J. Pharm. Pharmac., 1971, **23**, 837–841 *Received May* 18, 1971

Administration of two or more related drugs to investigate the effect of molecular modification and formulation on drug absorption, metabolism and excretion**

A. H. BECKETT AND L. G. BROOKES*

Department of Pharmacy, Chelsea College (University of London), Manresa Road, London, S.W.3, U.K.

Two or more related drugs of the amphetamine class were simultaneously administered to man under acidic urine control, their urinary excretion being examined by gas-liquid chromatography. This was shown to be a suitable procedure for determining the effect of molecular modification and formulation on drug absorption, metabolism and excretion.

Gas-liquid chromatography (g.l.c.) may be used to separate stimulant amines and others of closely related structure (Beckett, Tucker & Moffat, 1967). Many of these are excreted in the urine either substantially unchanged or as basic metabolites (Beckett & Rowland, 1965a,b; Beckett & Wilkinson, 1965; Beckett & Brookes, 1967; Beckett & Brookes, 1971; Beckett, Brookes & Shenoy, 1969; Beckett, Salmon & Mitchard, 1969). It should thus be possible to compare *under identical conditions* the rates of excretion of these compounds when they are administered simultaneously.

Under conditions of acidic urine to minimize kidney tubular re-absorption of bases, direct comparison of the effect of molecular modifications on drug absorption and excretion is facilitated, provided mutual interference of the drugs on these processes does not occur. The urinary excretion of some such amines (Ia–i) thus administered was investigated.

METHODS

Gas-liquid chromatography

The conditions were described by Beckett, Brookes & Shenoy (1969); identification of the drug and metabolite peaks from urine extracts was made by comparison with authentic samples and acetone, acetyl or propionyl derivatives.

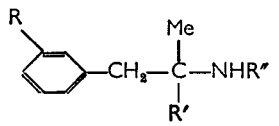
Dosage regimens

The subjects were healthy males (age 25–45) with maintained acidic urine. The drugs were administered orally in aqueous solution, alone and with one or more drugs of the same group; primary amines (Ia–d; dose = 10 mg base); *N*-methyl derivatives (Ie–g; dose = 10 mg base); *N*-ethyl derivatives (Ih, i; 20 mg HCl salt of each).

Phentermine and ethylamphetamine were administered together on separate occasions as formulated products (as the resin bonded drug in gelatin capsules or in

* Present address: Upjohn International, Crawley, Sussex.

** This work forms part of a thesis by L. G. Brookes, accepted for the degree of Ph.D. in the University of London, 1968.



I

	R	R′	R″	
(a)	H	H	H	amphetamine
(b)	CF_3	H	H	norfenfluramine
(c)	H	Me	H	phentermine
(d)	H(p-Cl)	Me	H	chlorphentermine
(e)	H	H	Me	methylamphetamine
(f)	CF_3	H	Me	methylnorfenfluramine
(g)	H	Me	Me	mephentermine
(h)	H	H	Et	ethylamphetamine
(i)	CF_3	H	Et	fenfluramine

sugar-coated tablets, respectively) with an aqueous solution of the HCl salt of the other drug. The methods for 24 h sample collection, analysis and maintenance of an acidic urine were as described by Beckett, Brookes & Shenoy (1969).

RESULTS AND DISCUSSION

The retention times on analysis of the compounds and their derivatives are given in Table 1. Nicotine had a retention time of 8·5 min in the system used, so its presence in smokers' urine did not interfere.

There was no significant difference in the total urinary excretion of the compounds administered in aqueous solution when given singly or with other drugs, indicating that the drugs did not interfere with absorption, metabolism, distribution and excretion of each other (see Table 2 for urinary excretion of compounds administered).

Since, under acidic conditions of urine, the plasma concentration and rate of urinary excretion of a basic drug are related (Beckett, Salmon & Mitchard, 1969), the kinetic analysis of the absorption and fate of such drugs in man (Beckett, Boyes & Tucker, 1968a, b; Beckett & Tucker, 1968) under comparable conditions is now possible. In addition, oral administration of marker drugs in solution may be used, under identical conditions, to study the effect of formulation on the bio-availability of other drugs.

Urinary excretion profiles of the drug mixtures administered in aqueous solution are given in Figs 1–2A. From these and Table 2, it may be concluded that chemical substitution in the amphetamine molecule results in the following: (a) halogen substitution causes a marked decrease in total urinary excretion of the drug over a given time period, i.e. more extensive metabolism, together with a delay of up to

Table 1. *Retention times of the compounds examined.*

Column	Stationary phase	Operating temp (°C)	Column length (metres)	Retention times (min)								
				A	MA	EA	P	MP	CP	NF	MNF	F
Chromosorb G acid-washed DMCS treated 80–100 mesh	10% KOH 10% Apiezon L	160	1	2·6	3·6	4·4	3·2	5·2	9·0	1·9	2·4	3·0
				Acetone derivative								
				4·5						2·9		
Chromosorb G acid-washed DMCS treated 80–100 mesh	5%KOH 2% Carbowax 20 M.	165	2									
				Acetyl derivative								
				7·2	5·2	5·0	4·4	3·6	14·6	5·7	4·2	3·3
				Propionyl derivative*								
				20·1	14·6	14·0	12·3	10·1	41·2	15·8	11·8	9·0

* Operating temperature 140°

A=amphetamine; MA=methylamphetamine; EA=ethylamphetamine; P=phentermine; MP=mephentermine: CP=chlorphentermine; NF=norfenfluramine; MNF=methylnorfenfluramine; F=fenfluramine.

Table 2. *The 24 h urinary excretion of phentermine, amphetamine chlorphentermine and norfenfluramine, and of the* N-*ethyl or* N-*methyl derivatives of some of those compounds after oral doses of solutions of the drugs, with maintained acidic urine.*

		% Drug excreted (including any basic metabolite)			
		Following administration of drug mixtures		Following administration of single drug**	
Drug group	Compound	Range of values	Average*	Average	*
Primary amines	Phentermine	71·7–83·6	76·4 (5)	71·8	(6)
	Phentermine-formulated product	43·3, 50·0	46·7 (2)		
	(+)-Amphetamine	49·2–68·4	60·8 (8)	62·7	(9)
	Chlorphentermine	30·0–39·2	35·0 (4)	35·5	(5)
	(±)-Norfenfluramine	22·4–35·5	28·8 (4)	29·3	(6)
Secondary amines (*N*-methyl)	Mephentermine	68·0–72·2	70·0 (4)	71·4	(2)
	(+)-Methylamphetamine	52·5–58·0	55·4 (4)	60·0	(7)
	(+)-Methylnorfenfluramine	28·9–38·6	34·2 (4)	36·4	(2)
Secondary amines (*N*-ethyl)	(+)-Ethylamphetamine			37·7	(5)
	(±)-Ethylamphetamine	45·5–61·3	53·8 (5)	52·6	(4)
	(−)-Ethylamphetamine			78·9	(3)
	(±)-Ethylamphetamine — formulated product	30·2, 37·2	33·7 (2)		
	(±)-Fenfluramine	29·2–40·1	33·5 (8)	32·4	(6)

* Value in parentheses refers to number of subjects for each trial.

** Date reported by Beckett & Rowland (1965a, b), Beckett & Brookes (1967, 1971), Beckett & others, (1969) and Brookes (1968).

5 h in the peak excretion and in the initial appearance of the drug in the urine (cf. also observed for *p*-chloroamphetamine; Beckett, Mitchard & Salmon, unpublished results), and (b) substitution by a second methyl group on the α-carbon atom causes a significant increase in total excretion, i.e. reduces metabolism, possibly due to additional steric hindrance reducing deamination, but has no influence on the rate of absorption.

The effects of side-chain substitution or ring halogenation (or both) on *N*-alkylamphetamines are similar to those occurring for amphetamine. However, *N*-alkylation, while not influencing the rate of absorption, reduces the amount of drug excreted unchanged, i.e. increases metabolism, *N*-ethylation being more effective than *N*-methylation in this respect. These results are in agreement with those predicted from the buccal absorption test (Beckett & Triggs, 1967; Brookes, 1968), although *N*-alkylation has little effect on the urinary excretion of norfenfluramine, the elimination profile of which, being analogous to that of fenfluramine (Fig. 2B), shows a two-phase exponential decline. The initial phase for the first 8–10 h parallels that of the non-halogenated molecule, ethylamphetamine, but the second phase is of significantly longer half-life (Fig. 2B), indicating slow drug release from an undetermined body compartment. A similar two-phase elimination profile for *p*-chloroamphetamine has recently been obtained (Beckett & others, unpublished results). Also, Jun & Triggs (1970) noted, from blood level studies, that chlorphentermine undergoes multi-compartment distribution.

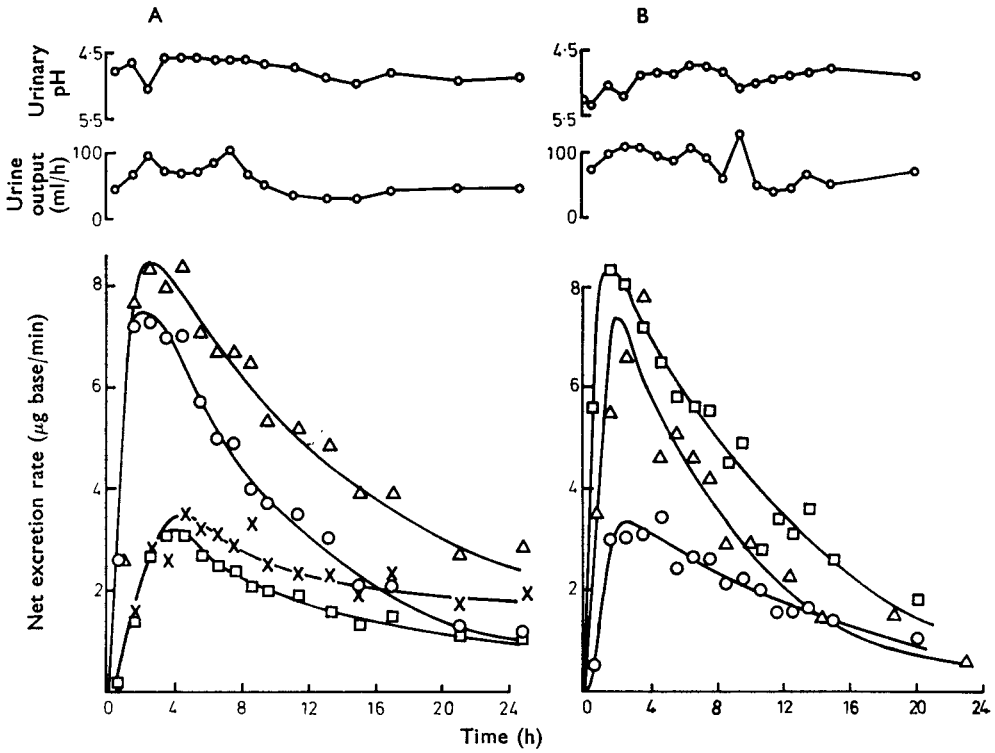


FIG. 1. A. Urinary excretion of amphetamine, phentermine, norfenfluramine and chlorphentermine with corresponding urinary pH and urine output after the simultaneous oral administration (10 mg base of each) of (+) amphetamine (as sulphate), and phentermine, (±)-norfenfluramine and chlorphentermine (as hydrochlorides) to a subject under acidic urine control. —△— Phentermine. —○— Amphetamine. —X— Chlorphentermine. —□— Norfenfluramine.

B. Urinary excretion of mephentermine, methylnorfenfluramine and methylamphetamine, with corresponding urinary pH and urine output, following the simultaneous oral administration (10 mg base of each) of mephentermine sulphate, (±)-methylnorfenfluramine and (+)-methylamphetamine hydrochlorides in aqueous solution to a subject under acidic urine control. —□— Mephentermine. —△— Methylamphetamine. —○— Methylnorfenfluramine.

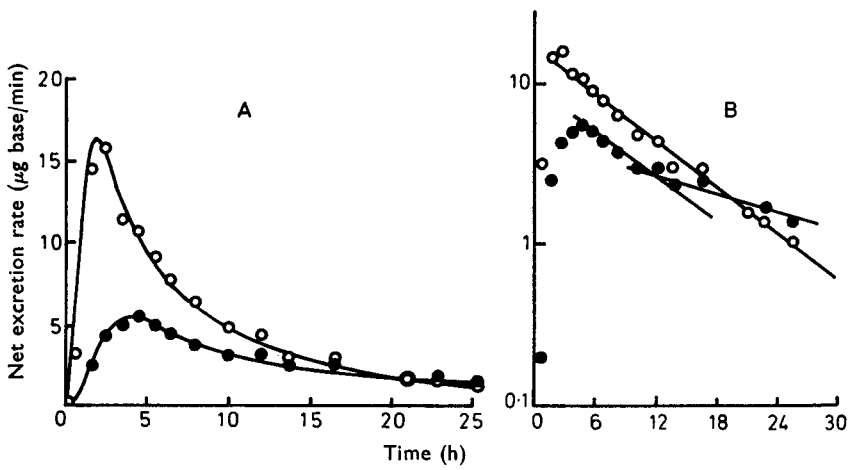


FIG. 2. Urinary excretion of fenfluramine and ethylamphetamine from a subject under acidic urine control, after the simultaneous oral administration of 20 mg each of (±)-fenfluramine (—●—) and (±)-ethylamphetamine (—○—) hydrochlorides in aqueous solution.

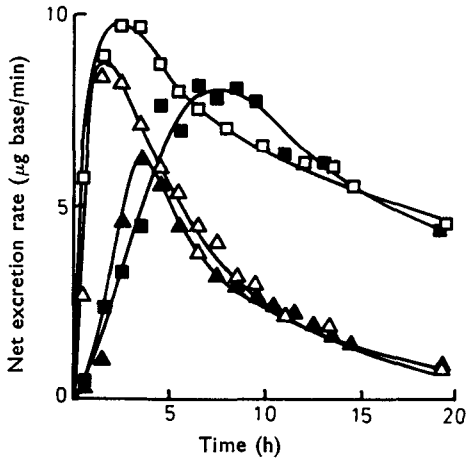


FIG. 3. Urinary excretion of ethylamphetamine and phentermine from a subject under acidic urine control following the oral co-administration of 12 mg (±) ethylamphetamine hydrochloride and 15 mg phentermine in either aqueous solution or formulated product. Dosage regimens: (1) Ethylamphetamine HCl in solution + phentermine capsule. (2) Phentermine in solution + ethylamphetamine HCl tablet. Solutions: —△— ethylamphetamine; —□— phentermine. Formulated products: —▲— ethylamphetamine (sugar-coated tablet); —■— phentermine (hard gelatin capsule containing phentermine-resin complex).

The use of a "marker drug" to illustrate the effects of formulation on the urinary excretion profiles of ethylamphetamine and phentermine is shown in Fig. 3. Resin-bonding with subsequent encapsulation of phentermine, and sugar-coated tableting of ethylamphetamine, delay the time of maximum excretion, and significantly reduce the rate and total amount of drug absorption from the gut, possibly due to the reduced bio-availability of drug caused by incomplete *in vivo* disintegration of the formulated preparations.

Acknowledgements

We are indebted to our colleagues who acted as subjects for this investigation. Companies who provided gifts of the compounds used have been acknowledged previously (Beckett & others, 1967).

REFERENCES

BECKETT, A. H., BOYES, R. N. & TUCKER, G. T. (1968a). *J. Pharm. Pharmac.*, **20**, 269–276.
BECKETT, A. H., BOYES, R. N. & TUCKER, G. T. (1968b). *Ibid.*, **20**, 277–282.
BECKETT, A. H. & BROOKES, L. G. (1967). *Ibid.*, **19**, 42S–45S.
BECKETT, A. H. & BROOKES, L. G. (1971). *Ibid.*, **23**, 288–294.
BECKETT, A. H., BROOKES, L. G. & SHENOY, E. V. B. (1969). *Ibid.*, **21**, 151S–156S.
BECKETT, A. H. & ROWLAND, M. (1965a). *Ibid.*, **17**, 628–639.
BECKETT, A. H. & ROWLAND, M. (1965b). *Ibid.*, **17**, 109S–114S.
BECKETT, A. H., SALMON, J. A. & MITCHARD, M. (1969). *Ibid.*, **21**, 251–258.
BECKETT, A. H. & TUCKER, G. T. (1968). *Ibid.*, **20**, 174–193.
BECKETT, A. H., TUCKER, G. T. & MOFFAT, A. C. (1967). *Ibid.*, **19**, 273–294.
BECKETT, A. H. & TRIGGS, E. J. (1967). *Ibid.*, **19**, 31S–41S.
BECKETT, A. H. & WILKINSON, G. R. (1965). *Ibid.*, **17**, 107S–108S.
BROOKES, L. G. (1968). Ph.D. Thesis (London).
JUN, H. W. & TRIGGS, E. J. (1970). *J. Pharm. Sci.*, **59**, 306–309.