Reprint from

10th Cologne Workshop On Dope Analysis
7^{th} to 12^{th} June 1992
- Proceedings -

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Sport und Buch Strauß, Köln, 1993

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In: M. Donike, H. Geyer, A. Gotzmann, U. Mareck-Engelke, S. Rauth (eds.) 10th Cologne Workshop On Dope Analysis 7^{th} to 12^{th} June 1992. Sport und Buch Strauß, Köln, (1993) 141-151

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Probenecid as Masking Agent in Dope Control - Inhibition of the Urinary Excretion of Steroid Glucuronides

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Abstract

Most of the endogenous androgenic and synthetic anabolic steroids as well as their metabolites are excreted in urine as glucuronides. Since probenecid suppresses the elimination of organic acids the administration of 2-3 g of this substance leads to a substantially reduction of the urinary excretion of both the endogenous androgenic and the synthetic anabolic steroids. Due to the weak diuretic effect of probenecid the urinary concentrations of the glucuronidated steroids are reduced more obviously than their urinary excretion rates. Ratios of epimeric steroids are not changed by the administration of probenecid, e.g. the ratio testosterone/epitestosterone. The excretion of non-conjugated metabolites of synthetic anabolic steroids like 6ß-hydroxy-metandienone is not influenced by probenecid.

Introduction

Probenecid is a uricosuric agent which increases the excretion of urates and is used in the treatment of gout (1,2,3). Probenecid is also used as an adjunct to penicillin therapy. It reduces the tubular excretion of penicillin and increases the plasma concentration up to the fourfold (4,5,6). This effect is due to a competitive inhibition of active transport mechanisms of organic acids in the proximal tubulus (7).

The use of this uricosuric agent in sports was first detected 1987 in a routine dope control test of 5 athletes. In addition to the presence of probenecid unusually low concentrations of endogenous steroids in the urine samples were observed. The mean of the urinary androsterone concentration of the 5 athletes was 34 ng/ml whereas the normal concentration of androsterone in urines of athletes ranges from 375 ng/ml to 5323 ng/ml (95% confidence interval of 1789 athletes).

The purpose of this investigation was to study the influence of probenecid first on the concentration and second on the excretion rates of endogenous androgenic and synthetic anabolic steroids.

Material and Methods

Protocol: Excretion studies with probenecid (BENEMIDE^R, Theraplix s.a., Paris, France) were carried out with 4 healthy adult male volunteers (V1-V4 years/kg: V1 36/74; V2 32/64; V3 31/61; V4 23/118). Volunteer 4 was a bodybuilder who has self administered anabolic steroids for several years, especially nandrolone-decanoate, and stopped taking steroids 46 days prior to the administration of probenecid.
Probenecid was administered orally in doses of 2x1 g respectively in a dose of 3x1 g for the bodybuilder in 1 hour intervals.
In a second study probenecid was applied in combination with metandienone (Sigma, St. Louis, USA). Volunteer 1 orally took 24 mg of metandienone and 2 g of probenecid whereas volunteer 2 took 12 mg of metandienone and 2 g of probenecid. Excretion studies with the same volunteers and the same amounts of metandienone but without probenecid were carried out 2 months later. Urine samples were collected before and after the administration of the drugs.

Assay: The sample preparation and the measurement of the urinary steroids and probenecid were performed according to the method of DONIKE et al. (8) for free and conjugated steroids.
In the present study the glucuronides of the following endogenous steroids and metabolites of anabolic steroids were analysed:
Endogenous androgenic steroids: androsterone (AND), etiocholanolone (ETIO), 11ß-hydroxy-androsterone (11OHAN), 11ß-hydroxy-etiocholanolone (11OHET), testosterone (TEST), epitestosterone (EPIT).
Metabolites of synthetic anabolic steroids: 5α-estran-3α-ol-17-one (norandrosterone), 5ß-estran-3α-ol-17-one (noretiocholanolone), 17α-methyl-androst-1,4-diene-6ß,17ß-diol-3-one (6ß-hydroxy-metandienone).
As internal standard 17α-methyltestosterone was used for the conjugated fraction and 17α-methyl-5α-androstane-3ß,17ß-diol for the free fraction. The calculations of the concentrations of the steroids were performed by means of calibration mixtures containing all substances of interest.

Results and Discussion

After the application of probenecid the parent compound is detectable in urine in the screening procedure for free and conjugated anabolic steroids. The EI spectrum of probenecid mono-TMS is shown in Figure 1.
The administration of probenecid leads to significant decreases of the urinary concentrations of the measured endogenous steroid glucuronides. This effect is shown in Figure 2 for androsterone. The lowest concentrations are found between 4 and 5 hours after the application of probenecid. The mean of the androsterone concentration is reduced from 3052.5 $\pm$1197.3 ng/ml to 105.9 $\pm$49.9 ng/ml. The main reason for the decrease of the concentrations is a depressed excretion of the steroids. The curves of the excretion rates of androsterone (Fig.3) look similar to the concentration curves (Fig. 2) but for the excretion rates individual variations of the effects of probenecid are more obvious. The mean of the excretion rates before the application is 143.1 $\pm$55.3 μg/h. After 4-5 hours the excretion is reduced to 14.7 $\pm$8.7 μg/h (Fig. 3).

The relative decreases of the concentrations and excretion rates of the analysed endogenous steroids 4-5 hours after the administration of probenecid are shown in Tables 1 and 2. The concentrations of all measured steroids are reduced to about 10% of the pretest levels (Tab. 1), whereas the excretion rates are reduced to about 20% of the levels before the administration of probenecid (Tab. 2). The reason for the higher depression of the concentrations in comparison to the excretion rates is a weak diuretic effect of probenecid, resulting in an increased urine flow.

Table 1: Concentrations of steroids 4-5 h after the administration of probenecid (volunteer 1-3: 2 g; volunteer 4: 3 g) relative to the pretest levels in percent. (n.a. = not analysed)

volun-teer	AND [%]	ETIO [%]	EPI [%]	TEST [%]	11OHAN [%]	11OHET [%]
1	4.7	4.7	6.5	5.3	6.8	4.7
2	4.7	5.1	10.0	9.2	6.9	8.3
3	2.8	2.9	5.8	8.1	6.3	9.2
4	2.1	2.1	18.7	14.1	n.a.	n.a.

GARDENER et al. (9) observed the same tendency of probenecid on total 17-ketosteroid excretion to urine, but they reported a reduction of only 50% which may be due to the nonselective analytical techniques used by these authors in 1951.

Table 2: Excretion rates of steroids 4-5 h after the administration of probenecid (volunteer 1-3: 2 g; volunteer 4: 3 g) relative to the pretest levels in percent. (n.a. = not analysed)

volunteer	AND [%]	ETIO [%]	EPI [%]	TEST [%]	11OHAN [%]	11OHET [%]
1	11.8	11.8	16.3	13.3	17.0	11.8
2	12.2	13.2	26.0	23.9	17.9	21.5
3	11.3	11.7	23.4	32.6	25.4	37.1
4	5.0	5.0	44.5	33.6	n.a.	n.a.

As shown in Tables 1 and 2 excretion and concentrations of epimeric steroids like androsterone and etiocholanolone, 11ß-hydroxy-androsterone and 11ß-hydroxy-etiocholanolone and epitestosterone and testosterone are depressed to the same extent. Therefore the ratios between epimeric steroids are not influenced by the application of probenecid. This is shown in Table 3 for the ratio testosterone/epitestosterone which is important in dope control to monitor testosterone abuse. The small changes of the testosterone/epitestosterone ratios are due analytical variations in the quantitation of the very low concentrations after the probenecid application and by small circadian changes of the ratios. The high testosterone/epitestosterone ratio of volunteer 4 can most probably be attributed to longterm abuse of anabolic steroids (10).

Table 3: The change of concentrations and ratios of testosterone and epitestosterone after the administration of probenecid

volunteer	time after administration [h]	EPI [ng/ml]	TEST [ng/ml]	TEST/EPI
1	0.0	49.0	91.6	1.8
	4.5	3.2	4.9	1.5
2	0.0	30.1	115.0	3.8
	4.0	3.1	10.6	3.4
3	0.0	77.3	85.9	1.1
	6.0	4.5	7.0	1.5
4	0.0	14.4	106.3	7.3
	4.0	2.7	15.0	5.5

The administration of probenecid reduces also the excretion of glucuronidated metabolites of anabolic steroids. This is shown for volunteer 4 who had self-administered nandrolone (see protocol) and excreted the main metabolites norandrosterone and noretiocholanolone at a constant level of 15 - 20 μg/h.
The administration of 3 g of probenecid reduced the norandrosterone excretion to 10% of the initial level (Tab. 4). After 24 hours the excretion rates of both androsterone and norandrosterone reached nearly pretest levels.
Because of the weak diuretic effect of probenecid the change of the concentrations is more evident than the change of the excretion rates. Four hours after the administration an approximately twenty-fivefold reduction of the norandrosterone concentration is found. Figure 4 obviously demonstrates the decrease of the peak heights of the registered ions for both the metabolites of the anabolic steroid nandrolone and for the endogenous steroids androsterone and etiocholanolone.

Table 4: The change of excretion rates of norandrosterone and androsterone after the administration of 3 g of probenecid.

time [h]	norandrosterone [μg/h]	androsterone [μg/h]
0	20.0	132.7
2.5	10.9	56.5
4	1.9	6.5
10	6.1	49.6
12	5.4	36.7
21	12.2	105.0
23.5	15.4	195.5

The excretion of non-conjugated metabolites of anabolic steroids like 6ß-hydroxy-metandienone is not affected by probenecid.
The administration of 12 mg and 24 mg of metandienone without probenecid and in combination with 2 g of probenecid leads to similar excretion profiles of 6ß-hydroxy-metandienone with maximum levels between 8 and 10 hours after the administration (Fig. 5).

The two additive effects of probenecid, increase of urine flow and reduction of the excretion of conjugated steroids, may lower the concentrations of the metabolites of many misused anabolic steroids to such an extent that the resulting concentrations remain under

the detection limit of the applied analytical method. Therefore the inhibition of the renal excretion by probenecid represents a pharmacological manipulation of the urine samples used in dope control and was banned by the Medical Commission of the International Olympic Commitee in 1988.

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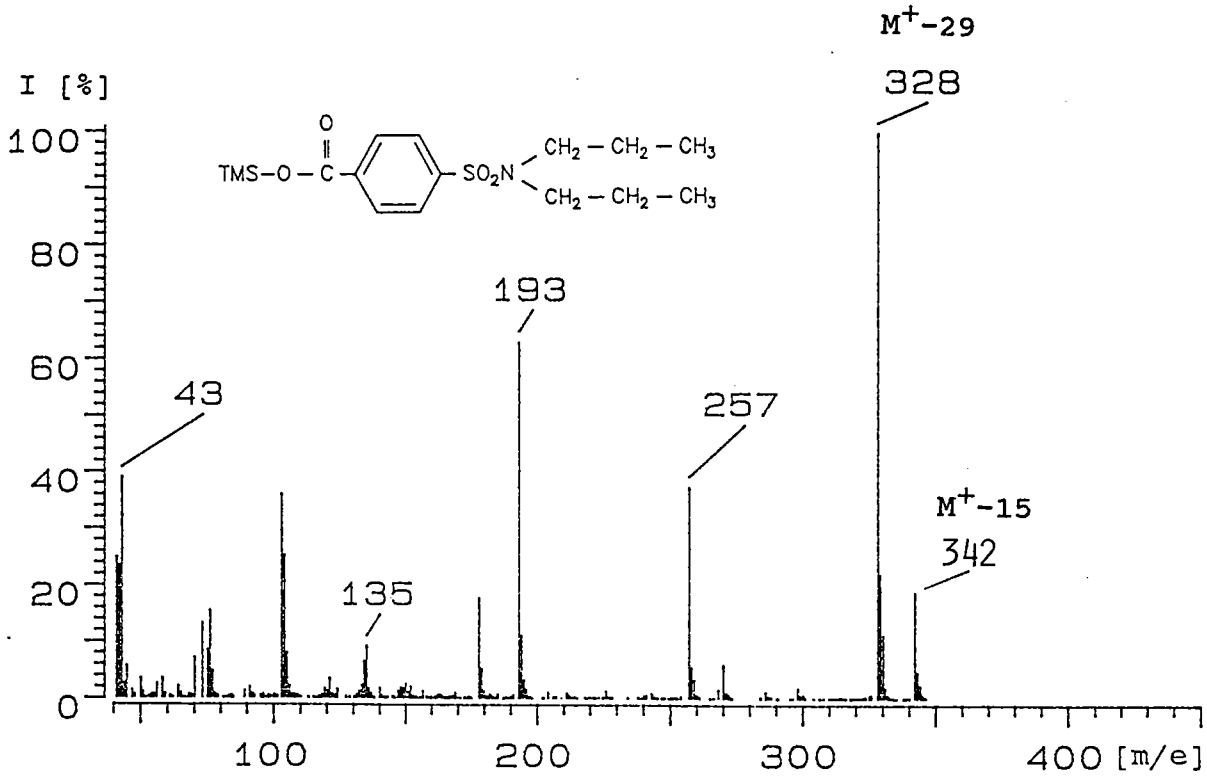

Fig. 1 : EI spectrum of probenecid-O-TMS. (M^+ = 357)

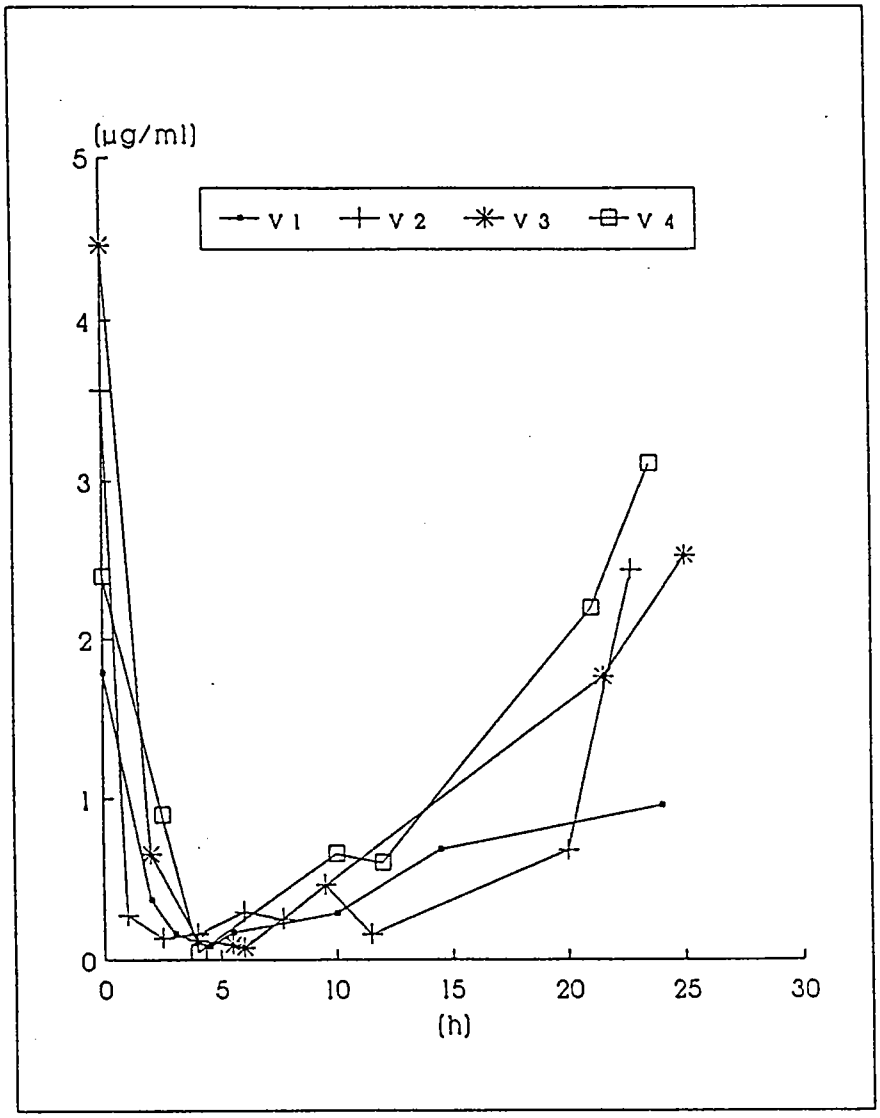

Fig. 2 : The effect of probenecid on the urinary concentration of androsterone glucuronide in 4 healthy volunteers (V1-V4).

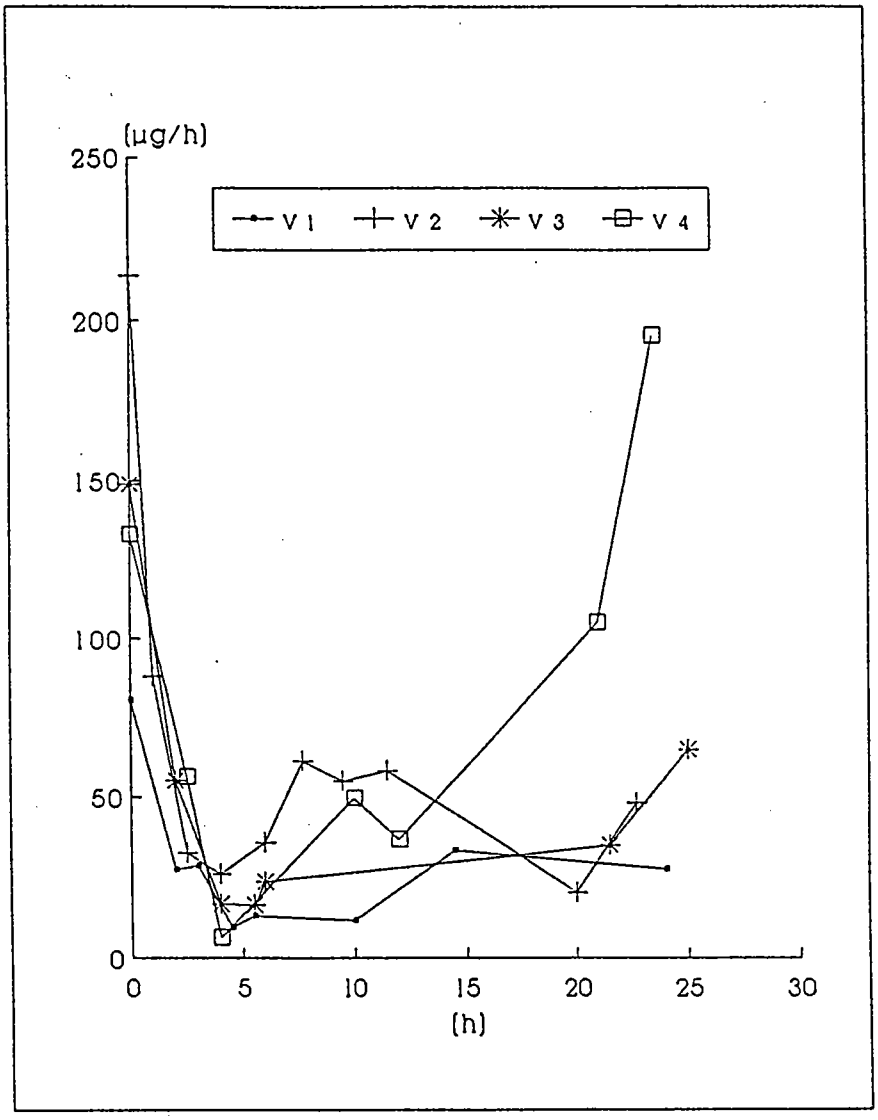

Fig. 3 : The effect of probenecid on the urinary excretion rates of androsterone glucuronide in 4 healthy volunteers (V1-V4).

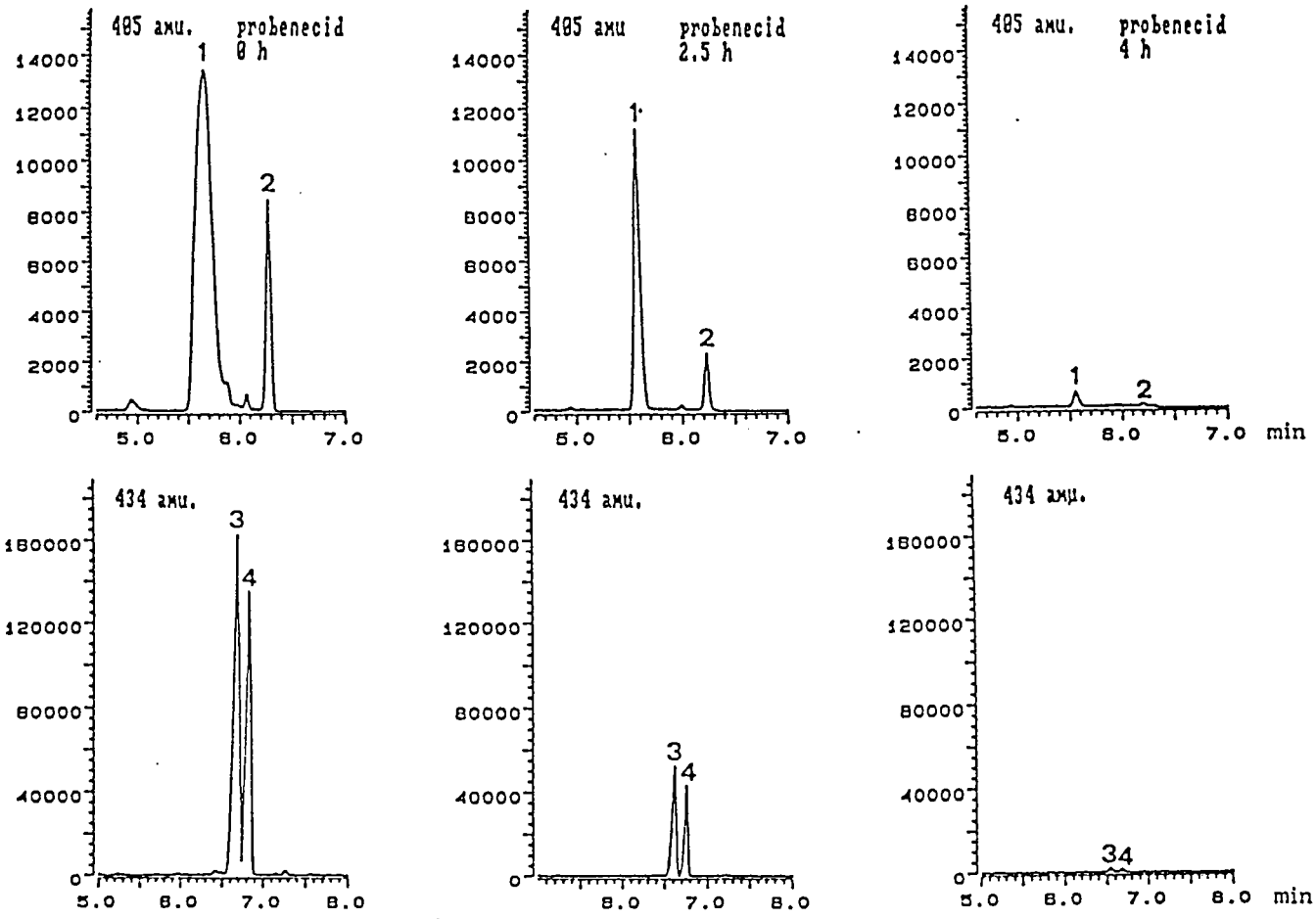

Fig. 4 : The effect of 3 g of probenecid on the urinary excretion of norandrosterone (1), noretiocholanolone (2), androsterone (3) and etiocholanolone glucuronide (4) demonstrated by the obvious decrease of the peak intensities of the registered ions.

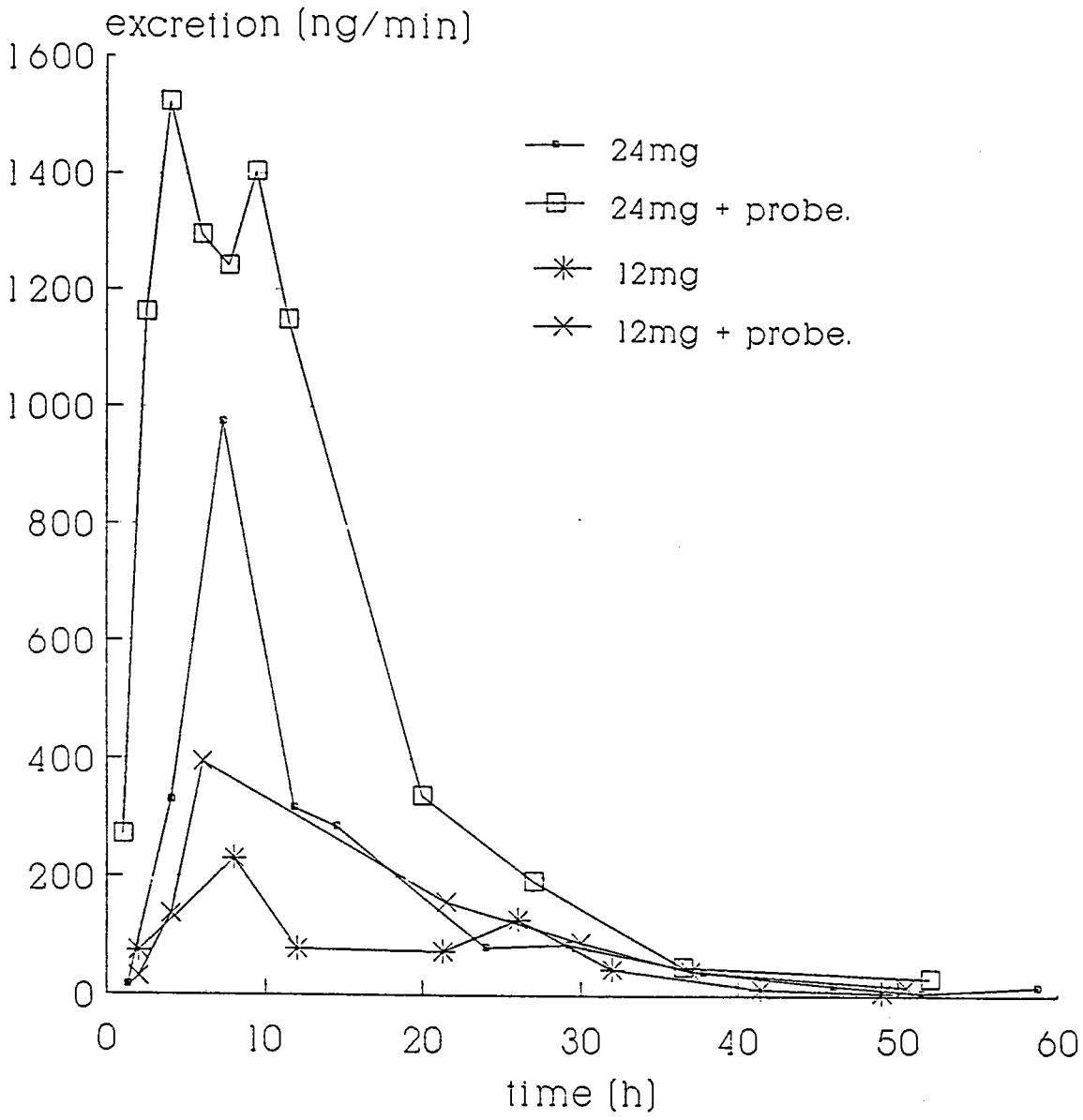

Fig. 5 : The excretion of 6-OH-metandienone after the administration of metandienone (24 mg; 12 mg) and after same amounts of metandienone + 2 g of probenecid (+ probe).