydrolysis with ß-glucuronidase from E.coli. The side activities may stem from bacterias in the phosphate buffer. We could show, that 5-androsten-3ß,17ß-diol, after incubation with an old phospatebuffer was converted to TEST [5].
To avoid suspicious results for TEST/EPIT ratios we recommend to heat the buffer preparation before the use.



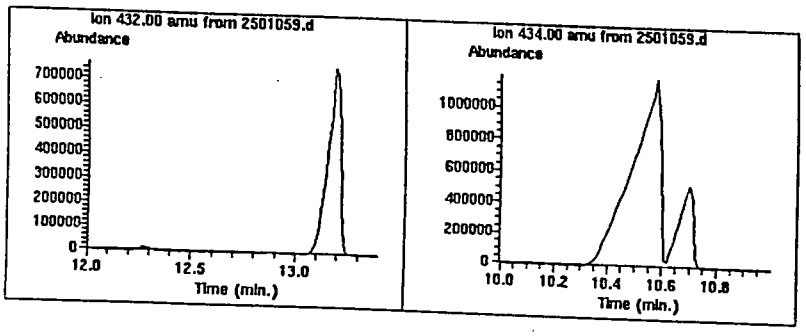
Substanz	RT	Flaeche	ng/ml				
Androsteron	10.48	1043988	2735.1	Methyltest.(446)	14.96	260168	496.5
Etiocholanolon	10.66	762579	1990.1	D4-OH-Androst.	13.46	63027	261.1
Epitestosteron	12.31	32283	34.5	D3-Epitesto.	12.28	12437	14.7
Testosteron	13.17	47353	46.3	D3-Testosteron	13.13	88142	92.5
				D4-Etioch.	10.57	174477	500.0
OH-Androsteron	13.50	239654	1024.3				
OH-Etiochol.	13.74	82277	386.9	Test/Epitest	1.467		
Androstandiol1	10.81	16967	68.5	d3T/d3Epit	7.087		
Androstandiol2	10.94	55035	212.9	Andr./Etio.	1.369		
DHEA	11.80	36741	86.0	Andr./Test.(c)	59.043		
Pregnandiol	15.49	304370	232.9				
TH-Cortisol	18.43	398575	1427.5	D4-Etioch+S	17.08	77	0.04
allo-THC	18.51	391790	1403.2	ISTD 301/446	1.246		
Dihydrotest.	12.47	369	1.9				
Pregnantriol-3	-99.99	-9999	-21.1	Prtriol-3(117)	16.10	61815	1034.6
Pregnantriol-2	-99.99	-9999		Prtriol-2(143)	17.36	2257	

Fig. 1: Screening printout of a "normal steroidprofile" with the ion chromatograms of the endogenous steroids and internal standards.



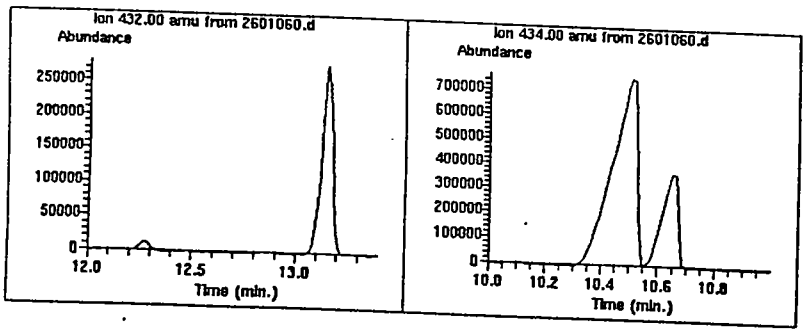
0 h

TEST/EPIT	=	1.5
AND/TEST	=	42.3
AND/ETIO	=	1.3
TEST [ng/ml]	=	81



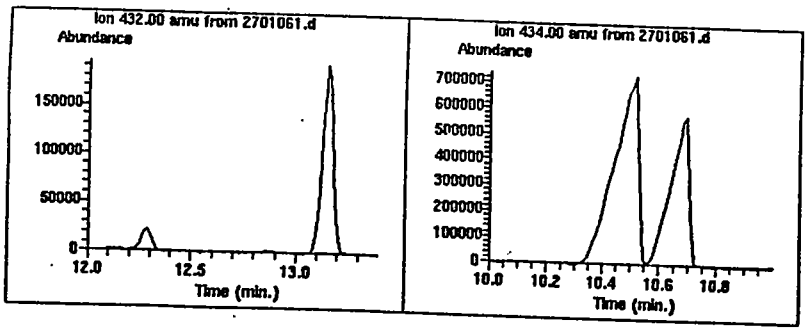
4 h

TEST/EPIT	=	65.2
AND/TEST	=	7.1
AND/ETIO	=	4.6
TEST [ng/ml]	=	2916



6 h

TEST/EPIT	=	18.8
AND/TEST	=	11.8
AND/ETIO	=	3.3
TEST [ng/ml]	=	964



8 h

TEST/EPIT	=	8.0
AND/TEST	=	16.2
AND/ETIO	=	1.7
TEST [ng/ml]	=	664

Fig. 2: Alterations of the steroidprofile after oral application of 40 mg testosterone undecanoate (Andriol ^{R})

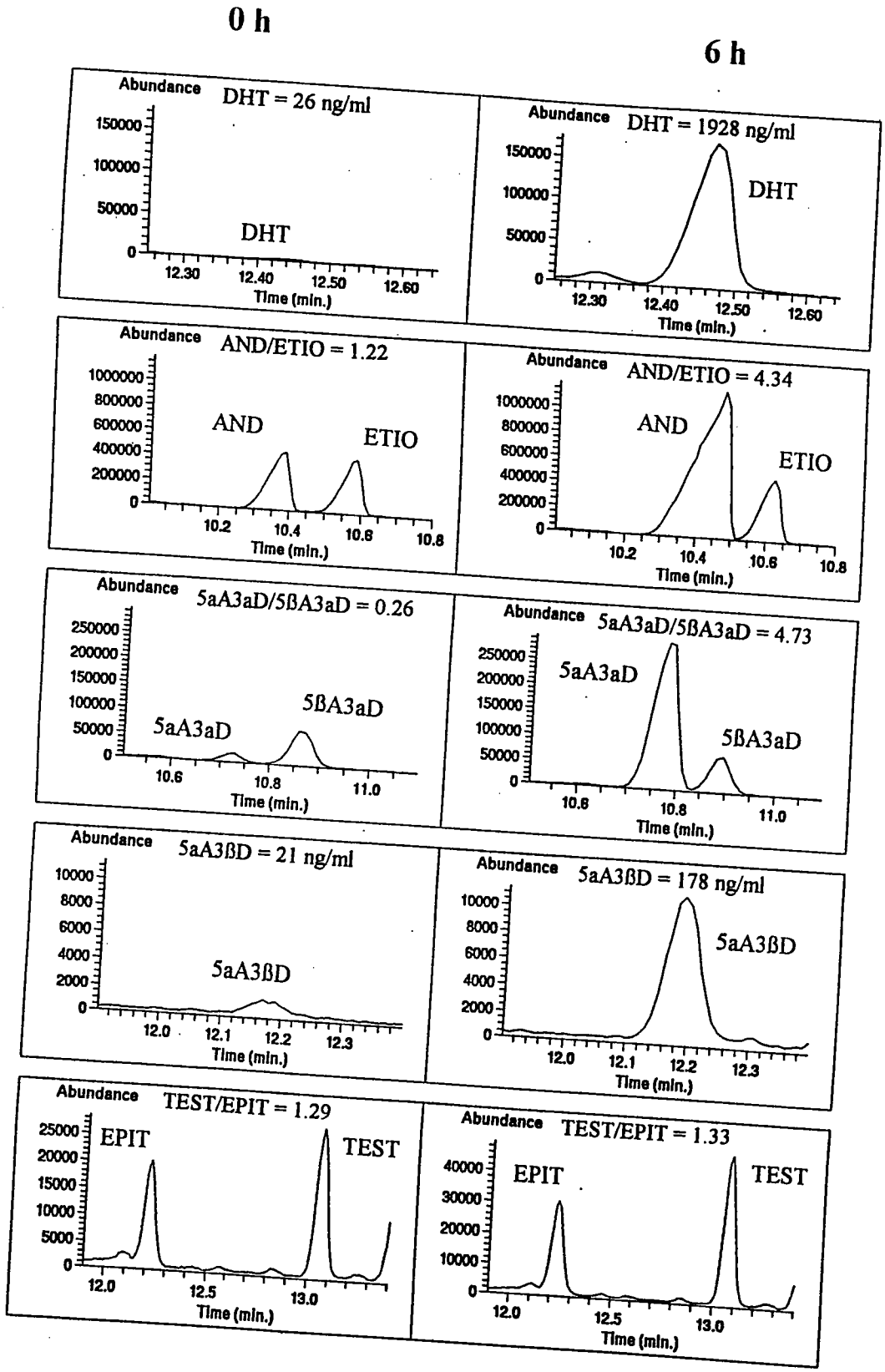


Fig. 3: Alterations of the steroidprofile before and 6 hours after the sublingual application of 25 mg dihydrotestosterone.

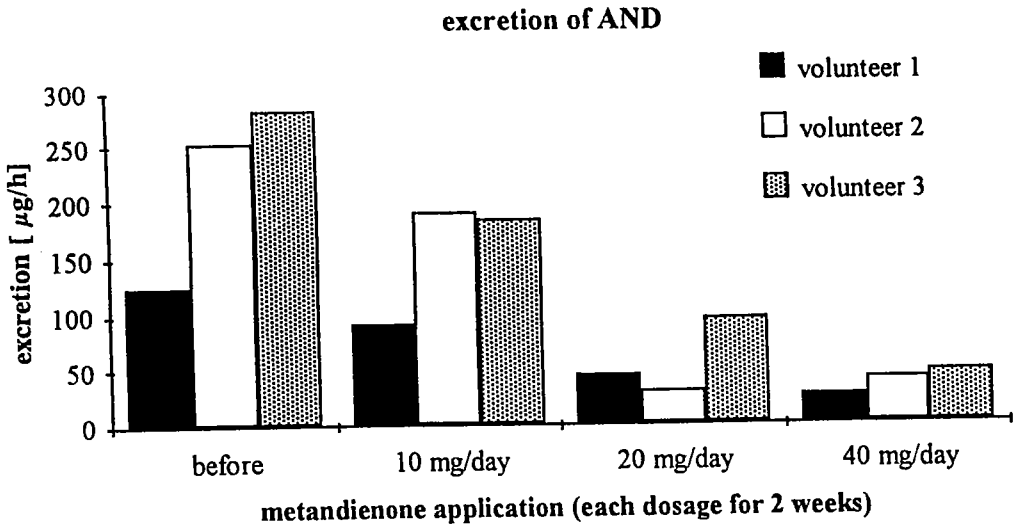


Fig. 4: Decrease of the excretion of androsterone glucuronide after the application of metandienone for 6 weeks with increasing doses.

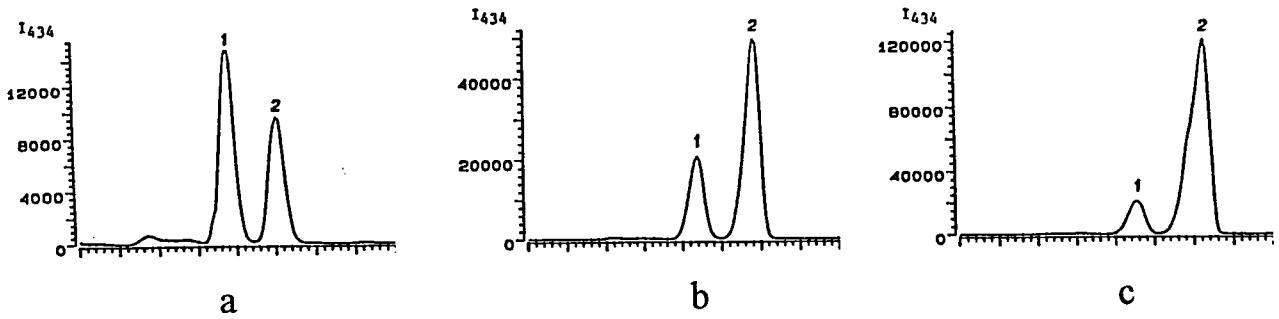
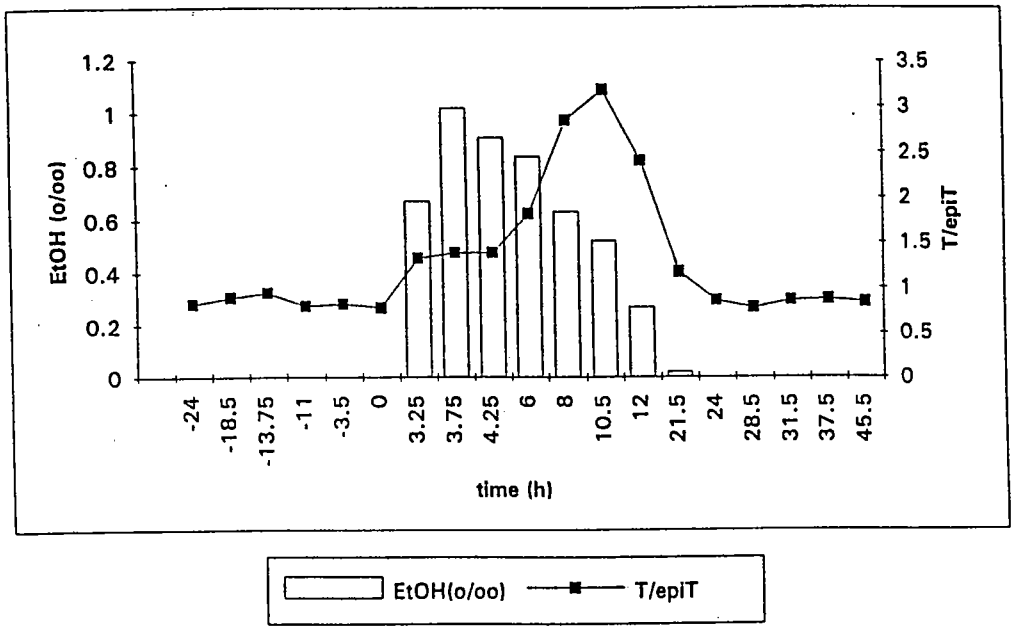


Fig. 5: Change of the ratio androsterone (1)/etiocholanolone (2) after the administration of stanozolol (20 mg/day orally for 8 weeks) was stopped.
a) with stanozolol b) 6 days after c) 15 days after

a)



b)

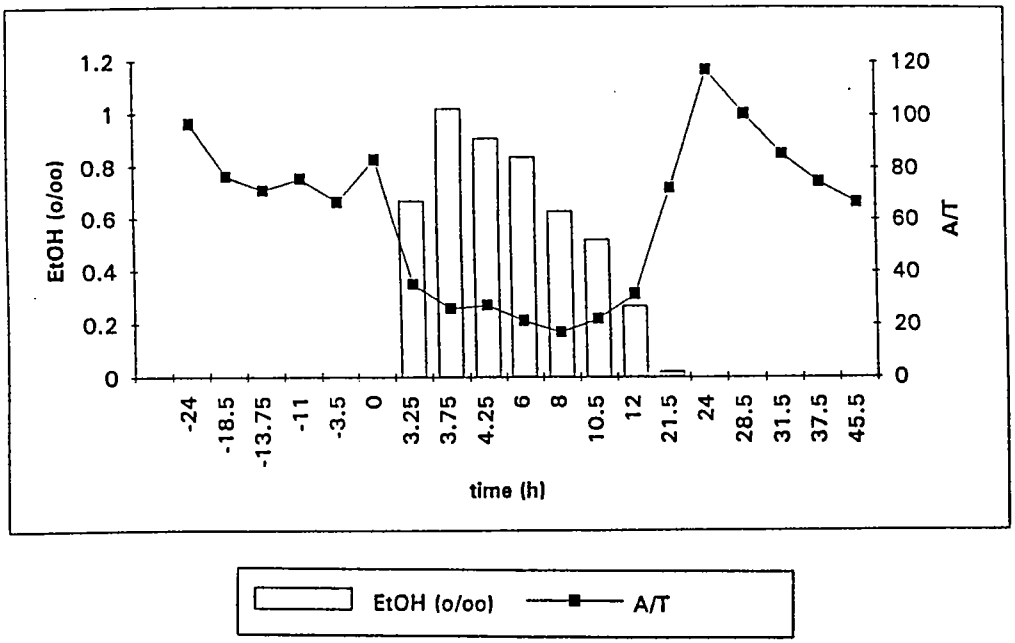


Fig. 6: Changes of the ratios testosterone/epitestosterone (a) and androsterone/ testosterone (b) of a male volunteer after application of 2 g of ethanol per kg bodyweight within 4 hours. Also presented are the found urinary ethanol concentrations (in bars).

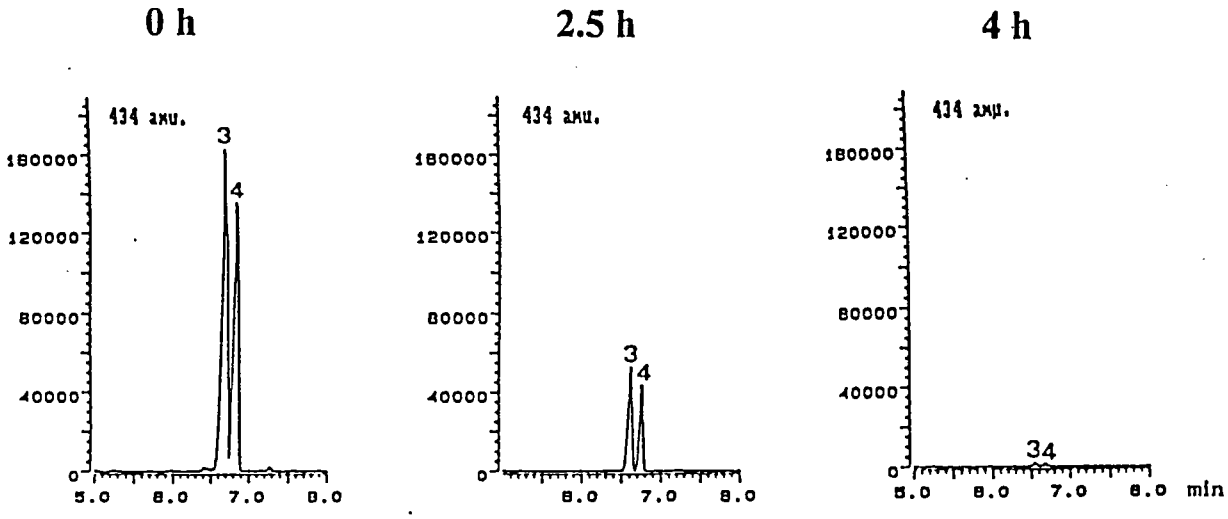


Fig. 7: Decrease of the concentrations of androsterone (3) and etiocholanolone (4) after the application of 3 g of probenecid.

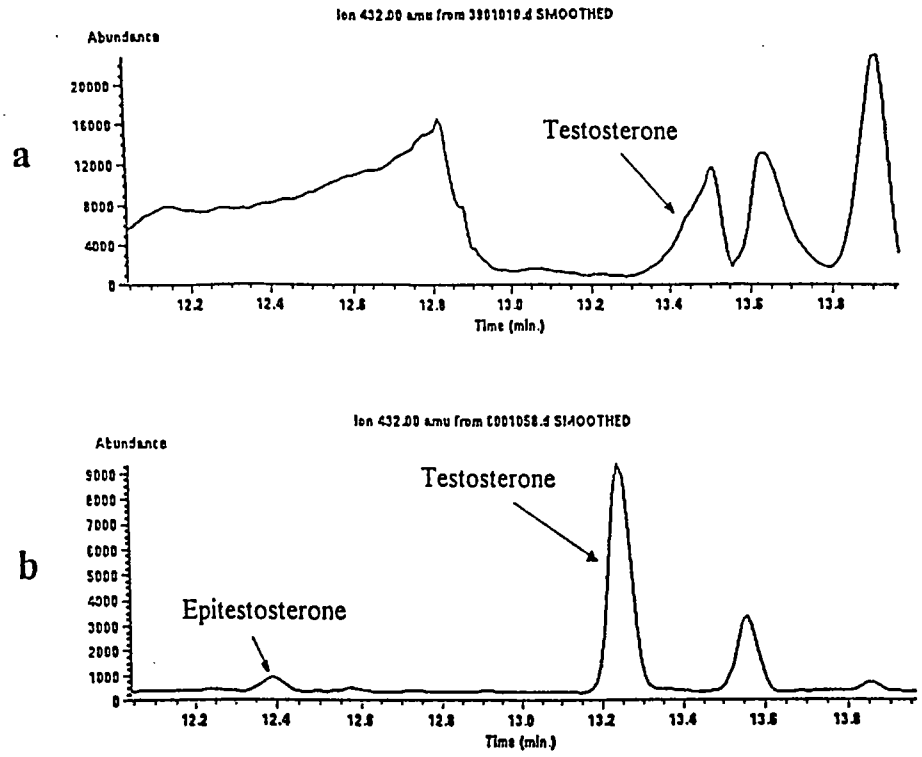
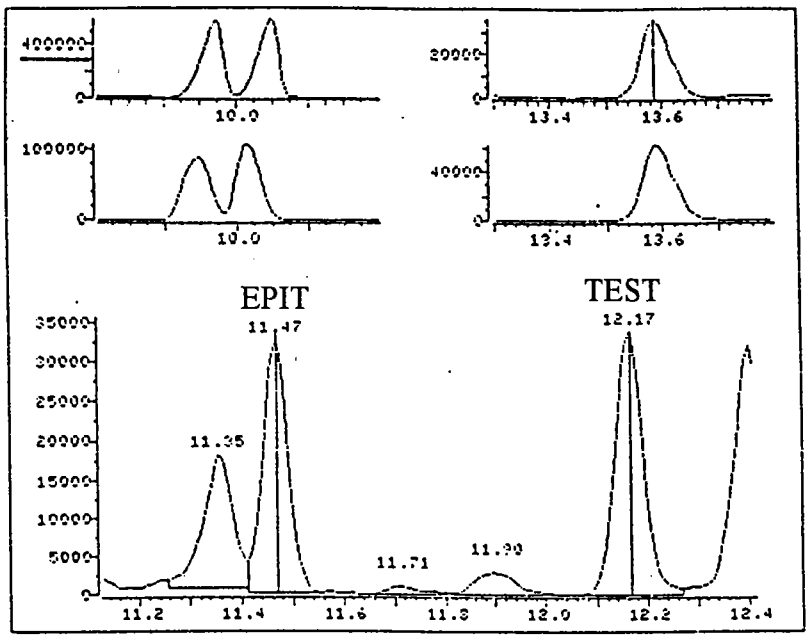
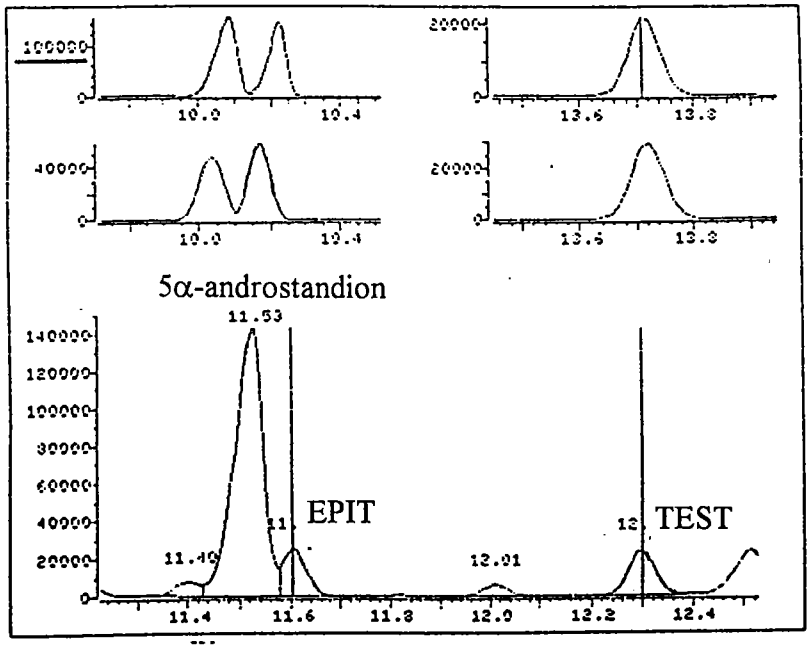


Fig. 8: Single ion chromatogram of ion m/z 432 in the region of testosterone and epitestosterone for an urine containing trimethoprim- and sulfamethoxazol metabolites. a) after ether extraction b) after n-pentane extraction.



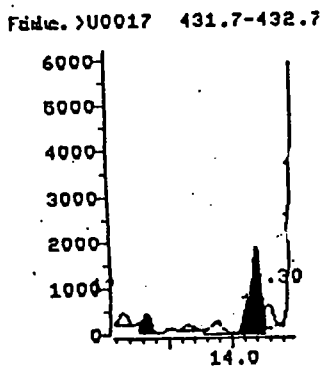
pH 6



48 h 30°C

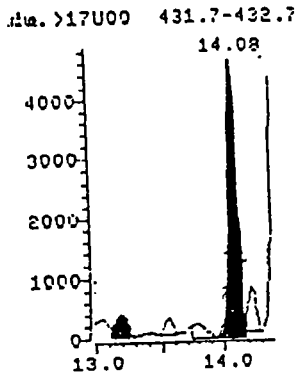
pH 7.5

Fig. 9: Changes of the steroidprofile by bacterial activities. 10 ml of a quality control urine were spiked with 0.5 ml bacteria urine. After 48 hours at 30° C high amounts of 5α-androstandion were found.



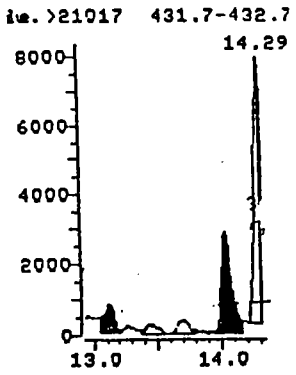
pH 5

combined fraction
Q T/E = 5.3

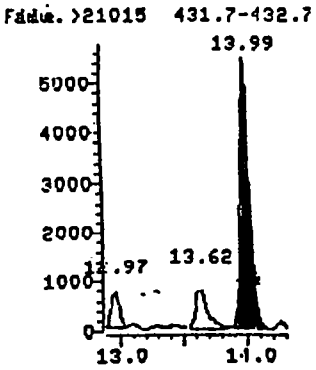


storage 6 months + 4°C
pH 8.9

combined fraction
Q T/E = 9.8

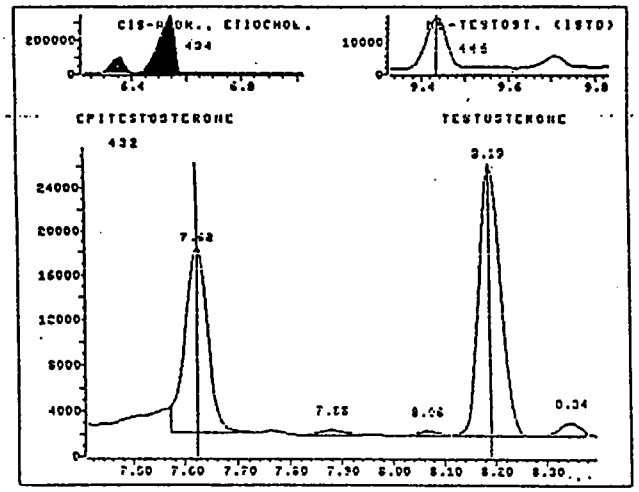


conjugated fraction
Q T/E = 4.9



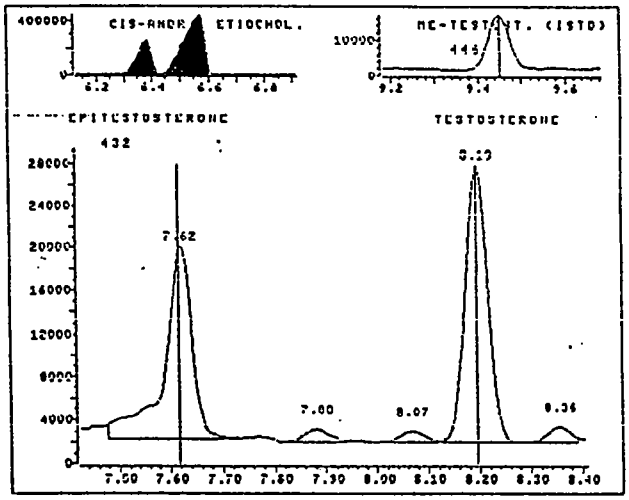
free fraction
with testosterone

Fig. 10: Formation of testosterone and increase of the ratio testosterone/epitestosterone (Q T/E) by bacterial activities in a female urine. High amounts of testosterone were found in the free fraction.



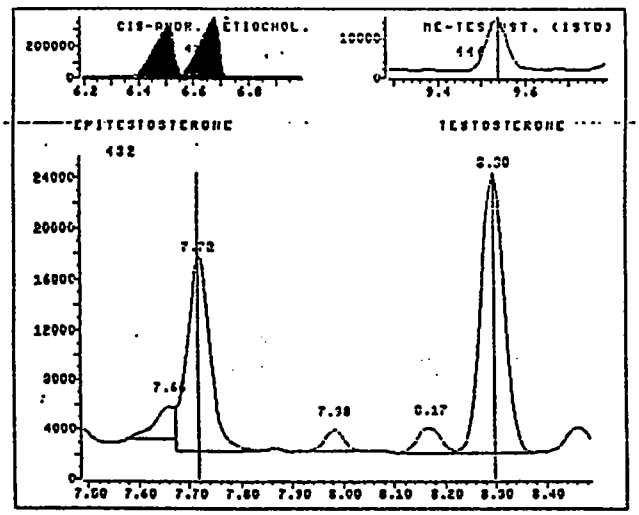
1 min 50°C

Q And/Etio = 0.21



3 min 50°C

Q And/Etio = 0.53



20 min 50°C

Q And/Etio = 1.00

Fig. 11: The dependence of the ratio androsterone/etiocholanolone (Q And/Etio) from the duration of the hydrolysis with ß-glucuronidase from E. coli at 50° C.

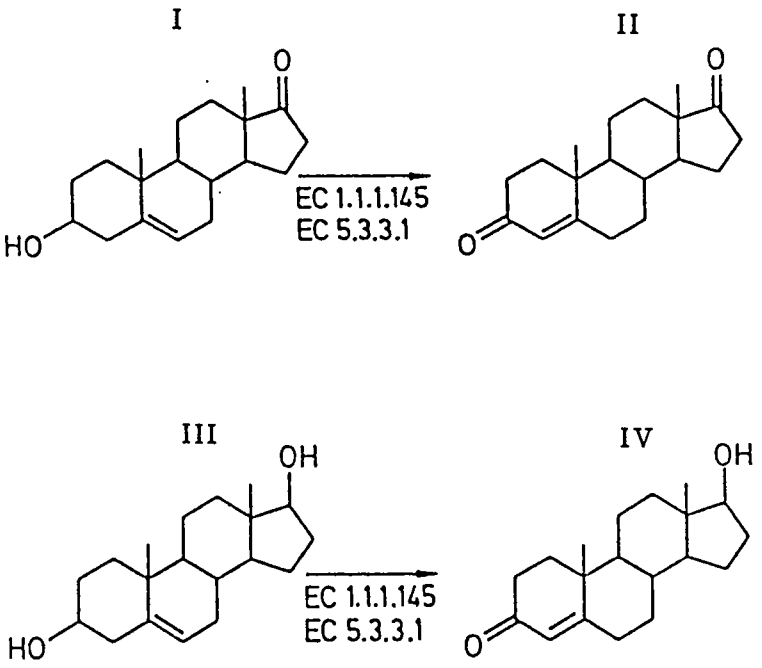


Fig. 12: Conversion of dehydroepiandrosterone (I) to 4-androstendion (II) and 5-androsten-3ß,17ß-diol (III) to testosterone (IV) by activities of a 3ß-hydroxy-Δ^5-steroid-dehydrogenase and steroid-Δ-isomerase.

References

1. Shackleton, C.H.L.: Profiling steroid hormones and urinary steroids - review. J. Chromat. 379 (1986) 91-156.
2. Vestergaard, P.; Sayegh, J.F.; Mowat, J.H.; Hemmingsen, L.: Estimation after multi-column liquid chromatography of common urinary neutral steroids with an application to the assay of plasma 17-oxosteroids. Acta Endocrin. 88 (1978)
3. Donike, M.; Bärwald, K.-R.; K.-R.; Klostermann, K.; Schänzer, W; Zimmermann, J.: Nachweis von exogenem Testosteron. in: Sport: Leistung und Gesundheit. Edt.: H.Heck, W.Hollmann, H.Liesen, R.Rost, Deutscher Ärzte Verlag Köln (1983) 293.
4. Zimmermann, J.: Untersuchungen zum Nachweis von exogenen Gaben von Testosteron Hartung-Gorre Verlag Konstanz, Dissertation Deutsche Sporthochschule Köln, 1986
5. Geyer, H.: Die gas-chromatographisch/massenspektrometrische Bestimmung von Steroidprofilen im Urin von Athleten. Dissertation, Deutsche Sporthochschule Köln, 1990
6. Nitschke, R.: Steroidprofile und Veränderungen biochemischer Parameter bei Hochleistungsradrennfahrern während zwei Rundfahrten. Dissertation, Deutsche Sporthochschule Köln, 1996.
7. Mareck-Engelke U., Geyer H. and Donike M.: Stability of steroid profiles (3) :The Circadian Rhythm of Urinary Ratios and Excretion Rates of Endogenous Steroids in Male. in: M.Donike, H.Geyer, A.Gotzmann, U.Mareck-Engelke (Eds.) Recent advances in doping analysis (2) Sport und Buch Strauß, Köln (1995).

8. Mareck-Engelke U., Geyer H. and Donike M.: Stability of steroid profiles (4) :The Circadian Rhythm of Urinary Ratios and Excretion Rates of Endogenous Steroids in Female and its Menstrual Dependency. in: M.Donike, H.Geyer, A.Gotzmann, U.Mareck-Engelke (Eds.) Recent advances in doping analysis (2) Sport und Buch Strauß, Köln (1995).
9. Mareck-Engelke U., Flenker U. and Donike M.: Stability of steroid profiles (5) : The Annual Rhythm of Urinary Ratios and Excretion Rates of Endogenous Steroids in Female and its Menstrual Dependency. in: M.Donike, H.Geyer, A.Gotzmann, U.Mareck-Engelke (Eds.) Recent advances in doping analysis (3) Sport und Buch Strauß, Köln (1996)
10. Geyer, H., Mareck-Engelke, U., Nolteernsting, E., Opfermann, G., Donike, M.: The matrix problem in calibration of the GC/MS for endogenous steroids. in: Donike, M., Geyer, H., Gotzmann, A., Mareck-Engelke, U. (eds.) Recent advances in doping analysis (2). Sport und Buch Strauß, Köln 1995.
11. Rauth, S.: Referenzbereiche von urinären Steroidkonzentrationen und Steroidquotienten - Ein Beitrag zur Interpretation des Steroidprofils in der Routinedopinganalytik. Dissertation, Deutsche Sporthochschule Köln 1994.
12. Southan, G.J.; Brooks, R.V.; Cowan, D.A.; Kicman, A.T.; Unnadkat, N.; Walker, C.J.: Possible indices for the detection of the administration of dihydro-testosterone to athletes. J. Steroid Biochem. Molec. Biol. 42 (1992) 87-94.
13. Geyer, H.., Schänzer, W., Schindler, U.: Changes of the urinary steroidprofile after sublingual application of dihydrotestosterone. in: Donike, M., Geyer, H., Gotzmann, A., Mareck-Engelke, U. (eds.) Recent advances in doping analysis (3). Sport und Buch Strauß, Köln 1996.
14. Donike, M., Ueki, M., Kuroda, Y., Geyer, H., Nolteernsing, E., Rauth, S., Schänzer, W., Schindler, U., Völker, E., Fujisaki, M.: Detection of dihydrotestosterone (DHT) doping - Alterations in the steroid profile and reference ranges for DHT and its 5α-metabolites. J. Sports.Med.Phys.Fitness, in press.
15. Donike, M.; Geyer, G.; Schänzer, W.; Zimmermann, J.: Die Suppression der endogenen Androgenproduktion durch Metandienon. in: I.-W.Franz, H.Mellerowicz, W.Noack (Eds.). Training und Sport zur Prävention und Rehabilitation in der technisierten Umwelt Springer Verlag Berlin Heidelberg 1985
16. Donike, M.; Geyer, G.; Kraft, M.; Rauth, S.: Longterm Influence of Anabolic Steroid Misuse on the Steroid Profile. in: P.Belotti, G.Benzi, A.Ljungqvist (eds.). Official proceedings. IInd International Athletic Foundation World Symposium on Doping in Sport, Monte Carlo 1989.
17. Geyer, H., Schänzer, W., Donike, M.:Probenecid as masking agent in dope control-Inhibition of the urinary excretion of steroid glucuronides.in: Donike, M., Geyer, H., Gotzmann, A., Mareck-Engelke, U., Rauth, S. (eds.) 10th Cologne workshop on dope analysis 7th to 12th june 1992. Sport und Buch Strauß, Köln 1993.
18. Geyer, H., Mareck-Engelke, U., Schänzer, W., Donike, M.: Simple purification of urine samples for improved detection of anabolic and endogenous steroids. in: Donike, M., Geyer, H., Gotzmann, A., Mareck-Engelke, U. (eds.) Recent advances in doping analysis. Sport und Buch Strauß, Köln 1994.
19. Kuoppasalmi, K., Leinonen, A., Kajalainen, U.: Detection of exogenous testosterone in doping analysis:methodological aspects. in: Sports medicine and exercise science. Proceedings of the Olympic scientific congress. Eugene, Oregon, 1984.