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RECENT ADVANCES IN DOPING ANALYSIS (2)

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Stability of Steroid Profiles (3): The Circadian Rhythm of Urinary Ratios and Excretion Rates of Endogenous Steroids in Male

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The stability of ratios and excretion rates of endogenous steroids in male urines was investigated. Five male (age: x=32±6 years) volunteers participated. Urine samples were collected every two hours (including during the night; altogether 12 samples) for 24 hours. The urines were prepared according to the screening procedure of conjugated anabolic steroids and analysed by GC/MS (1).
The following steroid glucuronides were measured and quantified: androsterone (A), etiocholanolone (E), testosterone (T), epitestosterone (epiT), 11ß-OH-androsterone (OHA), 11ß-OH-etiocholanolone (OHE), 5α-androstan-3α,17ß-diol (Adiol) , 5ß-androstan-3α,17ß-diol (Bdiol), pregnandiol (Pregnd) and tetrahydrocortisol (THF).
The results of selected steroid concentration ratios, their excretion rates and statistical evaluations are shown in Tables 1-4 and Figures 1-12.

References

(1) Donike M., Geyer H., Gotzmann A., Kraft M., Mandel F., Nolteernsting E., Opfermann G., Sigmung G., Schänzer W. and Zimmermann J.: Dope Analysis
In: Official Proceedings of the International Athletic Foundation World Symposium on Doping in Sport. P.Bellotti, G.Benzi, A.Ljungquist (Hrsg.) IAAF Florence (1988) 53-87.

(2) Mareck-Engelke U., Geyer H. and Donike M.: Stability of steroid profiles
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(3) Mareck-Engelke U., Geyer H. and Donike M.: Stability of steroid profiles (2) : Excretion rates from morning urines In: Recent Advances In Doping Analysis Proceedings of the11th Cologne Workshop On Dope Analysis, 7th to 12th March 1993 ,85-89

Conclusions

The ratio A/E is the most stable parameter (except for one volunteer). The variation is less than 10% (tab1,2 ; fig 1,2).
The ratios T/epiT, A/T, A/epiT and Adiol/Bdiol show coefficients of variation less than 30% for all volunteers.

The excretion rates of the endogenous steroids show strong intraindividual and interindividual variation (tab 3,4).
Maximum excretion rates were observed at daytime hours, minima at nighttime hours.
Maxima were stronger marked than minima.
Calculated were differences between excretion rates of all quantified steroids (max. and min.) and their corresponding mean values.
The calculated values for all steroids from all volunteers are presented in tab 5 and 6.
They are situated in a range characterised as: " mean ± 2.8 * st.dev."
These data are based on 12 data points per person and steroid.
The boarderlines of the subject-based reference ranges for persons under resting conditions within 24 hours (for n=12) may be defined as: L_O = mean $\pm$ 3.0 * st.dev.
The calculation factor t depends on the number of analysed and calculated samples from one individual. Decrease of sample numbers will lead to an increase of the calculation factor.

The ratio OHA/OHE decreases significantly during the night (fig.3).
The excretion of OHA and THF decreases significantly during the night (fig 4,5).
This circadianic character depends on the rhythm of ACTH. For all other steroids no common circadian rhythm is recognizable.

Between hormones which represent testicular activity (T, epiT) and those which represent adrenal activity (A,E) it was not possible to detect any difference referring to circadianic rhythm (fig 6-8).

For steroids with small variation in excretion during 24 hours (A,E,T,epiT) a small urine collecting interval (only a few hours or a morning urine) is representative for a 24 hour collected urine.
This is not possible for steroids with a large variation in excretion during 24 hours (steroids following the ACTH-rhythm: THF, OHA) (fig 9,10).

There is no correlation between excretion of endogenous steroids and flow of urine (fig 11,12).

Table 1: Stability of steroid profiles (male urines).

Coefficient of variation (%) of some selected steroid concentration ratios

V1-V5 = volunteer 1-5

	V1	V2	V3	V4	V5
A/E	8	8	8	9	26
T/epiT	21	18	25	9	12
A/T	30	18	26	14	15
A/epiT	16	22	15	12	13
Adiol/Bdiol	21	14	7	6	20
A/Adiol	24	7	34	11	32
A/Bdiol	17	16	30	10	35
E/Adiol	23	11	32	14	18
E/Bdiol	15	14	28	11	13
OHA/OHE	48	61	56	51	67
A/THF	25	49	66	50	48

Table 2: Stability of steroid profiles (male urines).

Statistics of some selected steroid concentration ratios

min = minimum value max = maximum value

st.de. = standard deviation c.v. = coefficient of variation (%)

V1-V5 = volunteer 1-5

	V1	V2	V3	V4	V5
A/E					
min	1.2	0.7	1.0	1.6	0.9
max	1.6	0.9	1.3	2.3	2.5
mean	1.4	0.8	1.1	2.0	1.5
st.dev.	0.11	0.06	0.09	0.17	0.38
c.v. (%)	8	8	8	8	26

	V1	V2	V3	V4	V5
T/epiT					
min	1.2	0.11	0.7	4.8	0.8
max	2.5	0.23	1.3	6.6	1.3
mean	1.8	0.15	0.9	5.4	1.0
st.dev.	0.36	0.03	0.23	0.49	0.11
c.v. (%)	21	18	25	9	12

Table 3: Stability of steroid profiles (male urines).

Coefficient of variation (%) of excretion rates

V1-V5 = volunteer 1-5

	V1	V2	V3	V4	V5
A	26	19	34	26	26
E	30	17	37	29	22
epiT	23	29	42	25	18
T	24	28	37	25	21
Adiol	23	22	37	25	26
Bdiol	31	17	34	26	25
OHA	48	46	46	52	50
OHE	40	30	48	56	56
THF	45	47	59	58	56

Table 4: Stability of steroid profiles (male urines).

Statistics of some selected excretion rates

min = minimum value max = maximum value

st.de. = standard deviation c.v. = coefficient of variation (%)

V1-V5 = volunteer 1-5

		V1	V2	V3	V4	V5
A (µg/h)	min	64	49	26	117	120
	max	136	97	153	258	288
	mean	85	71	99	169	189
	st.dev.	22	19	34	26	49
	c.v. (%)	26	19	34	26	26
E(µg/h)	min	45	68	23	55	82
	max	110	119	153	145	175
	mean	62	90	89	85	140
	st.dev.	18	15	33	25	31
	c.v. (%)	30	17	37	29	22
epiT (µg/h)	min	1.0	0.9	0.39	0.45	1.29
	max	2.2	2.2	3.0	1.1	2.38
	mean	1.6	1.6	1.6	0.76	1.71
	st.dev.	0.36	0.45	0.68	0.19	0.31
	c.v. (%)	23	29	42	25	18
T (µg/h)	min	1.4	0.14	0.52	2.4	1.08
	max	3.7	0.38	2.4	6.2	2.33
	mean	2.8	0.24	1.4	4.1	1.69
	st.dev.	0.65	0.07	0.51	1.01	0.35
	c.v. (%)	24	28	37	25	21

Table 5: Calculation factor (t) for the upper boarderline (u_o) of the subject-based reference range following the formula: u_o = mean + t * st.dev.
V1-V5 = volunteer 1-5

	V1	V2	V3	V4	V5
A	2.4	1.9	1.7	2.0	2.0
E	2.6	2.0	1.9	2.4	1.1
epiT	1.8	1.5	2.0	2.0	2.2
T	1.5	2.1	1.8	2.1	1.8
Adiol	2.1	1.9	1.8	1.6	1.6
Bdiol	2.7	1.8	1.5	1.9	1.3
11OHA	2.2	1.5	1.7	2.3	1.8
11OHE	2.3	1.3	1.9	2.6	2.0
Pregnd	2.6	1.3	1.6	1.8	1.2
THF	1.7	1.7	1.6	2.0	1.5
mean	2.2	1.7	1.7	2.1	1.7

Table 6: Calculation factor (t) for the lower boarderline (l_o) of the subject-based reference range following the formula: l_o = mean + t * st.dev.
V1-V5 = volunteer 1-5

	V1	V2	V3	V4	V5
A	1.0	1.6	2.1	1.2	1.4
E	0.9	1.4	1.9	1.2	1.9
epiT	1.5	1.5	1.7	1.6	1.4
T	2.1	1.5	1.7	1.7	1.7
Adiol	1.2	1.2	1.3	1.7	2.4
Bdiol	1.0	1.7	1.5	1.7	2.0
11OHA	1.3	1.5	1.8	1.1	1.4
11OHE	1.4	1.3	1.3	1.1	1.0
Pregnd	1.3	1.7	2.0	1.3	2.0
THF	1.1	1.5	1.5	1.2	1.3
mean	1.3	1.5	1.7	1.4	1.7

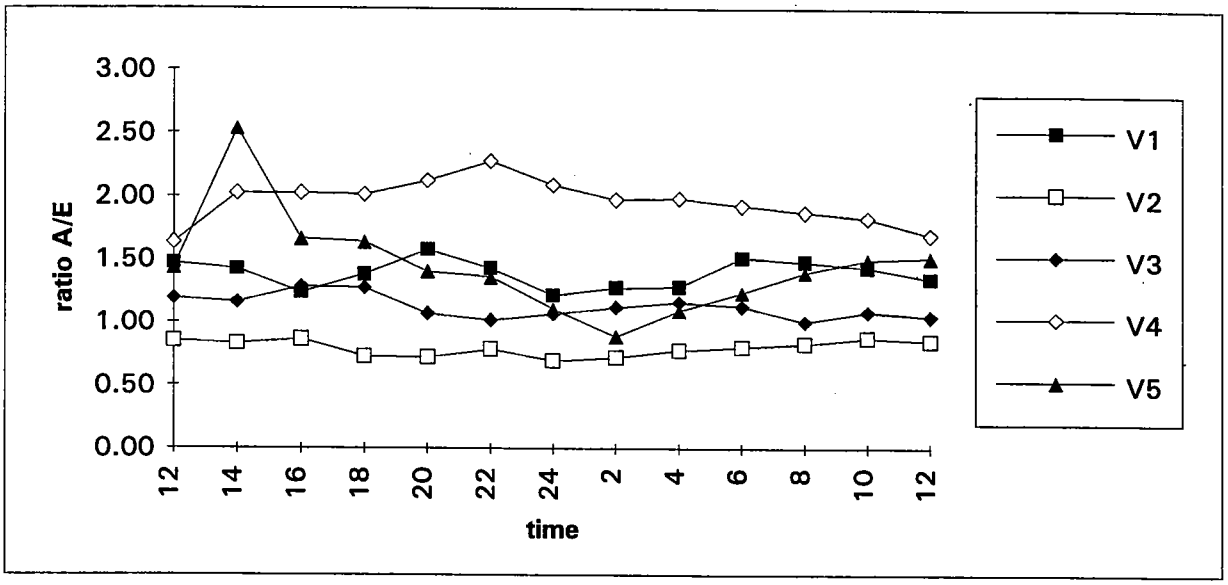

Fig 1: ratio (concentration) A/E in male urine, collected over 24 hours
V1-V5 = Volunteer 1-5

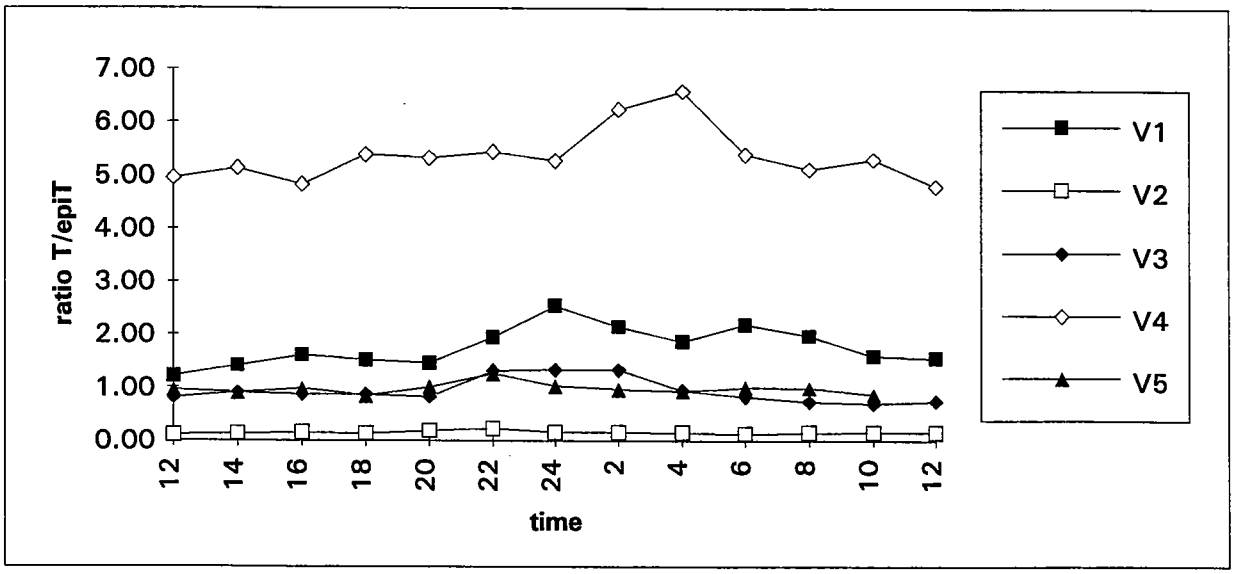

Fig 2: ratio (area) T/epiT in male urine, collected over 24 hours
V1-V5 = Volunteer 1-5

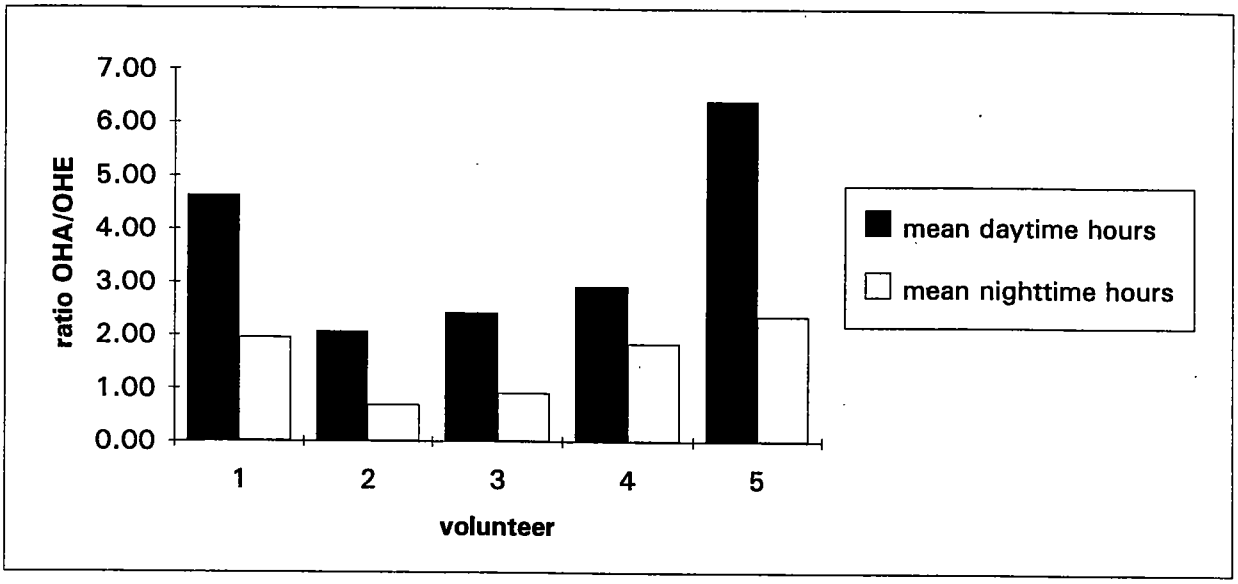

Fig 3: ratio (concentration) OHA/OHE in male urine, collected over 24 hours
V1-V5 = volunteer 1-5

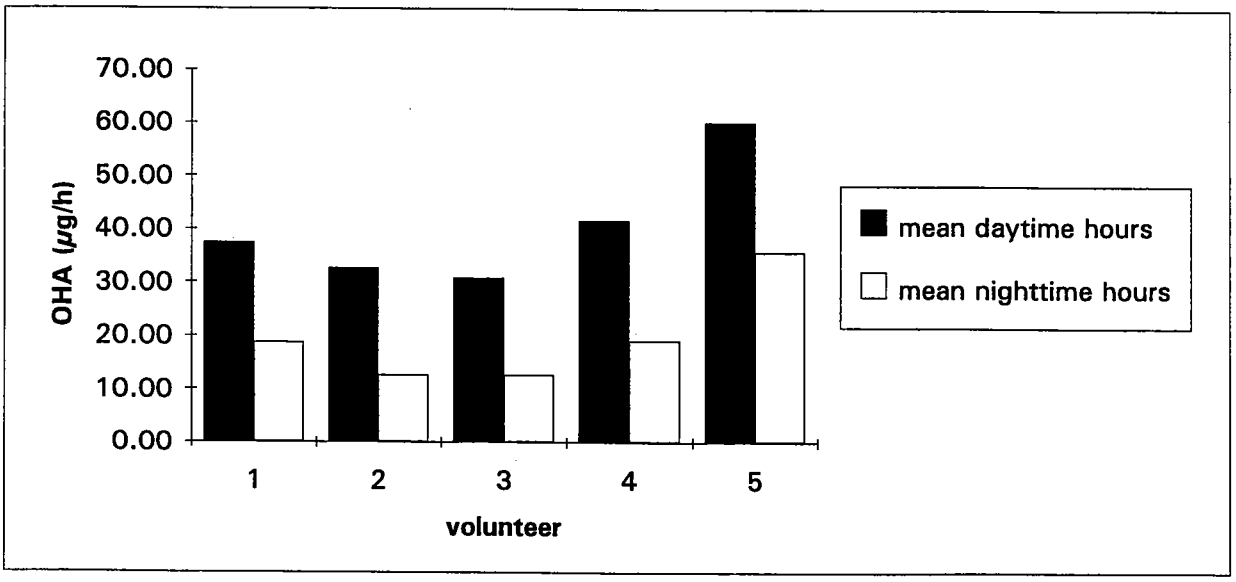

Fig 4: excretion OHA (µg/h) in male urine, collected over 24 hours
V1-V5 = volunteer 1-5

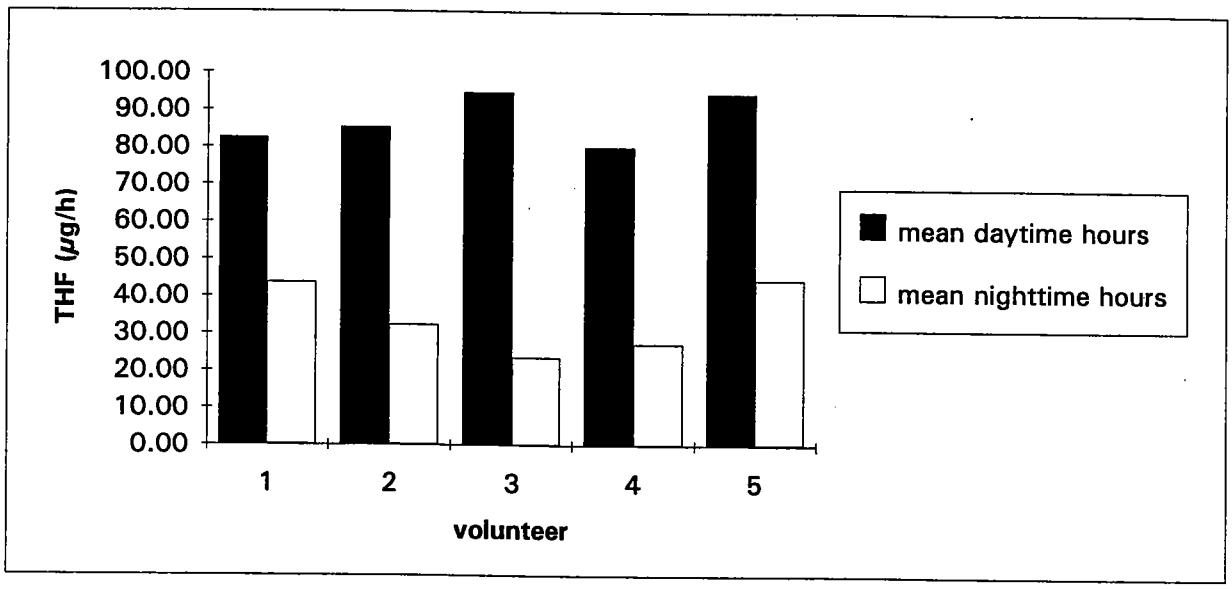

Fig 5: excretion THF (µg/h) in male urine, collected over 24 hours
V1-V5 = volunteer 1-5

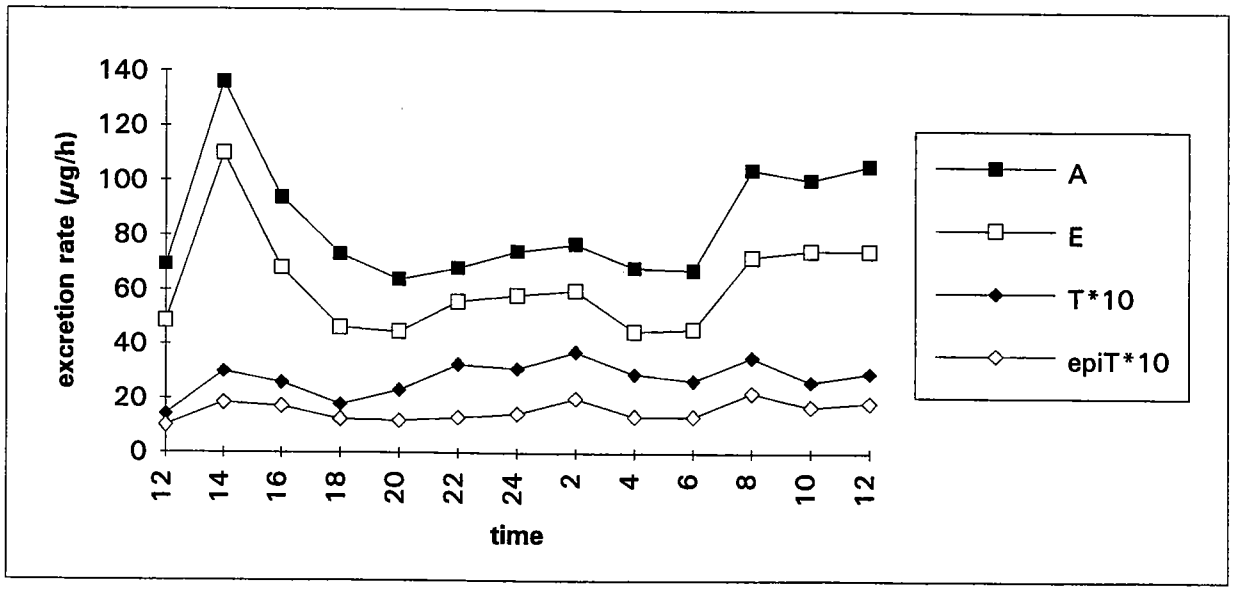

Fig 6: excretion rates of endogenous steroids in male urine, collected over 24 hours
volunteer 1

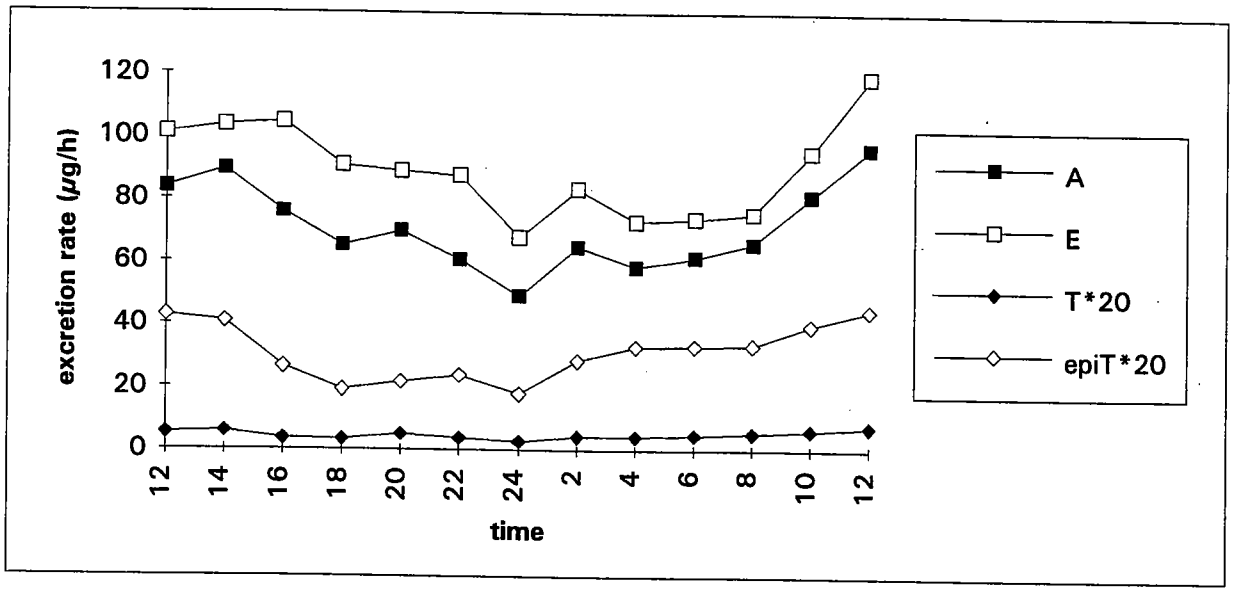

Fig 7: excretion rates of endogenous steroids in male urine, collected over 24 hours
volunteer 2

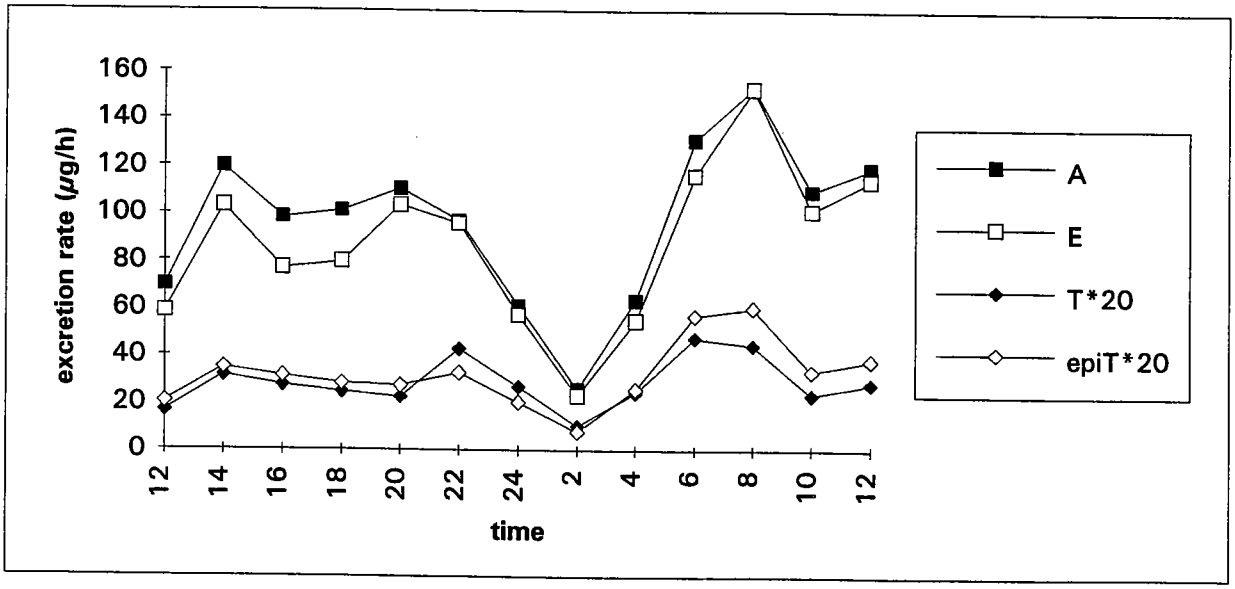

Fig 8: excretion rates of endogenous steroids in male urine, collected over 24 hours
volunteer 3

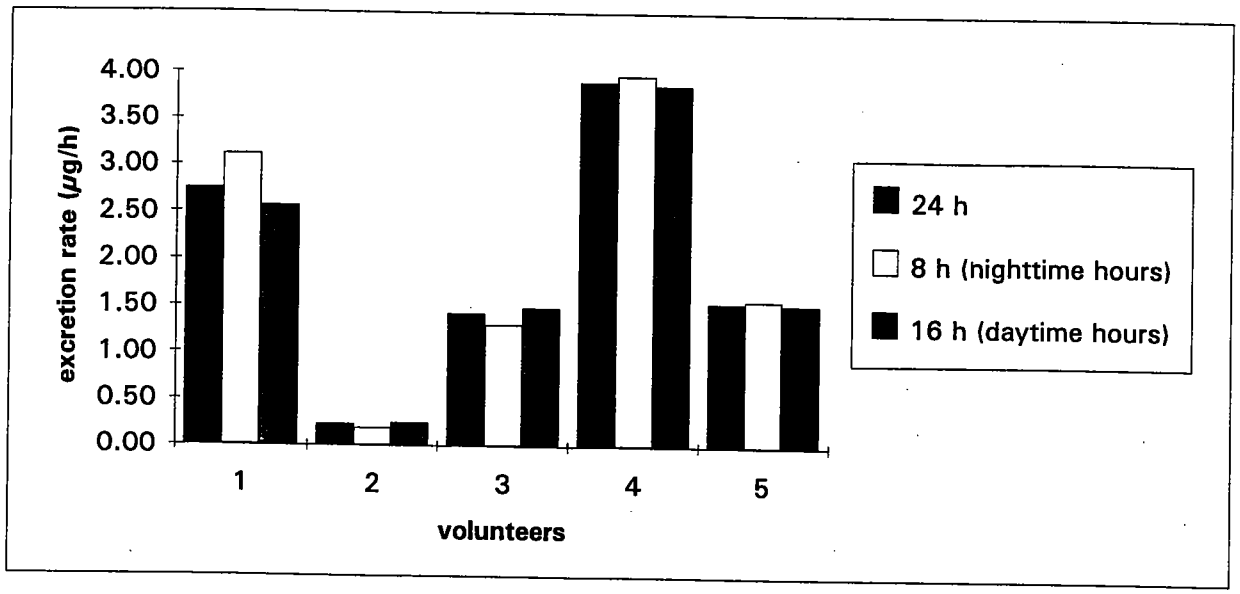

Fig 9: Testosterone excretion (µg/h)
comparison of daytime hours and nighttime hours with 24 hours collected urine
V1-V5 = volunteer 1-5

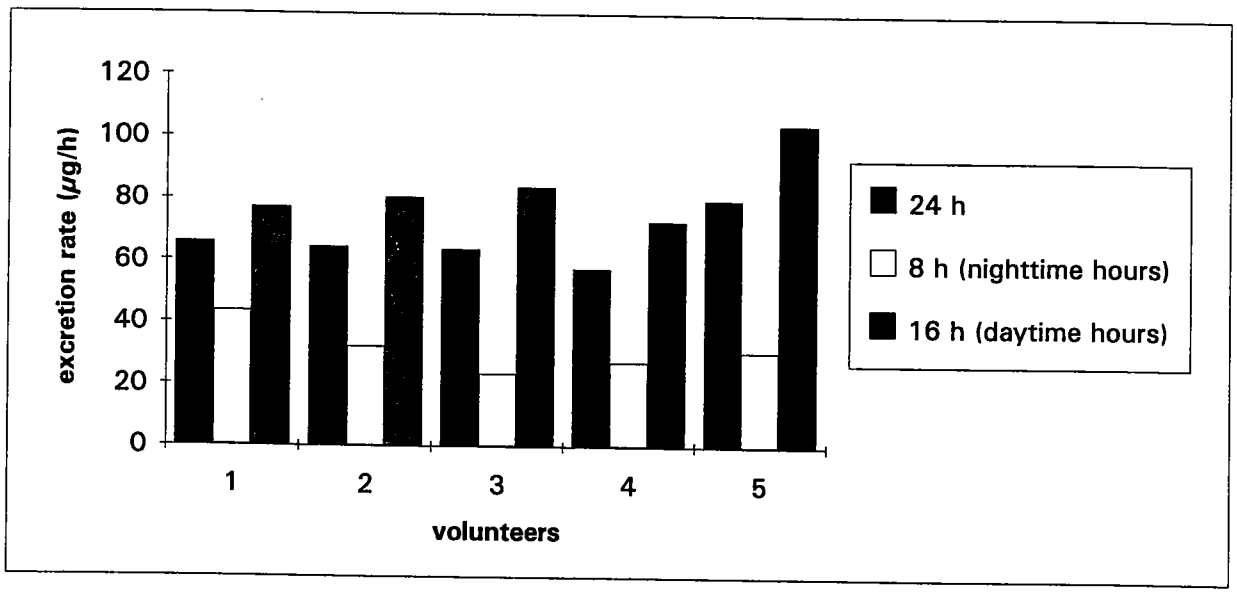

Fig 10: THF excretion (µg/h)
comparison of daytime hours and nighttime hours with 24 hours collected urine
V1-V5 = volunteer 1-5

The real calculated excretion amount for 24 hours is compared with values calculated for 24 hours from sleeping hours and wake hours.
Excretion rates obtained during the nighttime hours and daytime hours are added cumulatively and the values normalized to 24 hours.

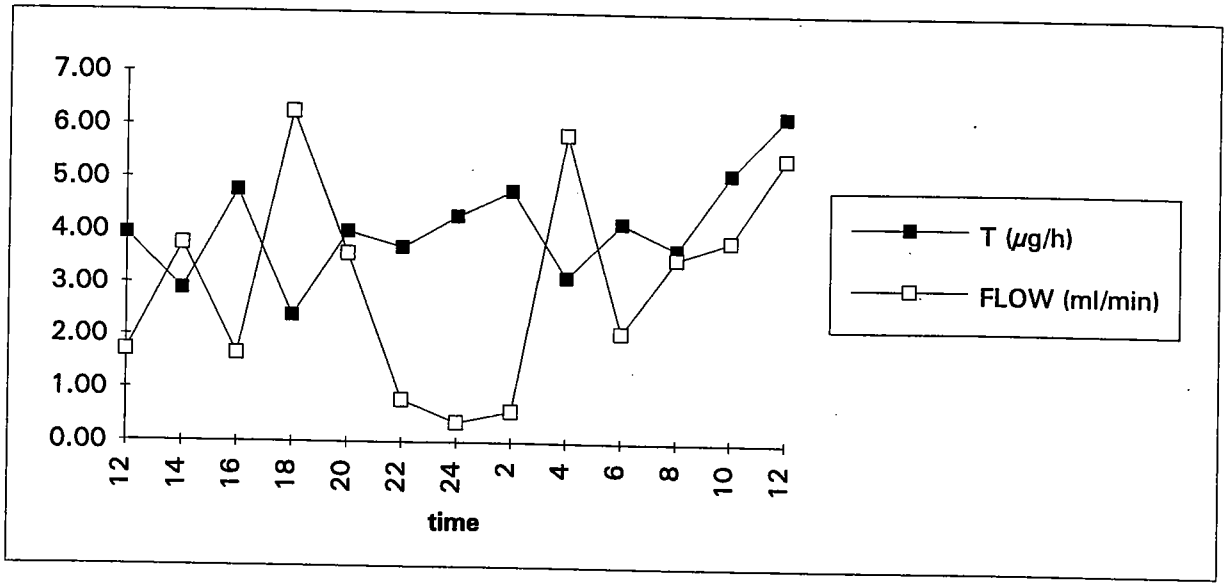

Fig 11: correlation between excretion of T (µg/h) and flow (ml/min) Volunteer 4

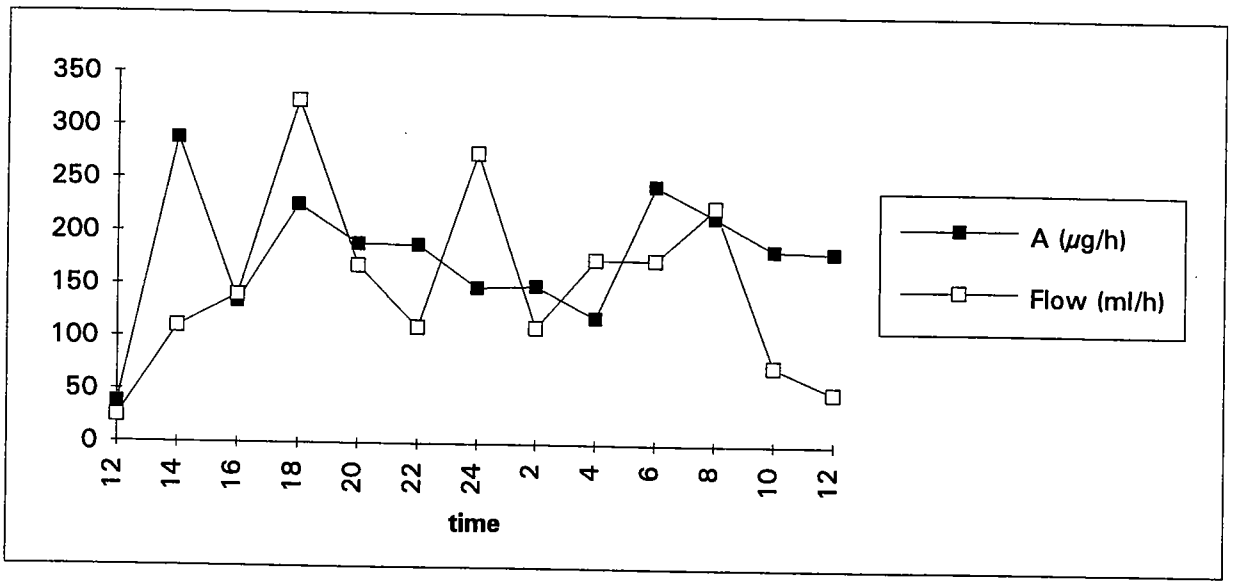

Fig 12: correlation between excretion of A (µg/h) and flow (ml/h) Volunteer 5