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The influence of diuretics on the excretion and metabolism of doping agents — I. Mephentermine

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Abstract: The urinary excretion of mephentermine and its major metabolite phentermine in human volunteers was followed over a period of several days after oral administration of mephentermine.

The excretion of both substances was affected by urinary pH. Maximum excretion was observed 2–4 h after administration and the total proportion of mephentermine excreted during 54 h was 57 to 83%. Based on urinary values, the biological half-life of elimination of mephentermine was 9.9 ± 2.6 h.

The ingestion of acetazolamide shortly after administration of mephentermine resulted in a decrease in excretion of both mephentermine and phentermine during one day; in some instances, the amounts of these substances in the urine were below the detection limit for a period of 3–9 h. The administration of frusemide only produced a urinary diluting effect during 2–4 h after administration.

Keywords: *Doping analysis; mephentermine; diuretic influence; frusemide; acetazolamide.*

Introduction

Although the continuing challenge encountered by doping analysts presented with samples from athletes is currently focussed on the misuse of anabolic steroids, glucocorticoids, testosterone or even caffeine [1], amphetamine and related compounds are still abused during sporting competitions. Since the latter group of substances can be readily detected in urine, attempts have been made to circumvent doping control and analysis by the use of diuretics, alkaline substances or drugs that possess both properties. It is well known that the excretion of sympathomimetic amines can be reduced or even suppressed by the intake of alkaline substances [2–4]. For example sodium bicarbonate is still used to depress the urinary excretion of stimulant amines. Diuretics such as frusemide can substantially reduce the urinary concentration of several drugs [5, 6]. Furthermore, the administration of therapeutic doses of acetazolamide, a potent carbonic anhydrase-inhibiting diuretic, not only results in an alkaline urine for several hours but also produces diuresis [4].

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The present work on mephentermine is the first of a series of studies undertaken to evaluate the effects of such substances on the urinary excretion of those doping agents which are listed as excluded substances, according to the International Olympic Committee (I.O.C.) and the International Cyclists Union (U.C.I.). Improved analytical methods have resulted in a reduction in the detection limits of doping agents and consequently in a lengthening of the 'drug detection time', that is the period during which a drug can still be detected in urine. Thus there is a need for the re-evaluation of the detection times of such drugs.

The present work reports the results of a study on the central nervous stimulant mephentermine (Wyamine®). This secondary amine is metabolized *in vitro* [7] and *in vivo* [8, 9] by oxidative dealkylation to yield the corresponding primary amine phentermine as its major metabolite. The excretion of both substances has been examined in the present work.

Experimental

Reagents

Mephentermine sulphate, phentermine hydrochloride and chlorphentermine hydrochloride were gifts of Wyeth (Philadelphia, PA, USA), C.E.R.T.A. (Brussels, Belgium) and Tropon Werke (Köln, FRG), respectively. Diethyl ether (analytical grade) was obtained from Merck (Darmstadt, FRG). The ammonia buffer was a saturated solution of ammonium chloride adjusted to pH 9.5 with a solution of ammonia.

Apparatus and operating conditions

A Varian model 3700 gas chromatograph equipped with a nitrogen-phosphorus specific detector (NPD) and connected to a Varian CDS 111 integrator was used for the gas chromatographic analysis. The 200 × 0.25 cm glass column was packed with 15% Apiezon L and 10% KOH on Chromosorb W HP 80–100. The oven temperature was 170°C, while the injector and detector temperatures were 230°C and 300°C respectively. Nitrogen (25 ml/min) was used as the carrier gas.

Procedure

200 μl of ammonia buffer and 100 μl of internal standard (chlorphentermine, 20 μg ml^{-1} in water) were added to 2.0 ml of urine in a glass-stoppered extraction tube. Two extractions (10 min) with 5 ml of diethylether were performed with a rotary shaker. After centrifugation the organic layers were combined, dried over Na_2SO_4 and transferred to a clean glass stoppered tube. Addition of several drops of diethyl ether saturated with gaseous HCl was followed by evaporation to dryness under vacuum at 40°C. The residue was dissolved in 200 μl of methanol and 1 μl was injected into the gas chromatograph.

Phentermine, mephentermine and the internal standard chlorphentermine gave sharp peaks with retention times of 3.75, 5.88 and 9.73 min respectively. Typical chromatograms obtained by processing control urine and urine after the administration of mephentermine are shown in Fig. 1.

The recoveries obtained by spiking urine with mephentermine and phentermine are summarized in Table 1.

Using different concentrations (2.5, 1, 0.5, 0.25 and 0.125 μg ml^{-1}) of mephentermine and phentermine in urine, standard graphs were obtained by analysing each urine sample in quadruplicate. The statistical data for slope, y-intercept and the correlation

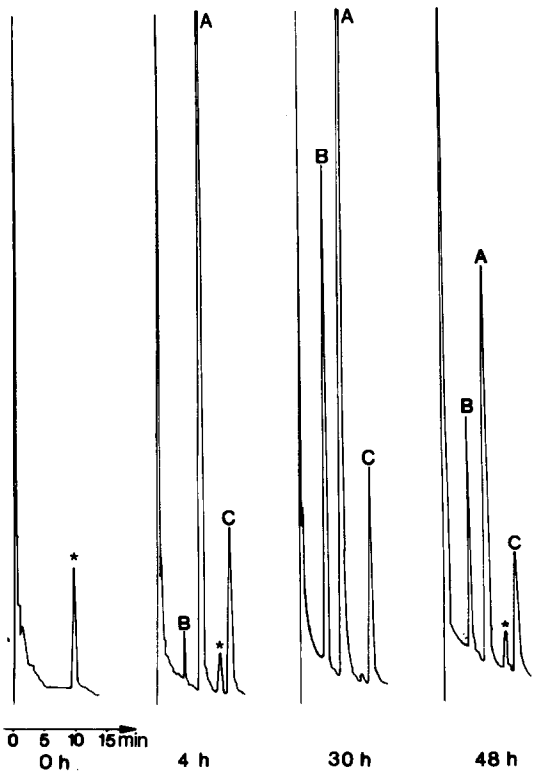

Figure 1
Typical chromatograms after administration of mephentermine (A = mephentermine; B = phentermine; C = internal standard chlorphentermine; * nicotine).

Table 1
Recoveries of phentermine and mephentermine from urine

Concentration added to urine* ($\mu g\ ml^{-1}$)	Concentration of phentermine (base) found ($\mu g\ ml^{-1}$)	Concentration of mephentermine (base) found ($\mu g\ ml^{-1}$)
1	0.97 ± 0.04	0.97 ± 0.04
0.5	0.47 ± 0.002	0.45 ± 0.01
0.25	0.24 ± 0.01	0.25 ± 0.004
Recovery (%)	96.2 ± 3.1	94.7 ± 4.8

* Four determinations were performed for each concentration; results are expressed as mean ± S.D.

coefficient were, respectively, 0.873, 0.0 and 0.9991 for phentermine and 0.738, −0.022 and 0.9993 for mephentermine. The routine detection limit was 0.100 μg ml^{-1} for each compound.

Human investigations

In the first series of experiments 12.5 mg Wyamine® (equivalent to 9.6 mg of mephentermine base) was given orally to five human volunteers. One month later the same subjects received the same dose of Wyamine® followed by 250 mg of acetazolamide (Diamox®) after 2 h.

The study was continued two months later in three subjects by administration of the same dose of Wyamine® followed by 40 mg of frusemide (Lasix®) after 2 h. Urine was collected at intervals up to 54 or 72 h after administration of mephentermine and either used immediately or stored deep-frozen for later analysis. Urinary pH and volume were measured. All samples were analysed in triplicate and appropriate dilutions made where necessary.

Results and Discussion

Urinary excretion under normal conditions

The time for peak excretion of mephentermine and the percentage excretion of both mephentermine and phentermine during a 54 h period in five subjects under conditions where urinary pH was not controlled are given in Table 2. The calculated elimination half-life of mephentermine was 8–9 h; results for subject V were not included in the calculation. The proportion of the dose excreted under normal conditions varied between 70 and 80%. The low value in subject I is probably due to the higher urinary pH values during the first few hours of the experiment in addition to interindividual variation. It is well known that the urinary excretion of a weak organic base depends largely on the lipid solubility of the undissociated form, which is related to the pK_a of the base and the pH of the medium. The influence of pH on the excretion of mephentermine is clearly illustrated in Fig. 2. A change from pH 7.5 (9 h) to pH 6.5 (12 h) with relatively constant urinary output (about 50 ml h^{-1}) resulted in an increase in the excretion rate from 55 to 132 μg h^{-1}. The influence of pH on the excretion of the metabolite

Table 2
Urinary pH, mephentermine excretion peak time, percentage of the dose excreted (54 h) and $t_{1/2}$ after oral administration of mephentermine under normal conditions

	Subject				
	I	II	III	IV	V
Mean pH*	6.48	6.21	6.09	5.32	6.53
Peak time (h)	3	3	2	3	4
Mephentermine (%)	42.2	68.7	59.8	67.6	55.6
Phentermine (%)	15.1	9.7	19.8	15.1	14.8
Total (%)	57.3	78.4	79.6	82.8	70.5
$t^1/_2$ (h)	8.9	9.1	7.9	9.1	14.5

* Calculated as $\frac{\Sigma pH_i \, \Delta t_i}{54}$.

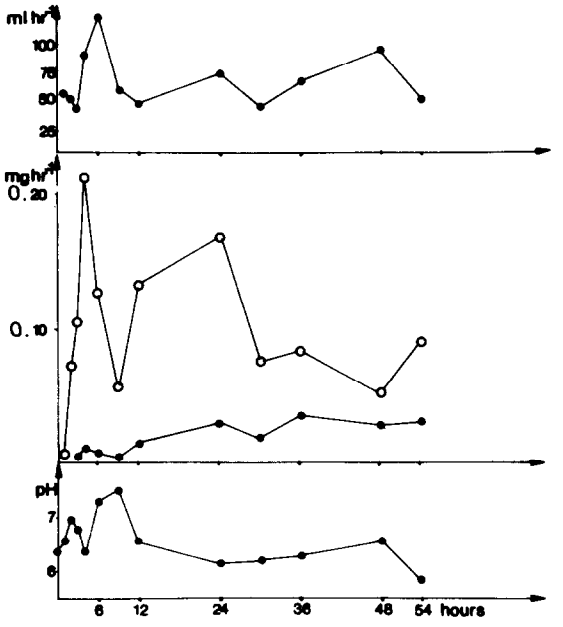

Figure 2
Urinary pH, urinary volume, mephentermine (○) and phentermine (●) excretion after administration of mephentermine under normal conditions in subject V.

phentermine was less obvious although the same trend in the excretion of both compounds occurred.

The excretion rate of mephentermine reached a maximum 2–4 h after administration of the drug. With an acidic urine (Subject IV) the peak elimination of phentermine occurred 9 h after administration of mephentermine; these results were similar to those obtained by Beckett and Brookes [9] under acidic conditions. However, under normal conditions of urinary pH (Subjects I, II, III and V) the peak phentermine excretion occurred 12–24 h after the administration of mephentermine.

Urinary excretion after administration of acetazolamide

The influence of the diuretic acetazolamide on the excretion of mephentermine and phentermine is shown in Fig. 3.

Generally the alkaline effect reached a maximum 2 h after administration of 250 mg of acetazolamide and persisted for about 22 h. The diuretic effect, however, was more subject to interindividual variation and resulted either in an increased urinary output over a maximum of 10 h or in a volume peak during a short time period (1 or even 4 h after the intake of the diuretic).

In all subjects, the suppression of mephentermine excretion lasted at least 22–28 h after the administration of acetazolamide. Because of its shorter diuretic effect, the alkaline effect of acetazolamide is mainly responsible for the decrease in mephentermine excretion. Moreover, in three of the five subjects the mephentermine concentrations dropped below the sensitivity limit of the analytical method 3–9 h after the administration of acetazolamide. It is noteworthy that the excretion of mephentermine was not suppressed to levels below the detection limit in 2 subjects, in whom a sharp urinary

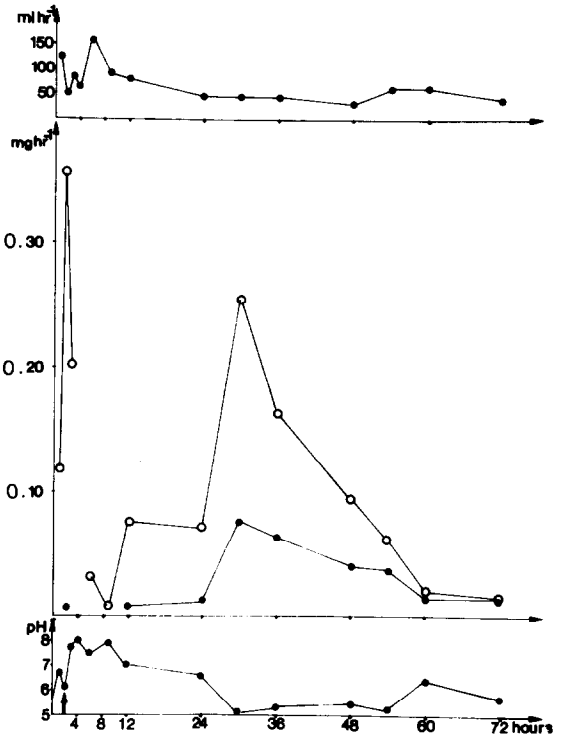

Figure 3
Urinary pH, urinary volume, mephentermine (○) and phentermine (●) excretion after administration of mephentermine followed by acetazolamide (↑) in subject II.

volume peak was produced shortly after the intake of acetazolamide. This effect will be investigated further.

Under normal conditions a maximum of 20% of mephentermine was excreted as phentermine in 24 h. Therefore, it is clear that phentermine could not be detected in all subjects up to 22 h after the intake of acetazolamide.

The excretion of both mephentermine and phentermine, together with the mean pH value for all subjects, is summarized in Table 3. The elimination half-life (measured by the 'sigma-minus' method) of mephentermine in the post-effect phase (24–72 h) was 8.7 ± 0.9 h (n = 5); this was similar to values found under normal conditions. Generally, the excretion rate of mephentermine reached a maximum in the post-effect phase 30–36 h after administration. The influence of acetazolamide on mephentermine excretion was neutralized 52 h after the intake of acetazolamide, as shown in Tables 3 and 4. During this period, however, more metabolite was excreted after administration of acetazolamide than under normal conditions (Table 3). Indeed, the higher reabsorption of the non-ionized, lipophilic mephentermine under strong alkaline conditions could lead to relatively higher metabolism. However, another contribution to this phenomenon could be the smaller influence of pH on the excretion of phentermine. This problem is currently under investigation.

Urinary excretion after administration of frusemide

The influence of the potent diuretic frusemide on the excretion of mephentermine was studied in three subjects. From the results it appeared that the duration of the diuretic

Table 3
Urinary pH, percentage of the dose excreted, $t_{1/2}$ and mephentermine peak excretion time in the post effect phase (after administration of acetazolamide)

Subject	Mean pH*	Peak excretion time (h)	$t^1/_2$ (h)	Percentage of the dose excreted as (a) phentermine (b) mephentermine After 12h	24h	48h	54h	72h
I	6.2	30	8.9	(a) 0.2	3.5	18.0	20.6	26.3
				(b) 4.9	14.5	33.3	35.3	37.6
II	6.0	30	7.7	(a) 0.4	2.1	17.0	19.5	22.2
				(b) 10.3	19.1	57.0	60.9	63.9
III	6.0	30	8.8	(a) 0.7	3.9	23.1	26.7	33.6
				(b) 10.5	19.7	44.1	46.7	50.3
IV	5.8	30	8.6	(a) 0.2	2.9	15.8	18.3	22.6
				(b) 8.8	17.9	42.2	44.9	48.2
V	6.1	36	10.0	(a) —	1.9	14.7	17.5	22.3
				(b) 5.1	13.8	40.1	44.0	48.4

* Calculated as $\frac{\Sigma pH_i \, \Delta t_i}{72}$.

Table 4
Comparison of the cumulative excretion* of mephentermine: (a) under normal conditions; (b) after administration of acetazolamide; and (c) after administration of frusemide

Subject		12h	24h	54h	72h
I	a	13.7	38.2	57.3	—
	b	5.2	18.0	55.9	63.9
	c	20.3	39.1	59.8	72.0
II	a	40.8	58.4	78.4	—
	b	10.7	21.2	80.4	86.2
	c	33.2	55.5	77.6	85.5
III	a	35.9	64.0	79.6	—
	b	11.2	23.6	73.4	83.9
	c	33.2	47.8	64.0	67.9

* Values are expressed as total % of the dose excreted (mephentermine + phentermine).

effect of frusemide was relatively short. The administration of this diuretic in doses of 40 mg reduced mephentermine concentrations by a factor of 3–5 after 2 h. The urinary levels of mephentermine appeared to return to normal within 4 h.

From the results it appeared that the administration of frusemide 2 h after administration of mephentermine had no effect on the detectability of mephentermine. The rules of doping (I.O.C. and U.C.I.) state that the urinary concentration is not important in determining illicit use, but the presence of a doping agent is in itself sufficient to establish a positive result. From the results of the present work the use of frusemide in an attempt to dilute mephentermine below the detection limit and thus to circumvent doping analysis, is of questionable value.

The observed effects of frusemide on mephentermine excretion in human volunteers are negligible compared with those found in the horse after the administration of

phenylbutazone, morphine and fentanyl followed by intravenous administration of frusemide (0.5 mg/kg), where dilution effects of respectively 10-, 13- and 18-fold were found [10]. Frey *et al.* [11] showed that the urinary concentration of amphetamine in the horse was not significantly affected by bumetanide, another acidic diuretic. The results obtained in the present work in man are in good agreement with these findings and with other studies [12] in the horse, which have shown no substantial effects of frusemide on urinary concentrations of procaine or methylphenidate, both lipophilic basic drugs.

Conclusions

Although the experimental conditions in the present work were different from real sport conditions, sportsmen should be aware of the long detection time of mephentermine. Indeed, with 2 ml of urine, mephentermine and its metabolite were detectable in urine at least 54 h after the administration of therapeutic doses. Although the total renal excretion of mephentermine during 54 h after administration was not influenced by acetazolamide or frusemide (Table 4), in some instances urinary concentrations dropped below the sensitivity limit 3–9 h after administration of acetazolamide. However, administration of frusemide in an attempt to 'dilute out' prohibited substances had no effect on the detectability of the basic drug mephentermine.

It is recommended that special attention should be paid to alkaline urine samples and that sporting organizations should consider banning diuretics, especially acetazolamide.

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