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The relation between blood levels and urinary excretion of amphetamine under controlled acidic and under fluctuating urinary pH values using [^{14}C]amphetamine

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Plasma, blood cell and urine levels of amphetamine were determined after the oral administration of *S*-(+)-[^{14}C]amphetamine sulphate to two subjects under conditions of controlled acidic and fluctuating urinary pH. The decline in plasma concentration of the drug was more rapid under the controlled acidic conditions than under conditions of fluctuating urinary pH. Under controlled conditions, the concentration time profiles of drug and metabolite in urine or plasma (as opposed to body levels), were suitable for kinetic analysis. The apparent rate of urinary excretion of amphetamine was proportional to its plasma concentration only under the controlled acidic urinary conditions. Amphetamine was cleared from blood more rapidly than could be accounted for by glomerular filtration under acid conditions, but when urinary pH fluctuated, clearance of the drug could be accounted for by this route.

Changes in urinary pH in man produce fluctuations in the rate of excretion of amphetamine (Beckett & Rowland, 1965). However, the excretion profile of this drug under normal conditions of fluctuating pH can be predicted accurately from pH values and volumes of urine collected after an oral dose. This has been done by kinetic analysis of the data. For this purpose, distribution and excretion rate constants were obtained by analysis, using an analogue computer, of data obtained previously under controlled conditions (Beckett, Boyes & Tucker, 1968a, b). In these calculations it was assumed that when reabsorption of amphetamine from the kidney tubule was negligible, the rate of urinary excretion was proportional to its plasma concentration, which in turn was proportional to the total amount present in the body (excluding the gut). The prediction of body levels and related plasma profiles of drug-time relations under conditions of changing urinary pH could not be verified at the time, because no suitable, sensitive analytical technique was available to determine amphetamine in human blood after a normal dose.

The purpose of the present work has been to determine the time course of plasma and blood cell concentrations of amphetamine, in addition to urine levels, after an oral dose under controlled and uncontrolled conditions to produce further support for the above assumptions and methodology. An additional objective was to demonstrate the advantage of studies under controlled acid urinary pH in establishing the relative importance of metabolic routes irrespective of whether determined using blood or urine data. It was also proposed to investigate whether amphetamine passed into the urine by routes other than by glomerular filtration.

EXPERIMENTAL

Apparatus and Materials

[^{14}C]Levels were determined on a Tri-Carb liquid scintillation counter (Packard Instrument Company Inc., Model 500D) or by using a radio-chromatogram scanner (Panax Equipment Limited, Model RTLS 1). Chromatographic determinations were made on a Perkin Elmer F11 gas chromatograph with a flame ionization detector and pH values were measured with a Pye Dynacap pH meter.

The *S*-(+)-[^{14}C]amphetamine (α-methyl-[β-^{14}C]phenethylamine) sulphate (3 μCi/mg) was supplied by Smith, Kline and French Research Laboratories, Philadelphia.

Method

Trials. Two male volunteers (21 and 23 years) were given *S*-(+)-[^{14}C]amphetamine sulphate (15 mg; 45 μCi) in aqueous solution by mouth. Urine samples were collected at 30 min intervals for 4 h and then at 60 min intervals for a further 8 h; finally a 24 h sample was collected. Blood samples were taken from the median cephalic vein at times midway between those of urine samples. The blood samples were oxalated and centrifuged (3000 *g* × 10 min) to separate plasma from the blood cells. All samples were stored at 4° until analyses had been completed. Two trials were conducted. In the first, the urinary pH was not modified, whilst in the second, an acid urinary pH (5·0 ± 0·2) was maintained as previously described (Beckett & Tucker, 1966). The pH and volume of each urine sample was accurately measured at the time of collection.

Extraction procedure. The pH of an aliquot (5 ml) from each urine sample was adjusted to pH 1 by adding 0·1 ml 6N HCl. The acidic and neutral metabolites of amphetamine were then extracted from the urine by freshly distilled diethyl ether (3 × 2·5 ml). Analar dioxan (1 ml) was added to the combined ethereal extracts in a Tri-Carb counting vial and most of the ether was removed by evaporation on a water bath at 43°, to give fraction A. The aqueous phase was adjusted to pH 12 by the addition of 0·5 ml 20% NaOH, was re-extracted with ether and the extract treated as described above to give a solution of [^{14}C]amphetamine in dioxan (Fraction B).

The procedure for extracting the drug from plasma (2 ml aliquot) and blood cells (1 ml aliquot) was similar to that described for urine. The blood cells were disintegrated by ultrasonic treatment before extraction.

Radioactive analysis. Scintillation fluid was prepared by mixing naphthalene (60 g), PPO (4 g), dimethyl POPOP (0·2 g), methanol (100 ml), toluene (100 ml), ethylene glycol (20 ml) with dioxan to 1 litre. The scintillant solution (10 ml) was added to the vials before counting. Both fractions (A and B), described above, were counted for each sample of urine, plasma and blood cells. In addition, a sample (0·2 ml) of the aqueous phase remaining after fractions A and B had been removed was counted (fraction C).

Background counting corrections were determined on samples of biological fluid (i.e. urine, plasma or blood cells) which had been extracted and prepared as described above. Corrections for dilution and quenching effects were made by the channel ratio method.

The concentration of metabolites were expressed as the concentration of amphetamine metabolized.

Chromatography. The components giving the activity in the three fractions (A, B and C) were investigated by thin-layer chromatography. Glass plates (20 × 20 cm)

were coated with a layer (0·5 mm) of silica gel G (nach Stahl), activated at 105° for 1 h and stored in a desiccator over silica gel until required. Spots of each fraction and also of [^{14}C]amphetamine in ether as reference were applied and the chromatograms developed in the solvent systems shown in Table 1. The plates were then scanned with a radiochromatogram scanner fitted with a continuous gas flow Geiger tube, and the Rf values of the regions of activity were determined.

Fraction B from several samples of urine was analysed by the gas-liquid chromatographic method of Beckett & Rowland, 1965.

The glomerular filtration rates of both subjects were determined by measuring the clearance of endogenous creatinine from the plasma, and the clearance rate of amphetamine from the plasma was calculated in each trial. Total blood volumes and haematocrit levels were also measured.

RESULTS AND DISCUSSION

Chromatographic studies showed that the radioactivity measured in fraction B was associated entirely with a component having the same Rf value (Table 1) as amphetamine. Determinations of the concentration of amphetamine by gas-liquid chromatography and by the radioactive technique gave similar results. Thus the radioactivity measured in fraction B in subsequent work was interpreted as representing amphetamine levels.

Table 1. *Rf values for the radioactive component present in fraction B and* [^{14}C] *amphetamine after thin-layer chromatography in various solvent systems*

Solvent system	Rf values	
	[^{14}C]amphetamine	Fraction B
Chloroform–diethylamine (9:1)	0·58	0·58
Chloroform–acetone–diethylamine (5:4:1)	0·70	0·68
Chloroform–ethanol–ammonia (100:15:1)	0·08	0·07
Benzene–1,4-dioxan–acetic acid (45:12·5:2)	0·03	0·04

The rate of excretion of amphetamine in urine showed urinary pH dependent fluctuations which agreed with earlier studies made under conditions of fluctuating urinary pH (Beckett & Rowland, 1965). However, as expected, plots of plasma concentration against time, although showing some irregularities, did not exhibit definite fluctuations associated with the changes in urinary pH (Fig. 1). Under controlled acidic conditions, there were no fluctuations in the rate of urinary excretion of the base, and the profiles of rate of urinary excretion and plasma concentration against time were similar (Fig. 2). For instance, peak levels of amphetamine were reached in both plasma and urine 1$\frac{1}{4}$ h after administration of the dose; also after absorption was complete (a period of about 4 h being indicated from both urine and plasma data) there was an exponential decrease in both plasma and urine concentrations, as shown by the linear log plots (Fig. 3) in which almost parallel lines were obtained for the two systems. Under uncontrolled conditions, no such relation could be obtained for urine, and the log concentration against time plot of plasma levels gave a completely different relation from that established under controlled conditions (Fig. 3).

The results indicate that under acidic but not under fluctuating urinary conditions the rate of urinary excretion of amphetamine is *directly* proportional to its plasma concentration.

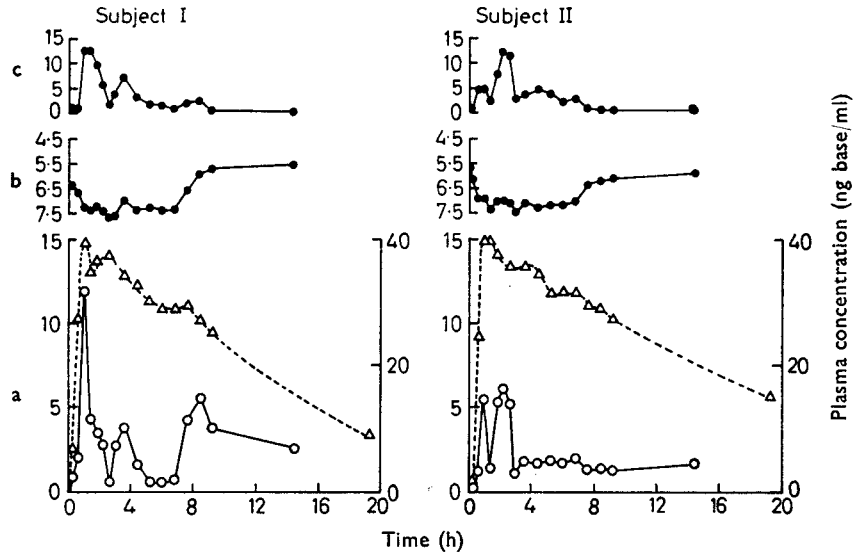

FIG. 1. Urinary excretion and the corresponding plasma levels of [^{14}C]amphetamine after oral administration of 15 mg (+)-[^{14}C]amphetamine sulphate to subjects I and II under conditions of fluctuating urinary pH. a. Rate of excretion. b. Urinary pH. c. Urine flow (ml/min). —○— Urinary excretion. -- △ -- Plasma concentration.

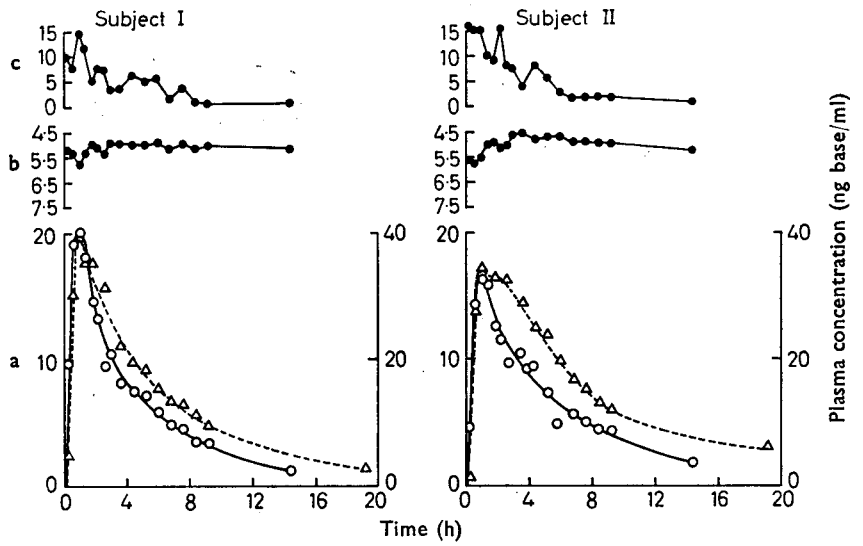

FIG. 2. Urinary excretion and the corresponding plasma levels of [^{14}C]amphetamine after oral administration of 15 mg (+)-[^{14}C]amphetamine sulphate to subjects I and II under conditions of acidic urinary pH. Symbols as in Fig. 1.

The urinary excretion rates of amphetamine, ether soluble acidic and neutral metabolites (Fraction A) and ether insoluble acidic metabolites (Fraction C) gave a very complicated pattern under conditions of fluctuating urinary pH (Fig. 5). However, when an acidic urine was maintained, a clear relation emerged between the rates of excretion of amphetamine and the two above-mentioned metabolite fractions (Fig. 6).

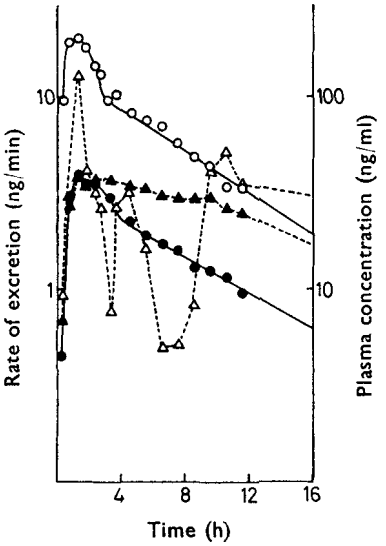

FIG. 3. Urinary excretion and plasma concentration of amphetamine after oral administration of 15 mg (+)-[^{14}C]amphetamine sulphate under conditions of acid controlled and fluctuating urinary pH. Subject I. Controlled urinary pH: —○—Rate of excretion. —●— Plasma concentration. Fluctuating urinary pH: —△— Rate of excretion. - - ▲ - - Plasma concentration. Subject II behaved similarly.

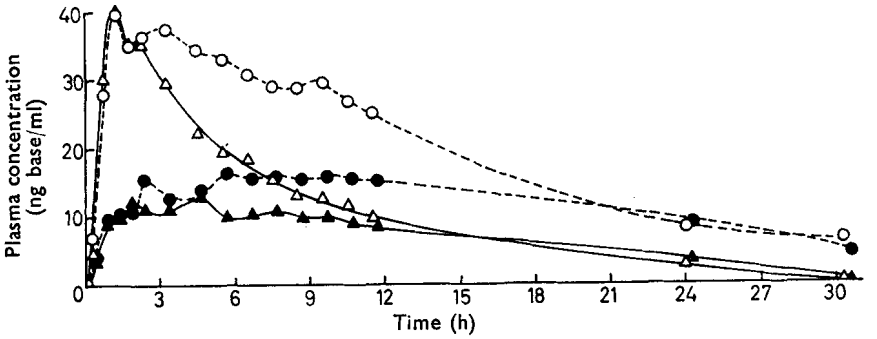

FIG. 4. Plasma concentrations of [^{14}C]amphetamine and its metabolites after the oral administration of 15 mg (+)-[^{14}C]amphetamine sulphate. Subject I. Controlled urinary pH: —△— Amphetamine base. —▲—Acidic and neutral, ether-soluble metabolites urinary pH. Uncontrolled urinary pH: - - ○ - - Amphetamine base. - - ● - - Acidic and neutral, ether-soluble metabolites.

As expected, the recoveries of unchanged drug and metabolite fractions from urine were very different under the two conditions (Table 2), but there was much less difference between the corresponding plasma levels. However, plasma levels fell more rapidly under the controlled acidic conditions (Fig. 4). Under acid conditions there was a lower concentration of neutral and acidic ether soluble metabolites in the plasma (Fig. 4). Ether insoluble acidic metabolites were only detected in urine (Figs 5 and 6).

Calculations of peak blood levels of amphetamine (48 and 40 ng/ml, acidic conditions, and 52 and 47 ng/ml, uncontrolled conditions, for subjects I and II respectively) from the plasma and blood cell data (Table 3) indicate that at peak concentration only 1/40th of the administered dose is present in the blood. This indicates rapid

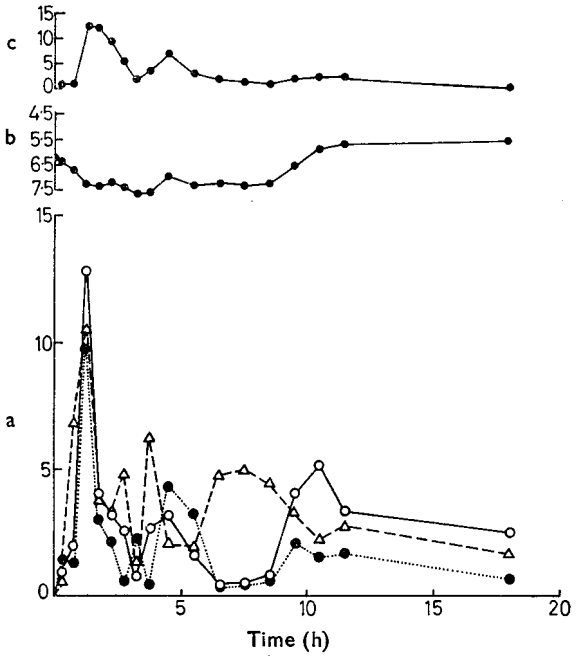

FIG. 5. Urinary excretion of [^{14}C]amphetamine and its metabolites after the oral administration of 15 mg (+)-[^{14}C]amphetamine sulphate under conditions of fluctuating urinary pH. Subject I. —○—Amphetamine base. – – △ – – Ether-insoluble metabolites. ---●--- Acidic and neutral ether-soluble metabolites. Subject II behaved similarly.

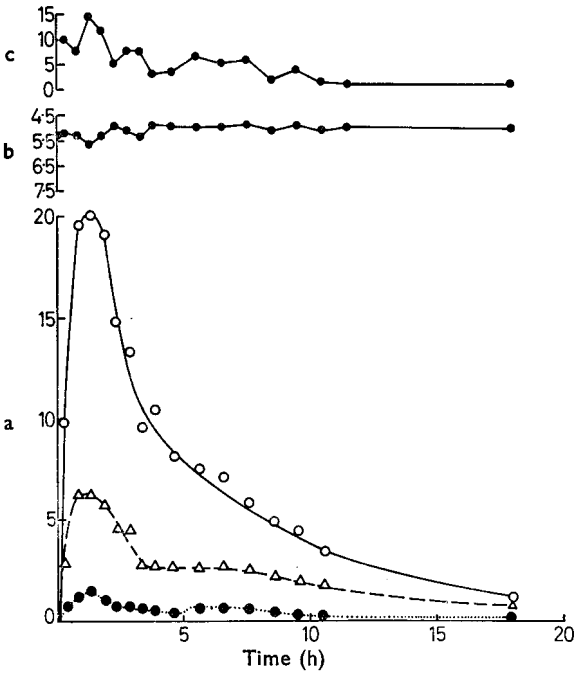

FIG. 6. Urinary excretion of [^{14}C]amphetamine and its metabolites after the oral administration of 15 mg (+)-[^{14}C]amphetamine sulphate under conditions of acidic urinary pH. Subject I. Symbols as in Fig. 5.

Table 2. *Urinary excretion of amphetamine and its metabolites—information from two subjects after each had received an oral dose* (15 *mg*) *of* (+)-[^{14}C]*amphetamine sulphate*

Condition of urine	Subject	Recoveries (%) in 24 h			
			Metabolites		
		Amphetamine base extracted into ether under alkaline conditions (pH 12)	Fraction A Metabolites soluble in ether under acidic conditions (pH 1)	Fraction C Metabolites remaining in aqueous phase after alkaline and acidic extractions	Total
Acid control	I	64·9	5·5	20·1	90·5
pH 5 ± 0·2	II	63·1	4·6	23·8	91·5
Uncontrolled urinary pH	I	34·6	17·4	35·5	87·5
pH 5·5–7·7	II	34·9	17·0	19·0	60·9

Table 3. *Results of haematological Investigation*

	Subject I	Subject II
Weight	68 kg	66·5 kg
Erythrocyte volume	1493 ml	2199 ml
Blood volume	4557 ml	5370 ml
Haemoglobin	12·0 g%	15·1 g%
Haematocrit value	36·0%	45%
Mean creatinine excretion in 24 h	1·65 g	1·80 g
Erythrocyte count	3,960,000	4,800,000

extravascular distribution. The maximum plasma concentration of amphetamine occurred 1¼ h after the dose was administered, whereas peak blood cell concentration occurred later. The concentration in the blood cell then remained at a consistently higher level than that in plasma, indicating that equilibrium is established only slowly between plasma and these cells (Fig. 7).

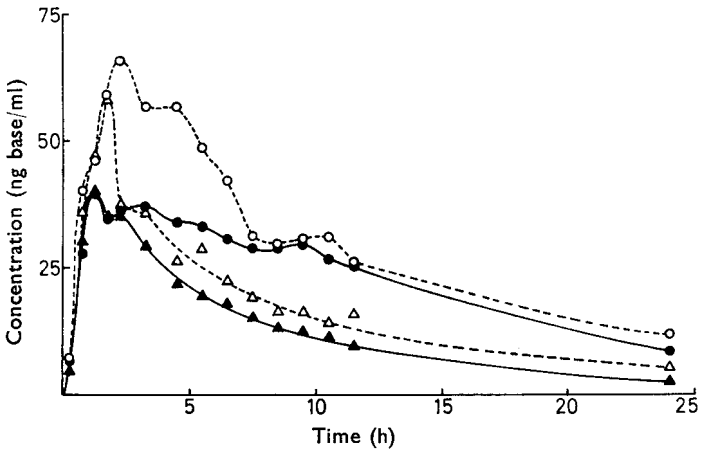

FIG. 7. Plasma and blood cell concentrations of [^{14}C]amphetamine after the administration of 15 mg (+)-[^{14}C]amphetamine sulphate under conditions of acid controlled and fluctuating urinary pH. Subject I. Controlled urinary pH: – – △ – – Blood cell.—▲— Plasma. Uncontrolled urinary pH: – – ○ – –Blood cell. —●— Plasma.

Table 4. *Amphetamine clearance from plasma. Subjects given an oral dose of* 15 mg (+)-[^{14}C]*amphetamine sulphate*

Condition of urine	Clearance of amphetamine from plasma (ml/min)	
	Subject I	Subject II
Acidic control	432–539	242–387
Fluctuating urinary pH	16–115	41–64

Glomerular filtration rate, (measured from creatinine clearance data) for subject I was 125, and for Subject II 126 ml/min.

The plasma clearance of amphetamine under acidic urine conditions was 400–550 ml/min whereas the glomerular filtration rate was only 125 ml/min (Table 4). The amphetamine clearance under uncontrolled conditions could be accounted for by glomerular filtration. The results indicate that about 75% of the drug must be transferred from the plasma into the tubules by routes other than glomerular filtration when the urine is acidic. Probably as urine flows down the kidney tubules, the drug passes from the blood to the urine because of the high concentration gradient of un-ionized drug across the lipid membrane.

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