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The influence of replacement of the *N*-ethyl group by the cyanoethyl group on the absorption, distribution and metabolism of (±)-ethylamphetamine in man

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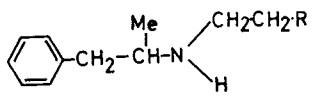
N-2-Cyanoethylamphetamine in man was metabolized rapidly and extensively, as indicated by the analysis of the drug and its metabolite in urine, under acidic and normal conditions. A comparison with the excretion and metabolism of ethylamphetamine under comparable conditions was made. The results were assessed using an analogue computer; a simple one compartmental model sufficed to describe the excretion and metabolism of ethylamphetamine and amphetamine but the additional programming of free amphetamine was necessary for cyanoethylamphetamine because of its very rapid metabolism to amphetamine.

Fenproporex (*N*-2-cyanoethylamphetamine, I; R = CN), claimed by the manufacturers as a non-stimulant anorectic agent, is structurally related to *N*-ethylamphetamine (I; R = H), which has stimulant properties in part attributable to its metabolism to amphetamine in man. The claim apparently implies that the replacement of the hydrogen in the ethyl chain of ethylamphetamine by the cyano-group has virtually eliminated *N*-dealkylation; experimental evidence on this point is lacking.

The metabolism and urinary excretion of *N*-2-cyanoethylamphetamine in man has been investigated and compared with results for *N*-ethylamphetamine already reported (Beckett, Brookes & Shenoy, 1969). The findings have been assessed pharmacokinetically by employing suitable biological models with the aid of an analogue computer.



I

MATERIALS AND METHODS

Analytical procedures

The urine samples were extracted (Beckett & Rowland, 1965a) and analysed by gas-liquid chromatography (g.l.c.) and thin-layer chromatography (t.l.c.) (Beckett & others, 1969).

Gas-liquid chromatography

The chromatographic columns used were those described by Beckett & others (1969); the operating conditions were as described for ethylamphetamine but the nitrogen flow rate was 35 ml/min and the oven temperature was 150°.

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Aletamine hydrochloride (1 ml; 10 μg/ml), used as internal standard, and NaOH (0·5 ml) were added to urine (5 ml) and the solution extracted with ether (6 × 2·5 ml). Calibration curves for *N*-2-cyanoethylamphetamine and amphetamine were constructed over the range 0·2–10 μg base/ml in urine and distilled water. Nicotine interfered with the assay procedures and hence the subjects were asked not to smoke during the experiment. Some of the ether extracts of urine were treated with acetone and acetic anhydride and examined by g.l.c. as described by Beckett & others (1969).

Thin-layer chromatography

Glass plates (20 × 20 cm) coated with a layer of 0·25 mm of silica gel G (Merck) activated at 105° for 1 h, were spotted with ethereal extracts described above, together with authentic samples of *N*-2-cyanoethylamphetamine and amphetamine in ether, and the chromatograms were developed at room temperature (24°) using solvent systems of Beckett & others (1969). Dragendorff's reagent (Stahl, 1962) and a solution of bromothymol blue in ethanol were used to visualize the spots.

Stability of N-2-*cyanoethylamphetamine*

(1) *N*-2-Cyanoethylamphetamine (10 μg/ml) was added to urine of pH 5·0 and 7·2 and stored at 4°. The drug content of both samples was determined every third day for two weeks. Amphetamine was stable under similar conditions (Beckett & others, 1969).

(2) Urine (5 ml) containing *N*-2-cyanoethylamphetamine (10 μg/ml) was adjusted to pH 12 (0·5 ml 20% NaOH) or pH 2 (0·2 ml 6 N HCl) and shaken on a mechanical shaker. After 2 h the samples were analysed for drug content.

(3) Buffer solutions simulating the pH of the stomach (pH 1·2–1·7) using Clark and Lub's potassium chloride–HCl buffer, duodenum (pH 4·7–6·5) and ileum (pH 6·1–7·3) using McIlvaine citric acid-phosphate buffer were prepared (Documenta Geigy, 1962) *N*-2-cyanoethylamphetamine (10 mg) in buffer solutions (25 ml) were incubated in flasks at 37·5° on a shaking water bath (80 oscillations/min) and samples (1 ml) withdrawn at 1, 2 and 4 h intervals were analysed for drug content.

Urinary excretion trials

Three healthy male subjects (age 25–45) were given 20 mg of (±)-*N*-2-cyanoethylamphetamine hydrochloride (equivalent to 16·71 mg of the base) in aqueous solution by mouth. The pH of the urine was maintained acidic (4·8 ± 0·2) by the administration of ammonium chloride (Beckett & Brookes, 1967). Two subjects were given the same dose of the drug but under conditions of fluctuating urinary pH.

The urine samples were collected at hourly intervals during the first 10–12 h and then at longer intervals up to 24 to 48 h. The pH and volume of each urine sample were measured at room temperature. All samples were stored at 4° until analysis had been completed.

The subjects who participated in these trials had previously been given oral doses of 20 mg of (±)-ethylamphetamine hydrochloride (see Beckett & others, 1969) and 13·63 mg of (+)-amphetamine sulphate (equivalent to 16·34 and 10·00 mg of the base respectively) in solution under conditions of acidic urinary pH, and two subjects 20 mg of (±)-ethylamphetamine hydrochloride under conditions of fluctuating urinary pH.

Pharmacokinetic studies

The general assumptions made by Beckett & Tucker (1968) when designing the

biological models to simulate the pharmacokinetics of amphetamine (Model I)* and methylamphetamine (Model II)* were adopted during the present investigations with amphetamine and ethylamphetamine. However, some modifications were necessary when cyanoethylamphetamine was considered.

Preliminary studies indicate that cyanoethylamphetamine was rapidly and extensively metabolized to amphetamine. The biological model proposed to study the pharmacokinetic parameters of ethylamphetamine (Model II of Beckett & Tucker) would not allow for such rapid and extensive biotransformation. Cyanoethylamphetamine was thought to be broken down on its first passage through the liver as was indicated for pentazocine (Beckett, Kourounakis & others, 1970) and acetylsalicylic acid (Harris & Riegelman, 1969). A second body compartment was introduced into the biological model to allow for the first pass phenomenon (Model III). The second compartment was assumed to include the liver and consequently the metabolic process was programmed from the second compartment. This model is similar to that discussed by Gibaldi & Feldman (1969) (see appendix).

The biological Model IV was also considered; this model required that a free dose of amphetamine was available for absorption at zero time. The distribution and elimination of the amphetamine should be the same as when amphetamine itself is given. Model IV does not specify how the amphetamine initially available is produced; it may be formed by a metabolic transformation on a first passage through the liver or by enzymic breakdown in the gastrointestinal tract, possibly by gut flora.

Computer simulations

A PACE TR20R (Electronic Associates Ltd.) analogue computer was used together with an X-Y recorder (Bryans Ltd.) and a digital voltmeter (Roband Ltd.). The appropriate pharmacokinetic model to describe absorption, distribution, metabolism and excretion of the drug was programmed. The experimental urinary excretion data of the unchanged drug and where relevant, of the metabolite were plotted on the X-Y recorder both as cumulative amounts and rates of excretion. The settings of the rate constant potentiometers were systematically varied in an attempt to fit the computed curve to the experimental data points. When the best agreement was obtained, the settings of the rate constant potentiometers were read from the digital voltmeter. The suitability of the biological model employed was judged on the basis of the fit obtained to the experimental data.

The pharmacokinetic parameters established for absorption, distribution and elimination of amphetamine in an individual were set on the appropriate potentiometers to govern the same processes of the metabolite formed from ethyl or cyanoethylamphetamine.

RESULTS AND DISCUSSION

Linear calibration curves were obtained for *N*-2-cyanoethylamphetamine and amphetamine in urine over the range stated; these curves were identical with those obtained for these drugs in distilled water. No substance interfering with the g.l.c. analysis was found in the urine.

Stability of N-2-*cyanoethylamphetamine*

N-2-Cyanoethylamphetamine was stable in urine of pH 5·0 and 7·2 at least for two

* The description and equations for these models are given by Beckett & Tucker (1968).

weeks at 4° and at least for 2 h at pH 2 and 12; it was recovered quantitatively from various buffer solutions after incubation at 37·5°. Therefore cyanoethylamphetamine will be chemically stable in the gastrointestinal tract and in the extraction procedure.

Structure of the metabolite from N-2-*cyanoethylamphetamine*

The identity of the unchanged drug and metabolite amphetamine from metabolic studies was shown by the identical retention times of the compounds and of their derivatives and of authentic samples. These were (min): column 1—amphetamine 2·2, cyanoethylamphetamine 24·3, amphetamine + acetone 4·2, amphetamine-acetyl derivative 10·2, cyanoethylamphetamine-acetyl derivative 114·0, aletamine 7·0; column 2—amphetamine acetyl derivative 7·2. The columns 1 and 2 have been described by Beckett & others (1969).

Identity of R_F values of the drug (and the metabolite amphetamine) with authentic samples was demonstrated in systems (a) 0·71 (0·54), (b) 0·85 (0·35) and (c) 0·73 (0·29) (see Beckett & others, 1969).

Urinary excretion trials

Acidic urine control. After an oral dose of cyanoethylamphetamine, peak excretion rates of the unchanged drug were reached within 1–2 h (Fig. 1) only 5–9% of the dose was excreted unchanged in urine while 34–56% was excreted as amphetamine (Table 1). The unchanged drug had a biological half life of about 2 h and was detected in the urine only up to 14 h. The peak excretion rates of metabolite amphetamine were reached within 1–4 h (Fig. 1) and the profile and half life were comparable to those when the metabolite itself was administered, suggesting rapid and extensive biotransformation of cyanoethylamphetamine in the gut or in the liver during the first pass.

Under comparable conditions, 45% of the dose of ethylamphetamine with a biological half life of about 5 h was excreted unchanged and only 10% of the dose was

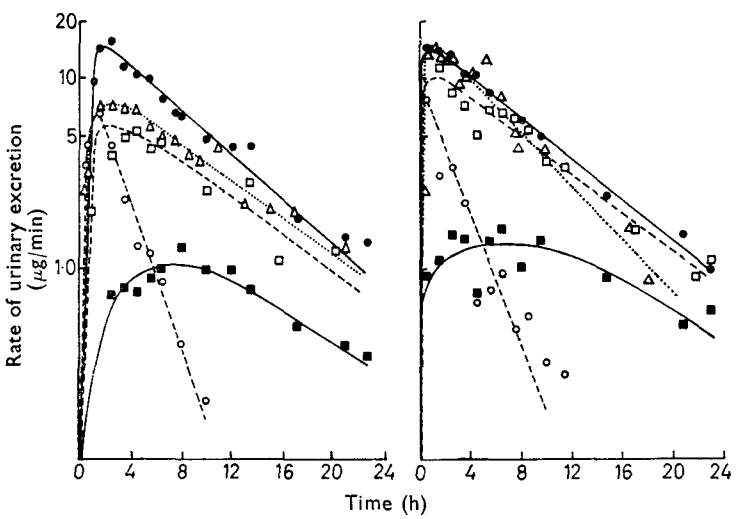

FIG. 1. Comparison of the urinary excretion of (a) amphetamine (△ . . . △) after the oral administration of 13·63 mg (+)-amphetamine sulphate, (b) ethylamphetamine (●——●) and metabolite amphetamine (■——■) after the oral administration of 20 mg (±)-ethylamphetamine HCl and (c) cyanoethylamphetamine (○ - - - ○) and metabolite amphetamine (□ - - - □) after the oral administration of 20 mg (±)-*N*-2-cyanoethylamphetamine HCl under acidic urine control. Subjects 1 and 2.

Table 1. *Comparative urinary excretion of the parent drug and metabolite amphetamine (A) over a period of* 24 *h in subjects receiving an oral dose of (a)* (±)-N-2-*cyanoethylamphetamine (CEA) and (b)* (±)-N-*ethylamphetamine (EA) as hydrochlorides under acidic and normal urinary pH.* Dose CEA—20 mg HCl ≡ 16·71 mg of base; EA—20 mg HCl ≡ 16·34 mg of base.

	Cyanoethylamphetamine % Dose excreted with				Ethylamphetamine** % Dose excreted with			
	Acid urine		*Normal urine		Acid urine		Normal urine	
Subject	CEA	A	CEA	A	EA	A	EA	A
2	8·0	47·8	about 3·0	47·4	45·9	12·7	24·2	10·4
1	8·7	34·2	—	—	45·5	7·3	12·6	4·4
3	5·4	55·9	—	—	42·3	8·4	—	—
4	—	—	about 3·0	29·3	—	—	—	—

* 48 h urine.
** Data from Beckett, Brookes & Shenoy (1969).

excreted as amphetamine, the peak excretion of which was at about 6–8 h (Fig. 1, Table 1). The slope of the curve of the metabolite amphetamine from peak rate of excretion to about 15 h is less than that for amphetamine given orally indicating that metabolism of ethylamphetamine to amphetamine was slower than that of cyanoethylamphetamine and that amphetamine was being produced from the former for at least 15 h.

The results demonstrate that the introduction of a CN group into the ethyl group of ethylamphetamine leads to rapid and extensive metabolic *N*-dealkylation of the compound. The peak excretion rate of amphetamine after a dose of 20 mg of cyanoethylamphetamine approached that reached after oral doses of 13·63 mg of amphetamine sulphate in solution; a direct relation between the rate of excretion and blood concentrations of amphetamine under conditions of acidic urine is known to exist (Beckett, Salmon & Mitchard, 1969).

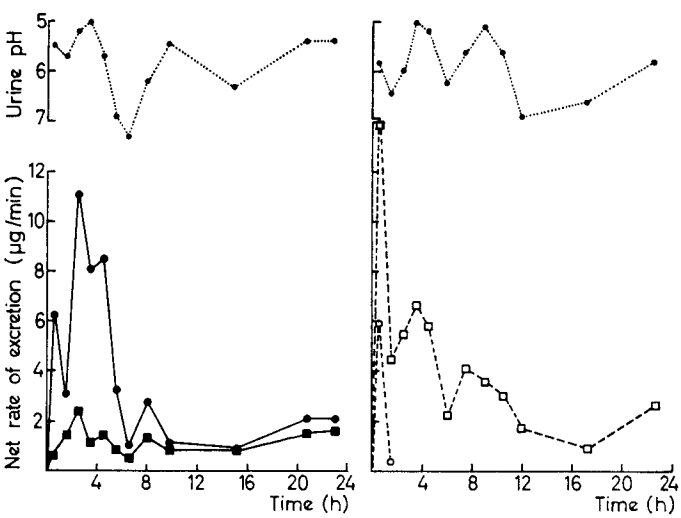

FIG. 2. Comparison of the urinary excretion of (a) ethylamphetamine (●——●) and metabolite amphetamine (■——■) after the oral administration of 20 mg (±)-ethylamphetamine HCl and (b) cyanoethylamphetamine (○ - - - ○) and metabolite amphetamine (□ - - - □) after the oral administration of 20 mg (±)-*N*-2-cyanoethylamphetamine HCl under uncontrolled urinary pH.

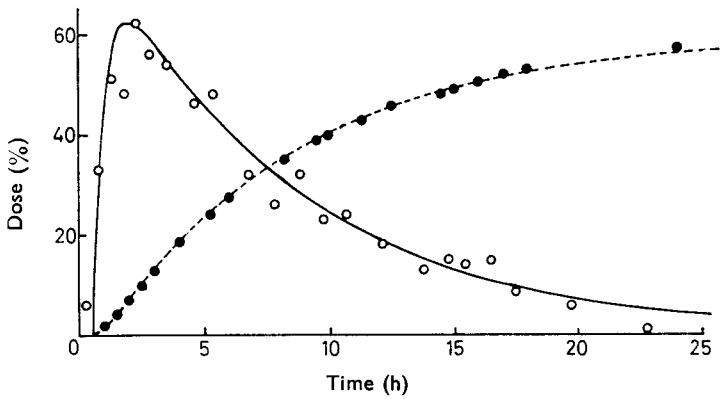

FIG. 3. Computer curves and experimental data points for the urinary excretion of (+)-amphetamine after oral administration of 13·63 mg of (+)-amphetamine sulphate under conditions of acidic urine (subject 1). ● Experimental points for cumulative urinary excretion (% dose). ○ Experimental points for dU/dt × 10 (% dose/h).

Uncontrolled urinary pH. Cyanoethylamphetamine was detected in the urine in measurable amounts only during the first 2 h after the dose; less than 3% was recovered unchanged but the metabolite amphetamine continued to be excreted even after 48 h (Fig. 2; Table 1). Under comparable conditions after an oral dose of ethylamphetamine both unchanged drug and metabolite amphetamine were excreted up to 24 h, the levels of unchanged drug being always higher than that of the metabolite. The results indicate that the metabolism of cyanoethylamphetamine to amphetamine is virtually complete within 4 h, the small amounts of unchanged drug present thereafter being completely reabsorbed since at normal pH values of the urine (pH 5·5–7·0) more of this drug (pKa 7·16) would be present in the lipid soluble unionized form than was ethylamphetamine (pKa 10·18).

Pharmacokinetic studies

Good agreement between computed curves and experimental data points was obtained for amphetamine (Fig. 3). The rate constants governing the biological processes (Table 2) compare favourably with those previously reported (Beckett & Tucker, 1966, 1968). Also, the computed curves satisfactorily fitted the experimental data points for the excretion of unchanged ethylamphetamine and its metabolite, amphetamine (Fig. 4); the pharmacokinetic parameters (Table 2) were

Table 2. *Pharmacokinetic parameters for amphetamine, ethylamphetamine and cyanoethylamphetamine*

Drug rate constants (h^{-1})	Amphetamine subject		Ethylamphetamine subject		Cyanoethylamphetamine subject	
	1	2	1	2	1	2
k_a	1·9750	2·9680	1·7480	6·3160	2·9700	2·1640
k_e	0·0752	0·1017	0·0667	0·0655	0·0319	0·0445
k_{m1}	0·0519	0·0434	0·0783	0·0743	0·1202	0·1007
k_{m2}			0·0179	0·0203	0·1434	0·2448
k_{mm}			0·0519	0·0434	0·0519	0·0434
k_{me}			0·0752	0·1017	0·0752	0·1017
k_{ma}					1·9750	2·9690
Free dose of amphetamine ..	—	—	—	—	25%	30%

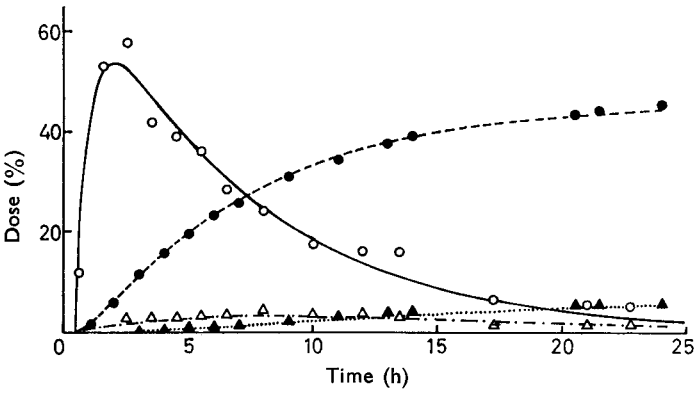

FIG. 4. Computer curves and experimental data points for the urinary excretion of ethylamphetamine and amphetamine after oral administration of 20 mg of (±)-ethylamphetamine hydrochloride under conditions of acidic urine (subject 1). ● Experimental points for cumulative urinary excretion of ethylamphetamine (% dose). ○ Experimental points for dU/dt × 10 of ethylamphetamine (% dose/h). ▲ Experimental points for cumulative urinary excretion of amphetamine (% dose). △ Experimental points for $dM_2U/dt \times 10$ of amphetamine (% dose/h).

comparable with those of methylamphetamine (Beckett & Tucker, 1968). However, the results indicated that de-ethylation was faster than demethylation and, as expected, the rates of absorption and excretion were slightly higher for ethylamphetamine.

The two compartment biological model (Model III) proposed to study the pharmacokinetics of cyanoethylamphetamine did not satisfactorily simulate the biological processes. However, using Model IV a good correlation between experimental and computed data of cyanoethylamphetamine was obtained if approximately 25% of the administered dose was considered as free amphetamine (Fig. 5). The rate of metabolism of the remaining cyanoethylamphetamine was markedly higher than for ethyl amphetamine (Table 2).

It is, at present, uncertain where or how the metabolite of cyanoethylamphetamine is derived; therefore it is an advantage that the origin of the amphetamine is not included in the design of Model IV. The pharmacokinetic studies emphasise the importance of the metabolism of cyanoethylamphetamine to amphetamine.

The rapid and extensive metabolism of cyanoethylamphetamine results in at least 70% of the dose being present in the body as free amphetamine within 3–4 h. The

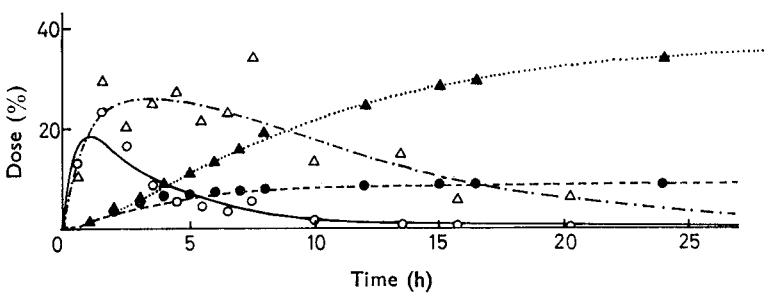

FIG. 5. Computer curves and experimental data points for the urinary excretion of cyanoethylamphetamine and amphetamine after oral administration of 20 mg of (±)-cyanoethylamphetamine hydrochloride under conditions of acidic urine (Subject 1). ● Experimental data points for cumulative urinary excretion of cyanoethylamphetamine (% dose). ○ Experimental data points for dU/dt × 10 of cyanoethylamphetamine (% dose/h). ▲ Experimental data points for cumulative urinary excretion of amphetamine (% dose). △ Experimental data points for $dM_2U/dt \times 10$ of amphetamine (% dose/h).

"apparent" lack of side effects in the clinical trials reported by Riviere (1968) is therefore difficult to explain.

Acknowledgement

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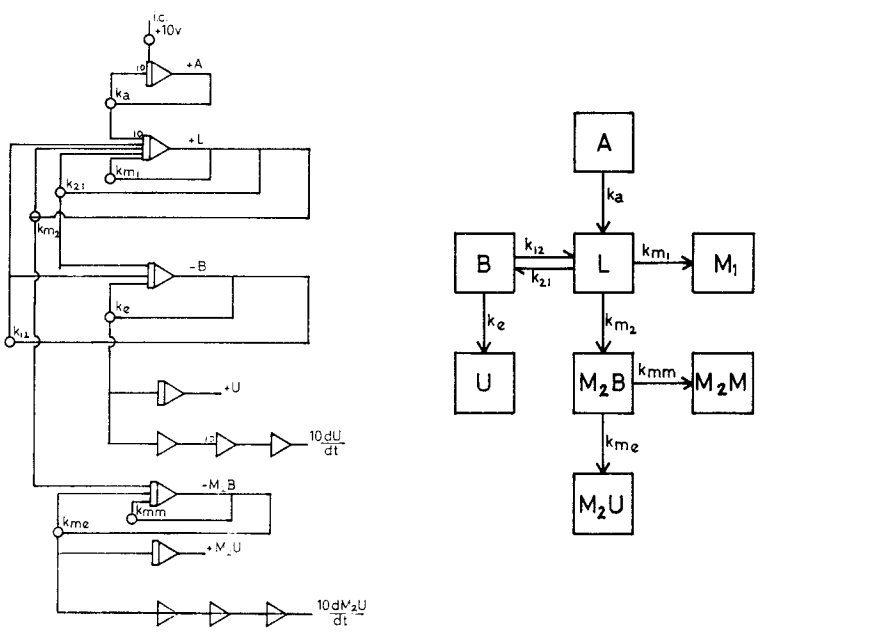
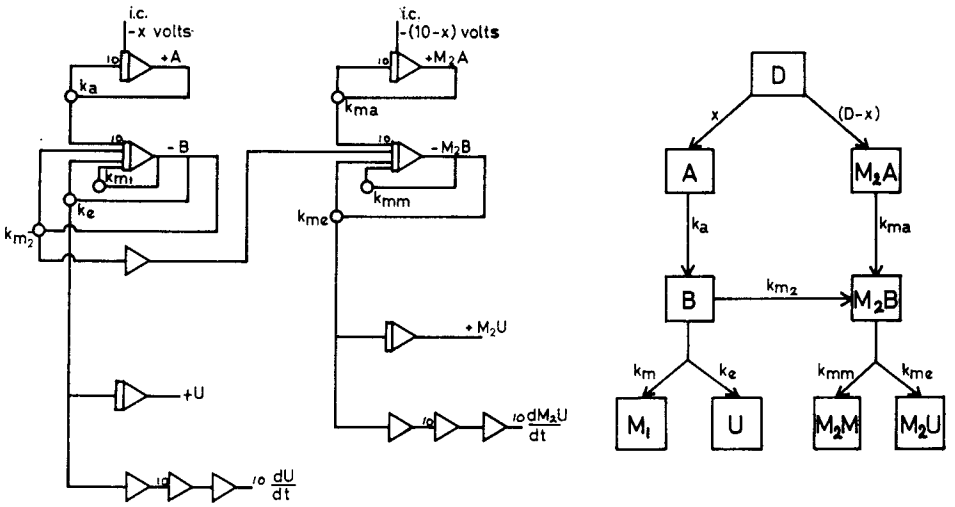
APPENDIX I



Model III.* Biological model for studying the pharmacokinetics of *N*-2-cyanoethylamphetamine and analogue computer program. 1 s of machine time equals 1 h of real time.

* For Models I and II, see Beckett & Tucker (1968).



Model IV. Biological model for studying the pharmacokinetics of cyanoethylamphetamine (x = proportion of the dose which enters the body as unchanged cyanoethylamphetamine) and analogue computer program for Model IV. 1 s of machine time equals 1 h of real time. 10 V = 100% dose. $10x \times V$ = % dose absorbed as unchanged cyanoethylamphetamine. $10(1-x)$ V = % dose absorbed as free amphetamine.

t	time in h after ingestion of dose
Lag time	the time interval between ingestion of dose and zero time
Zero time	time at which loss of drug from the gastrointestinal tract may be described as a first order process
D	dose of drug administered
A	amount of unchanged drug available for absorption
B	amount of unchanged drug in the body
U	amount of unchanged drug excreted in the urine
M_1	amount of unspecified metabolites formed
M_2B	amount of metabolite, amphetamine, in the body
M_2U	amount of metabolite, amphetamine, excreted in the urine
M_2M	amount of unspecified metabolites formed from the major metabolite, amphetamine
M_2A	amount of "metabolite", amphetamine, available for absorption
k_a	rate constant for the absorption of drug from the gastrointestinal tract
k_e	rate constant for the excretion of unchanged drug
k_{m1}	rate constant for an unspecified metabolic route
k_{m2}	rate constant for the formation of amphetamine from the parent drug in the body
k_{me}	rate constant for the excretion of metabolite, amphetamine
k_{mm}	rate constant for the formation of unspecified secondary metabolites from the primary metabolite, amphetamine
k_{ma}	rate constant for the absorption of "metabolite", amphetamine, from the gastro intestinal tract

Differential equations to describe model III are $dA/dt = -ka.A$; $dM_1/dt = km_1.L$; $dM_2B/dt = km_2.L - kmm.M_2B - kme\ M_2B$; $dL/dt = ka.A - k_{21}.L + k_{12}.B - km_1L - km_2L$; $dU/dt = ke.B$; $dB/dt = k_{21}.L - k_{12}.B - ke.B$; $dM_2M/dt = kmm.M_2B$; $dM_2U/dt = kme.M_2B$.

Differential equations to describe model IV are $dA/dt = -ka.A$; $dM_1/dt = km_1.B$; $dU/dt = ke.B$; $dB/dt = ka.A - km_1.B - km_2.B - ke.B$; $dM_2A/dt = -kma.M_2A$; $dM_2M/dt = kmm.M_2B$; $dM_2U/dt = kme.M_2B$; $dM_2B/dt = kma.M_2A + km_2B - kmm\ M_2B - kme\ M_2B$.