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Application of the analogue computer to pharmacokinetic and biopharmaceutical studies with amphetamine-type compounds

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The kinetics of absorption, metabolism, and excretion of (+)-amphetamine and (+)-methylamphetamine, after oral administration of "free" dosage forms to man, under controlled acidic urine conditions, have been examined using an electronic analogue computer. This device has also been used to determine the *in vivo* rate of release of the drugs from hard gelatin capsule dosage forms and prolonged-release preparations. *In vivo* drug release from the prolonged-release preparations was correlated with *in vitro* drug release data.

FOR many drugs, urinary excretion studies offer the most practical method of evaluating the *in vivo* absorption of the drug from its dosage form. Ideally, conditions are required which give maximal excretion of unchanged drug and which allow smooth curves to be drawn through urinary excretion rate versus time data points. The finding that the urinary excretion of many drugs is pH dependent and in some instances urine-volume dependent (Milne, Scribner & Crawford, 1958; Peters, 1960; Weiner & Mudge, 1964; Braun, Hesse & Malorry, 1963; Beckett & Rowland, 1965a) has important implications in this context. Since urinary pH varies between subjects and throughout the day (Elliot, Sharp & Lewis, 1959; and others), the rate of excretion of many drugs which are partially ionized over the normal range of urinary pH (4·5 to 8·0), will vary accordingly.

Maintenance of a constant acidic urinary pH with a basic drug, such as amphetamine, has therefore been advocated (Beckett & Tucker, 1966) for the *in vivo* evaluation of dosage forms. Then, the selective and passive reabsorption of the unionized drug species from the kidney tubules is minimized and meaningful results and comparisons are obtained.

The present paper is concerned with the pharmacokinetic interpretation of urinary excretion data for amphetamine and methylamphetamine after their administration to man, in various oral dosage forms, under controlled acidic urinary pH conditions. Specifically, the objective was to evaluate the *in vivo* release rates of drugs from prolonged-release formulations and to compare them with *in vitro* release rates. An analogue computer greatly facilitated the calculations.

THEORETICAL

The fundamentals and philosophy of the use of electronic analogue computers in pharmacokinetics have been discussed by Garrett & Alway

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(1963). Analogue computers, which use voltages to represent the variables, have been employed in pharmacokinetics either to plot the dependent variable as a continuous function of time when programmed on the basis of a specific equation (Taylor & Wiegand, 1962) or more commonly to build compartmental models by curve-fitting procedures (eg. Garrett, Johnston & Collins, 1963).

The following assumptions are made when using simple compartmental models to investigate the kinetics of the absorption and elimination of amphetamine and methylamphetamine in various dosage forms, under acidic urine conditions. (i) The rate of urinary excretion of the drug is proportional to its concentration in the plasma, which in turn is proportional to the total amount in the body, excluding the gut and metabolite compartments. (ii) Drug transfer from one compartment to another is irreversible. (iii) Transfer rate of drug from one compartment to another is directly proportional to the amount of drug in that compartment, i.e. drug release, absorption, metabolism, and excretion are apparent first-order processes with rate constants having units of reciprocal time. (iv) Compartments are uniform and homogeneous throughout the transfer processes. (v) The release of drug from dosage forms is the rate-determining step in drug absorption. (vi) There is no decomposition of the drug at the absorption site, no enterohepatic or salivary cycling, or diffusion of the drug from the blood into the stomach. (vii) The rate constant for drug absorption is unchanged along the intestinal tract. (viii) The drug is ultimately completely available for absorption, and is 100% absorbed. (ix) Excretion of unchanged drug by pathways other than via the kidney is negligible. (x) Absorption and elimination rate constants are independent of dosage form, as also are distribution processes.

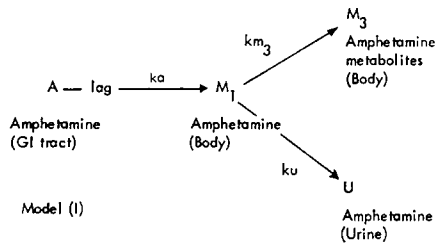
The validity of some of these assumptions will be discussed in relation to correlations observed of experimental results and computer simulations.

Although the absolute significance of kinetic data obtained under forced extremes of urinary pH is questionable, particularly as it is not known whether such conditions affect the distribution, and binding of the drug, such data is of value if used in a comparative sense, especially if the comparative performance of different drug formulations is being considered. The further assumption is made in these studies of drug formulations that the use of ammonium chloride to acidify the urine does not influence release of drug from the dosage forms.

PHARMACOKINETIC MODELS

Model (I): applicable to "free" forms of amphetamine.

Pharmacokinetic model (I) is proposed to describe the kinetics of absorption, metabolism, and excretion of (+)-amphetamine in man after oral administration of "solution" or "free pellet" forms of the drug (see Experimental for description of dosage forms) under controlled acidic-urine conditions.



Based upon this model, the following rate equations may be written (all symbols are defined in Appendix 1):

Post-lag time:

$$\frac{dA}{dt} = -\,ka.A \quad .. \quad .. \quad .. \quad .. \quad 1$$

$$\frac{dM_1}{dt} = ka.A - ku.M_1 - km_3.M_1 \quad .. \quad .. \quad 2a$$

$$= ka.A - ky.M_1 \quad .. \quad .. \quad .. \quad .. \quad 2b$$

$$\frac{dM_3}{dt} = km_3.M_1 \quad .. \quad .. \quad .. \quad .. \quad 3$$

$$\frac{dU}{dt} = ku.M_1 \quad .. \quad .. \quad .. \quad .. \quad 4$$

The analogue computer program for the solution of model (I) is shown in Fig. 1.

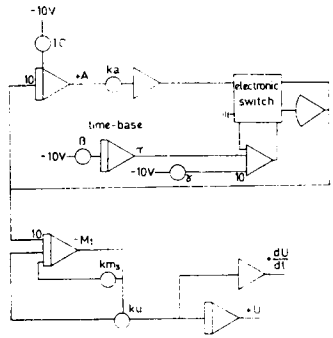
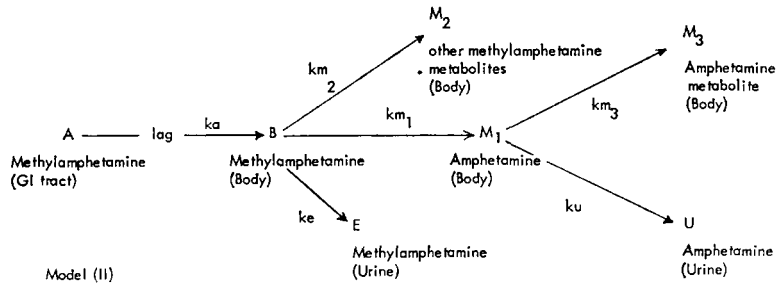


FIG. 1. Analogue computer program for Model (I). 1 sec of machine time equals 1 hr of real time.

Model (II): applicable to "free" forms of methylamphetamine.

Pharmacokinetic model (II) is proposed to describe the kinetics of absorption, metabolism, and excretion of (+)-methylamphetamine in man after oral administration of "solution" forms of the drug under controlled acidic urine conditions.



Differential equations to describe this model are:

Post-lag time:

$$\frac{dA}{dt} = -ka.A \quad .. \quad .. \quad .. \quad .. \quad 5$$

$$\frac{dB}{dt} = ka.A - ke.B - km_1B - km_2B \quad .. \quad 6a$$

$$= ka.A - kd.B \quad .. \quad .. \quad .. \quad .. \quad 6b$$

$$\frac{dM_2}{dt} = km_2.B \quad .. \quad .. \quad .. \quad .. \quad 7$$

$$\frac{dE}{dt} = ke.B \quad .. \quad .. \quad .. \quad .. \quad 8$$

$$\frac{dM_1}{dt} = km_1.B - ku.M_1 - km_3.M_1 \quad .. \quad .. \quad 9a$$

$$= km_1.B - ky.M_1 \quad .. \quad .. \quad .. \quad 9b$$

$$\frac{dM_3}{dt} = km_3.M_1 \quad .. \quad .. \quad .. \quad .. \quad 10$$

$$\frac{dU}{dt} = ku.M_1 \quad .. \quad .. \quad .. \quad .. \quad 11$$

The analogue computer program for the solution of model (II) is shown in Fig. 2.

Model (III): applicable to drug preparations of amphetamine.

Model (I) is modified to describe the kinetics of release, absorption, metabolism, and excretion of (+)-amphetamine, after administration to man of "capsule" and prolonged release preparation B forms of the drug (see Experimental for description of dosage forms), under controlled acidic urine conditions. The modification is the addition of a formulation compartment, D_m, containing the total dose at zero time and from which drug is released into the gastrointestinal tract by sequential first-order processes governed by rate constants kr_1 and kr_2.

In addition to the equations given for model (I), equation 12 also applies.

$$\frac{dD_m}{dt} = -kr.D_m \quad .. \quad .. \quad .. \quad .. \quad 12$$

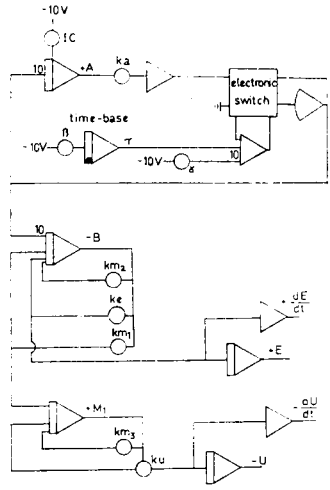


FIG. 2. Analogue computer program for Model (II). 1 sec of machine time equals 1 hr of real time.

where, kr is initially kr_1 and becomes kr_2 after the "break time". Equation 12 is modified to give equation 13.

$$\frac{dA}{dt} = kr.D_m - ka.A \qquad .. \quad .. \quad .. \quad 13$$

The analogue computer program for the simulation of model (III) is shown in Fig. 3. Since the electronic switch was required to change over from kr_1 to kr_2, lag time could not be programmed directly and was therefore estimated by manually setting the abscissa zero of the X–Y recorder.

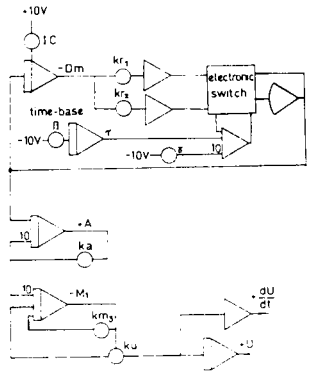


FIG. 3. Analogue computer program for Model (III). 1 sec of machine time equals 1 hr of real time.

Model (IV): applicable to drug preparations of methylamphetamine.

Model (II) was modified to describe the kinetics of release, absorption, metabolism, and excretion of (+)-methylamphetamine after administration to man of prolonged-release preparation D (see Experimental), under controlled acidic urine conditions. The basic model was extended by the addition of a formulation compartment, D_m, initially containing 80% of the total dose and from which the drug is released into the gastrointestinal tract by a first-order process governed by the rate constant kr.

In addition to the equations given for model (II), equation 12 also applies and equation 13 is substituted for equation 5. The analogue computer program required to simulate model (IV) was as shown in Fig. 2, with the addition of a formulation integrator feeding D_m into the gut integrator. An initial condition of 8 V, representing f_m, was set on the formulation integrator; similarly 2 V, representing f_l, was set on the gut integrator.

Experimental

DOSAGE FORMS

Solution: aqueous solutions of (+)-amphetamine sulphate or (+)-methylamphetamine hydrochloride. Dose: 15 mg; 5 mg. *Free pellets:* sugar pellets, coated with (+)-amphetamine sulphate. Dose: 15 mg; 3 × 5 mg (4 hrly). *Capsule:* as free pellets but pellets contained in a hard gelatin capsule. Dose: 15 mg; 3 × 5 mg (4 hrly); 5 mg. *Preparation B:* commercial prolonged-release product of (+)-amphetamine sulphate pellets each coated with a material forming a dialysing membrane and contained in a hard gelatin capsule. Dose: 15 mg. *Preparation D:* prolonged-release tablet product containing (+)-methylamphetamine hydrochloride distributed in a porous plastic matrix. Dose: 15 mg. Urinary excretion results obtained using other prolonged-release preparations (see Beckett & Tucker, 1966; Tucker, 1967) were not subjected to computer analysis since the data indicated incomplete *in vivo* availability of the drug from the dosage form).

In vitro EXPERIMENTS

Preparation B and rotating-bottle method. In vitro drug release was determined by the manufacturers of preparation B, according to Krueger & Vliet (1962). Samples equivalent to 80 mg of (+)-amphetamine sulphate were used. Release was determined in 40 ml vials containing 25 ml of digestive fluid, revolving end over end in a 37° ± 1° water bath at 30 rev/min. The amphetamine content of residues collected at appropriate time intervals was determined, after washing and extraction with alkaline chloroform, by non-aqueous titration with standard perchloric acid-glacial acetic acid, using crystal violet as the indicator.

Preparation D and rolling-bottle method. In vitro drug release was determined by the manufacturers of preparation D, according to the following procedure.

Tablets equivalent to 120 mg of (+)-methylamphetamine hydrochloride were placed in a $3\frac{1}{16}$ inch diameter bottle of 500 ml capacity containing 300 ml of distilled water. The bottle was rotated continuously at 25° ± 2·5° on rollers having a peripheral speed of 100 ± 7·5 ft/min. Aliquots were removed for analysis of released drug at appropriate time intervals.

Rotating-bottle method. Tablets equivalent to 60 mg of (+)-methylamphetamine hydrochloride were placed in a screw-cap bottle of 4 fl. oz. capacity containing 80 ml of distilled water (pH 5·2). The bottle was rotated end over end at 40 rev/min in a 37° ± 1° water bath. Samples (20 ml) of the elution fluid were removed at appropriate time intervals for analysis. At each time interval the volume of the elution fluid was maintained by addition of 20 ml distilled water from a control bottle. The 20 ml samples were allowed to cool to room temperature and diluted to 25 ml in volumetric flasks with 0·5N sulphuric acid.

An ultraviolet absorption curve was obtained from each diluted sample using a Beckman DK2 ratio recording spectrophotometer and 2 cm matched silica cells. (+)-Methylamphetamine content was determined using a calibration curve of absorption difference between a base line drawn between the minima at 255 and 262 mμ and the maximum at 257·5 mμ against methylamphetamine concentration over the range 0·1 to 1·0 mg equivalent base/ml 0·1N sulphuric acid (cf. Souder & Ellenbogen, 1958). 0·1N sulphuric acid was used as reference. The percentage of drug released up to each time interval was calculated, allowing for the amount which had been removed for analysis at earlier intervals, from the concentration of drug in each diluted sample.

Method using the BP tablet disintegration apparatus to determine dissolution rate. Tablets equivalent to 120 mg of (+)-methylamphetamine hydrochloride were placed in the glass cylinder of the apparatus containing 330 ml of distilled water (pH 5·2). The apparatus was operated at 37° ± 1°. Samples (20 ml) of the elution fluid were removed at appropriate time intervals for analysis. At each time interval the volume of the elution fluid was maintained by addition of 20 ml of distilled water from a control cylinder. The 20 ml samples were allowed to cool to room temperature and diluted to 25 ml in volumetric flasks with 0·5N sulphuric acid. Methylamphetamine was determined as described in the rotating bottle method above.

URINARY EXCRETION TRIALS

The dosage forms were given to healthy male subjects under conditions of constant acidic urinary pH (appprox. 4·7 ± 0·2). At least three subjects received each dosage form or regimen of amphetamine. Two subjects both received each form of methylamphetamine. Tables 2 and 3 show that subjects were chosen such that when comparisons were to be made between two forms or regimens, at least one subject, but usually two or three, received both forms. The protocol regarding time of

administration of drug, times of urination, measurement of urine pH, and dosage regimen for ammonium chloride has been described previously (Beckett & Tucker, 1966). Methylamphetamine and amphetamine in urine were determined by the method of Beckett & Rowland (1965b).

COMPUTER SIMULATIONS

A PACE TR20R (Electronic Associates Ltd) analogue computer was used together with an X–Y recorder (Advance Electronics Ltd) and a digital voltmeter (Roband Ltd). The appropriate pharmacokinetic model to describe absorption, metabolism, and excretion was programmed. The experimental urinary excretion data were plotted on the X–Y recorder, both as cumulative excretion and rate of excretion. In some cases, the absorption points derived from these data using the equation of Wagner & Nelson (1964) were also plotted. The settings of the rate potentiometers were then systematically varied in an effort to fit the computer generated curves to the experimental data points. When the best fit was obtained, the settings of the rate constant potentiometers were read from the digital voltmeter. The suitability of the model was judged on the basis of the fit obtained to the experimental data. Lag time was programmed using a suitable electronic switch (comparator relay). During initial curve fitting, the rate constant for elimination of unchanged drug, either kd or ky, was that estimated from the slope of the semi-logarithmic plot of rate of excretion versus time for each subject.

As well as for the fitting of single 15 mg (+)-amphetamine sulphate "free" dose data, model (I) was also used in an attempt to fit 3 $\times$ 5 mg (4 hrly) "free pellet" data obtained from two subjects. The appropriate computer program (see Fig. 1) was suitably modified; an initial condition of 3·3 V (representing the first 5 mg dose) was set on the gut integrator. At 4 hr (+ lag time) computer time, a second gut integrator, with the same initial condition, was made operational and its output fed into the body integrator. This was accomplished using the electronic switch operating between earth and the feedback of the second gut integrator. Since only one switch was available, the third dose was introduced by manually "holding" the computer at the beginning of the third dosage interval (+ lag time), plugging the output from a third gut integrator into the body integrator, then putting the computer back into the operate mode. It was also now necessary to estimate lag times manually.

In fitting the amphetamine excretion curves after the administration of methylamphetamine, it was assumed that although the ky value might be different from that determined after the administration of amphetamine, the ratio of the component rate constants, ku and km_3, would remain essentially constant for each subject.

Fitting of "capsule" and prolonged-release preparation data from appropriate subjects was made, as far as possible, using values of the rate constants for absorption, metabolism, and excretion, and the lag time, similar to those used in previous simulations of "solution" and "free pellet" data.

In vivo drug release rate was plotted by taking the output from the integrator representing the formulation compartment D. Hence, direct comparison of *in vivo* and *in vitro* drug release rates could be made.

Results

In vitro DATA

In vitro drug release data for the batches of preparations B and D supplied, are summarized in Table 1. In the spectrophotometric method

TABLE 1. PERCENTAGE DRUG RELEASED *in vitro* VERSUS TIME DATA FOR PROLONGED-RELEASE PREPARATIONS B (AMPHETAMINE) AND D (METHYLAMPHETAMINE).

Preparation	Method	Time (hr)								
		½	1	2	3	4	5	6	7	8
B (pellets)* lot BUK	Rotating bottle	—	23·2	36·1	—	56·7	63·8	—	75·8	81·1
D (tablets) lot 777–1316-21	Rolling bottle	32·1	44·5	59·9	70·6	76·6	86·3	89·4	92·4	—
	Rotating bottle†	38·0	50·8	65·5	73·6	79·0	82·7	85·8	89·1	—
	BP tablet disintegration apparatus‡	33·1	44·9	61·2	70·4	74·2	84·0	84·1	90·0	—

* Further experiments indicate that the release rate of amphetamine from this preparation is essentially independent of pH, the presence of enzymes, elution volume, and agitation rate (Dr. R. Goldman, personal communication).

† Average of three determinations } results obtained for drug release at each time in individual experi-
‡ Average of two determinations } ments were all within ±4% of the reported averages.

for the analysis of methylamphetamine, the calibration curves were linear over the concentration range examined. The measurements used as the basis of calibration were chosen to minimize errors due to interfering absorption from extraneous substances, i.e. other tablet ingredients also present in the elution fluid. Although not a significant problem, since the plastic matrices of the tablets did not disintegrate during the test, interference was sufficient to preclude the use of measurements employing a more conventional base-line.

Close agreement between the results using three methods for determining the release rate of methylamphetamine from preparation D was obtained (Fig. 4), when the logarithm of the percentage drug not released was plotted against time. A graph suggested that release of most of the drug from preparation D could be described as an apparent first-order rate process. Extrapolation of the plots to zero time indicates that about 20% of the dose was present in an essentially "free" form.

COMPUTER SIMULATIONS

"Solution" and "free pellet" forms, i.e. "free" drug

(+)-*Amphetamine.* Model (I) was fitted to the experimental data, and typical fitted cumulative and rate of excretion curves, along with the derived curve for the amount of amphetamine in the gastrointestinal tract, are shown in Fig. 5*a*. The fit obtained, using the same model, to the 3 × 5 mg "free pellet" data of subject 4 is shown in Fig. 5*b*; a similar result was obtained with the data of subject 5.

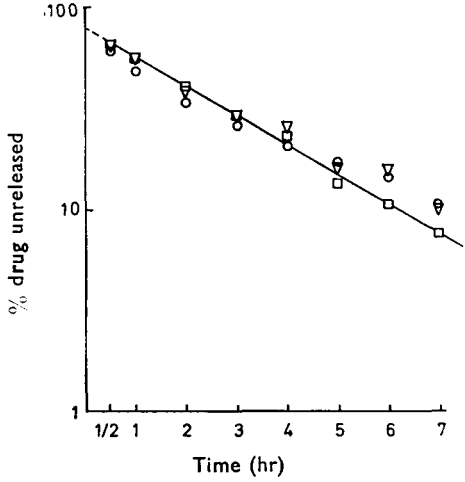


FIG. 4. Percentage methylamphetamine dose unreleased (logarithmic scale) *in vitro* from preparation D versus time, determined using three methods. □ Rolling bottle apparatus. ○ Rotating bottle apparatus. △ BP tablet disintegration test apparatus.

The kinetic parameters, obtained using the above method, for each subject are shown in Table 2. The ratio of rate constants ku/ky indicates the fraction of the dose eventually excreted unchanged in the urine, assuming there was no change in elimination half-life beyond 24 hr; the ratio km_3/ky indicates the fraction metabolized.

Excellent agreement was found among the experimental data in three of the five subjects who received the "free" forms of amphetamine, and the same data calculated by the computer. With subject 3, however, it was not possible to fit the peak hour of the rate of excretion data; data points were slightly higher (+1 to +2% dose/hr) over this period compared with the computer curve. A similar trend was observed in the

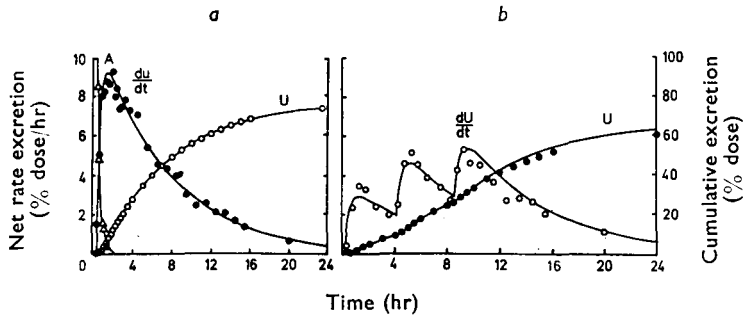


FIG. 5*a*. Computer curves and experimental data points for the urinary excretion of amphetamine, after oral administration of 15 mg (+)-amphetamine sulphate, in 'free pellet' form (Subject 5) (Model 1).

b. Computer curves and experimental data points for the urinary excretion of amphetamine, after a 3 × 5 mg dosage regimen of (+)-amphetamine sulphate orally in 'free pellet' form. (Subject 4) (Model 1).

TABLE 2. KINETIC PARAMETERS FOR THE RELEASE, ABSORPTION, METABOLISM AND EXCRETION OF (+)-AMPHETAMINE AFTER 15 MG DOSES OF THE SULPHATE IN VARIOUS DOSAGE FORMS.

Subject	Dosage form	Dose (as mg SO_4)	Lag time (hr)	kr (hr^{-1})	kr_2 (hr^{-1})	Break time	ka (hr^{-1})	ku (hr^{-1})	km_3 (hr^{-1})	ky (hr^{-1})	ku/ky	km_3/ky
1*	Solution	15	0·25	—	—	—	1·7	0·088	0·050	0·138 (5·02)	0·638	0·362
2*	"	15	0·6	—	—	—	3·0	0·117	0·024	0·141 (4·91)	0·830	0·170
3	"	15	0·25	—	—	—	2·8	0·102	0·058	0·160 (4·33)	0·638	0·362
4	"	15	0·2	—	—	—	2·8	0·107	0·044	0·151 (4·59)	0·709	0·291
4	Free pellets	15	0·3	—	—	—	2·7	0·107	0·045	0·152 (4·56)	0·704	0·296
4	"	15	0·1	—	—	—	2·8	0·108	0·031	0·139 (5·00)	0·777	0·223
5	"	15	0·4	—	—	—	2·8	0·108	0·029	0·137 (5·06)	0·788	0·212
4	"	3 × 5	0·1 0·1 0·1	— — —	— — —	— — —	2·8 2·8 2·8	0·107	0·050	0·157 (4·41)	0·682†	0·318
5	Free pellets	3 × 5	0·2 0·2 0·3	— — —	— — —	— — —	1·3 2·7 2·4	0·107	0·024	0·131 (5·29)	0·817†	0·183
6	Capsule	15	0·5	0·100	1·8	1·5	2·8	0·092	0·023	0·115 (6·03)	0·800	0·200
7	"	15	—	0·183	1·3	1·1	2·8	0·124	0·042	0·166 (4·17)	0·747	0·253
5	"	15	0·3	0·640	2·8	1·9	2·8	0·107	0·024	0·131 (5·29)	0·817	0·183
4	"	15	—	0·360	2·8	1·8	2·8	0·108	0·040	0·148 (4·68)	0·730	0·270
4	Prepn B (pellets)	15	0·6	0·074	0·339	2·0	2·8	0·107	0·041	0·148 (4·68)	0·723	0·277
4	Prepn B (capsule)	15	0·1	0·075	0·339	2·4	2·8	0·107	0·043	0·150 (4·62)	0·713	0·287
5	"	15	0·4	0·050	0·328	1·7	2·8	0·107	0·024	0·131 (5·29)	0·817	0·183
6	" ‡	15	0·2	0·163	0·285	1·6	2·8	0·092	0·023	0·115 (6·03)	0·800	0·200

* Experimental data of Beckett & Rowland (1965a).
† Values for total fraction excreted unchanged, determined by extrapolation of 24 hr experimental data to infinite time, were 0·646 and 0·762 respectively. All other values of ku/ky quoted are identical to extrapolated 24 hr experimental values.
‡ Assumed dose was 'cut-off' after 88·6% had been released.
Values in parentheses are the t/2 values, in hr, equivalent to the rate constant above.

data from subject 4. This difference was not readily apparent when fitting the cumulative excretion data and in the post-absorption period an excellent fit was obtained to both rate and cumulative plots.

Computer fits to 3 × 5 mg data were less satisfactory since trials indicated the possibility of a "dose-effect" in the distribution or elimination of the drug, or both. No significant differences in the elimination half-life were apparent when (+)-amphetamine sulphate was given in a single 15 mg dose ("solution", "free pellet", or "capsule" forms) and

when it was given in a divided 3 × 5 mg regimen (4 trials; 4 subjects) or a single 5 mg dose (3 trials; 3 subjects) in the same forms. However, a slightly smaller amount (proportionately *ca* 10% less) of unchanged amphetamine was excreted with the two latter regimens (see Table 3).

TABLE 3. RECOVERY OF UNCHANGED AMPHETAMINE FROM 3 × 5 MG AND SINGLE 5 MG DOSAGE REGIMENS RELATIVE TO RECOVERY FROM A SINGLE 15 MG DOSE.

Subject	Dose (equiv. mg SO_4)	Form	Relative recovery*
6	3 × 5	Capsule	88·3
6	5	"	92·0
7	3 × 5	"	87·0
5	3 × 5	Free pellets	95·0
5	5	Capsule	87·0
4	3 × 5	Free pellets	88·4
3	5	Solution	88·2

* Relative recovery calculated as: $\frac{\%\text{ dose excreted unchanged (total)}}{\text{mean }\%\text{ 15 mg single dose excreted unchanged (total)}} \times 100$

(+)-*Methylamphetamine.* For the two subjects, it was found that model (II) described the absorption, metabolism, and excretion of (+)-methylamphetamine, and good agreement was obtained between the experimental and the theoretical excretion curves of methylamphetamine and its metabolite, amphetamine. Fitted curves for one subject along with the derived curves for the gastrointestinal tract and body compartments, for both methylamphetamine and amphetamine, are shown in Fig. 6*a*. The solution curve 1 in Fig. 6*b* shows the computer fit to the

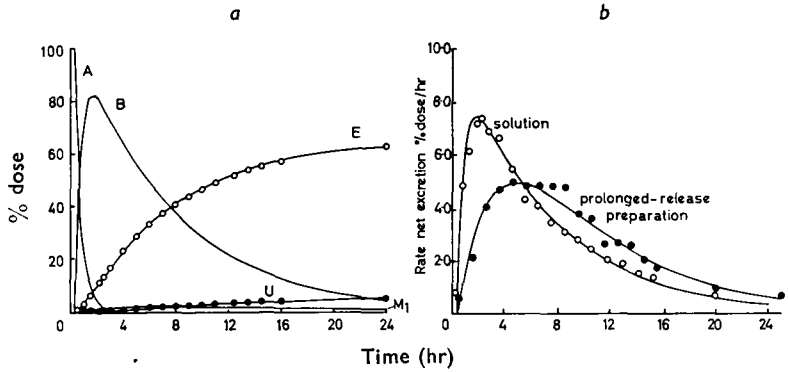


FIG. 6*a*. Computer curves and experimental data points for the elimination of methylamphetamine and amphetamine, after oral administration of a solution of 15 mg (+)-methylamphetamine hydrochloride (Subject 5) (Model II).

b. Computer curves and experimental data points for the urinary excretion of methylamphetamine, after oral administration of a solution of (+)-methylamphetamine hydrochloride and prolonged-release preparation D. (Subject 5) (Models II and IV).

rate of methylamphetamine excretion data in the same subject. A similar result was obtained for the second subject.

The individual kinetic constants are in Table 4. The ratios of rate constants, ke/kd, km_2/kd, indicate the fraction of the dose present in

TABLE 4. KINETIC PARAMETERS FOR THE RELEASE, ABSORPTION, METABOLISM AND EXCRETION OF (+)-METHYLAMPHETAMINE AFTER 15 MG DOSES OF THE HYDROCHLORIDE IN DIFFERENT DOSAGE FORMS

Subject	Dosage form	Lag time (hr)	kr (hr^{-1})	ka (hr^{-1})	ke (hr^{-1})	km_1 (hr^{-1})	km_3 (hr^{-1})	kd (hr^{-1})	ke/kd	km_1/kd	km_3/kd	ku (hr^{-1})	km_3 (hr^{-1})	ky (hr^{-1})	ku/ky	km_3/ky	km_1/kd × ku/ky
5	Solution	0·4	—	2·0	0·090	0·010	0·039	0·139 (4·99)	0·648	0·072	0·281	0·201	0·044	0·245 (2·83)	0·820	0·180	0·059
4	,,	0·3	—	3·1	0·087	0·011	0·039	0·137 (5·05)	0·635	0·080	0·285	0·215	0·061	0·276 (2·51)	0·779	0·221	0·062
5	Prepn D	0·6	0·270	2·0	0·093	0·010	0·035	0·138 (5·02)	0·674	0·073	0·254	0·201	0·044	0·275 (2·83)	0·820	0·180	0·060
4	,,	0·5	0·300	2·7	0·114	0·011	0·039	0·164 (4·23)	0·695	0·067	0·238	0·300	0·085	0·385 (1·80)	0·779	0·221	0·052

Values in parentheses are the t/2 values, in hr, equivalent to the rate constants above.

compartments E and M_2 respectively, at infinite time, assuming there to be no change in elimination half-life beyond 24 hr. The ratio km_1/kd indicates the fraction of the dose which is metabolized to M_1 (i.e. amphetamine) and the fate of this fraction is controlled by the ratios ku/ky and km_3/ky. Hence, the fraction of the dose found in the urine at infinite time, as unchanged methylamphetamine and amphetamine, is given by the ratios ke/kd and km_1/kd × ku/ky respectively.

"Capsule" and "Prolonged-release" drug preparations

(+)-Amphetamine. Model (III) was fitted to the experimental data and typical fitted cumulative and rate of excretion curves, along with the derived curve for the amount of amphetamine in the formulation, are shown in Fig. 7*a* ("capsule" data) and Fig. 7*b* ("preparation B" data). The kinetic parameters for each individual subject are shown in

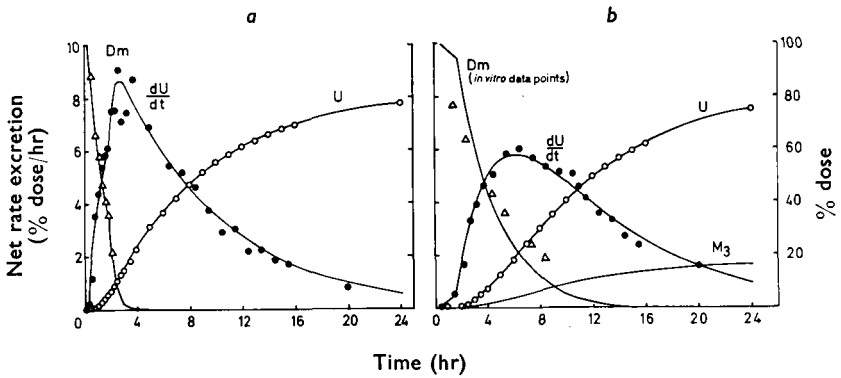


FIG. 7*a*. Computer curves and experimental data points for the urinary excretion of amphetamine, after oral administration of 15 mg (+)-amphetamine sulphate in 'capsule' form. (Subject 5) (Model III).

b. Computer curves and experimental data points for the absorption and elimination of amphetamine, after oral administration of prolonged-release preparation B. (Subject 5) (Model III).

Table 2. Since ka was relatively large, its value was not critical and was assumed to be 2·8 hr^{-1} in each instance (cf. "solution" and "free pellet" data). By assuming two sequential release rates, governed by kr_1 and kr_2, good computer fits to the data were obtained. Only for the "capsule" data in two subjects, could simulations be made using a single release rate constant.

The values for the release constants determined in fitting the preparation B data for subject 6 are arbitrary since it is assumed that the preparation ceases releasing drug after loss of 88·6% of the dose. The recovery of unchanged amphetamine in this trial, relative to that after single 15 mg "capsule" doses to the same subject, indicated that only 88·6% of the dose was available for absorption. Therefore, the output from the D_m compartment was "cut-off" (manually) after 8·86 V had passed into the gut integrator. It could also be argued that only 88·6% of the dose was available at zero time or that reduced availability was spread over a period of time, in which cases slightly different values for the release rate constants would be obtained.

(+)-*Methylamphetamine.* Model (IV) was fitted to the experimental data for "preparation D". The fitted cumulative excretion curve, for one subject, along with derived curves for the amount of methylamphetamine in the maintenance form and in the gut is shown in Fig. 8.

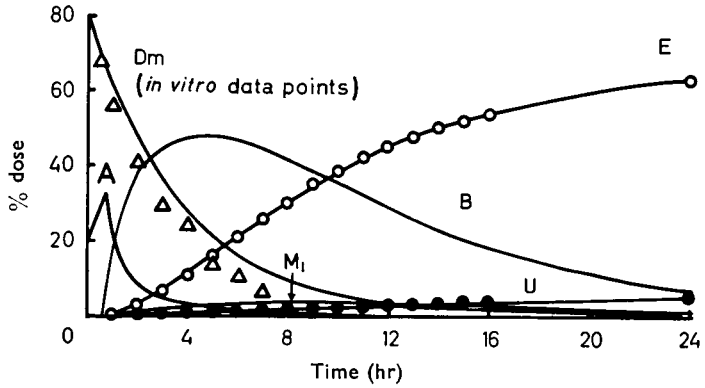


FIG. 8. Computer curves and experimental data points for the absorption and elimination of methylamphetamine and the elimination of amphetamine, after oral administration of prolonged-release preparation D. (Subject 5) (Model IV).

Predicted body levels of methylamphetamine and amphetamine are also shown. The prolonged release curve in Fig. 6*b* shows the computer fit to the rate of methylamphetamine excretion data in the same subject. A similar result was obtained for the second subject. The kinetic parameters for the two subjects used are shown in Table 4. Excellent fits to the data were obtained, assuming 20% of the dose to be in a "free" form and using a single release rate constant. This is consistent with the *in vitro* release data in Table 1.

Preliminary inspection of the semi-logarithmic plot of the methylamphetamine excretion data for preparation D in subject 4 indicated

that the elimination half-life of the drug was significantly lower than that observed after the "solution" dose in the same subject (see Table 4). In subject 5, however, the corresponding half-life was almost identical after the "solution" and prolonged-release forms.

In vivo/in vitro CORRELATIONS

In Fig. 9 the percentage of drug released *in vivo* from the maintenance forms of preparations B and D is plotted against the percentage of drug released *in vitro* after the same time intervals. The rotating bottle *in vitro* data were used for preparation B and the rolling bottle data for

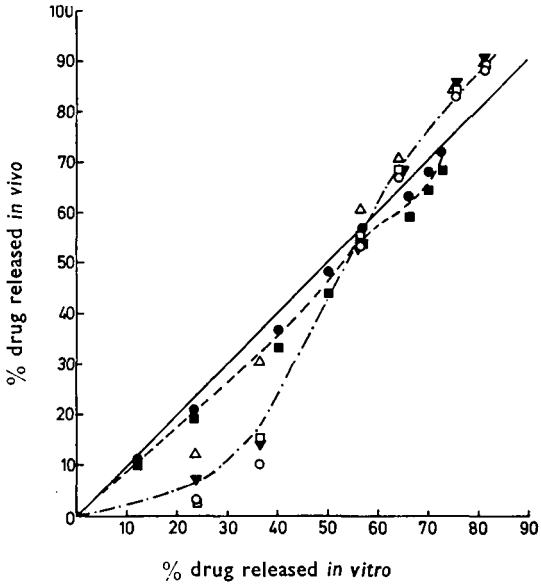


FIG. 9. *In vitro/in vivo* drug release correlations with two prolonged-release preparations: Preparation B containing amphetamine ○□▼△. Preparation D containing methylamphetamine ●■. ○▼● subject 4. □■ subject 5. △ subject 6.

preparation D (see Table 1). If complete correlation between *in vivo* and *in vitro* results were obtained the experimental points would lie on the solid line shown.

Discussion

"SOLUTION" AND "FREE PELLET" DATA

In general there was good agreement between the theoretical urinary excretion curves, based on models (I) and (II), and the experimental values. Thus, Figs 5 and 6 indicate the suitability of the models to describe the kinetics of absorption, metabolism, and excretion, in man, of the amphetamine preparations considered; they also show the usefulness of the analogue computer in analysing urinary excretion data. One

observation however, served to emphasize that although the models apparently described *in vivo* situations reasonably well, physiological reality remains much more complex. Thus, the inability to simulate peak rate of amphetamine excretion levels in two of the five subjects, despite correct computer-experimental correlation for the remaining excretion, indicates that consideration of the body as a single, homogeneous compartment may not always be justified. Rate constant, k, values determined using simple models of the type described, will always be hybrid constants including distribution as well as absorption, metabolism, and excretion. Based on the experimental data available, the use of more sophisticated models would necessitate the introduction of more variables and unknowns.

Inter- and intra-subject variation was apparent in the absorption phase parameters, lag time and ka, for both amphetamine and methylamphetamine (see Tables 2 and 4), although such variation was not great, especially when compared with effects specifically due to formulation. Lag times have previously been reported by several authors (Levy & Hollister, 1964, 1965; Moore, Portmann & others, 1965; Wilkinson, 1966; and others), and several explanations have been suggested, for example, accumulation of drug in the gastrointestinal wall before entry into the blood stream (Levy & Jusko, 1965), and stomach emptying rate limiting the absorption process. The time taken for filtered drug to pass from the kidney glomerulus to the bladder could also contribute to the observed lag time.

In the post-absorption phase, the elimination of amphetamine could be described by simultaneous first-order processes, namely excretion and metabolism. Inter-subject variation occurred in the values of the rate constants for these processes (see Table 2), but, like intra-subject variations, such variations were relatively small. Thus, in the subjects studied, the value for ku, the excretion constant, showed very little variation, i.e. about 0·1 hr^{-1}. A two to two and a half-fold variation was apparent in the value for the metabolic constant, km_3.

The total recovery of unchanged amphetamine and its elimination half-life (t/2), after single 15 mg doses (5 subjects; 11 trials, when "capsule" data are included) ranged from 63·8–81·7% of the dose (mean 74·3%) (see ku/ky values in Table 2) and from 4·17–6·03 hr (mean 4·88 hr), respectively (see Table 2). Recoveries and half-lives were reproducible within a single subject, e.g. subject 4 (4 trials, including "capsule" data) in whom total recoveries ranged from 70·4–77·7% of the dose (mean 73·0%) and the elimination half-life from 4·56–5·00 hr (mean 4·71 hr). Furthermore, no obvious progressive changes were apparent in the above parameters on repeated administration of the drug.

The elimination of methylamphetamine in the post-absorption phase was describable by three simultaneous first-order processes, namely excretion, metabolism to amphetamine, and metabolism to unmeasured metabolite(s). The total recoveries in urine of unchanged methylamphetamine and its metabolite amphetamine were similar in the two subjects used and were independent of the dosage form (see ke/kd and km_1/kd × ku/ky values in Table 4). Table 4 also shows that, with the

exception of the value obtained with preparation D in subject 4 the elimination half-life of unchanged drug was almost identical in each trial. Furthermore, the results were consistent with those of Beckett & Rowland (1965c), using solution forms of the drug. Using models(II) and (IV), the formation and elimination of amphetamine produced by *N*-demethylation of methylamphetamine, could be described by two consecutive first-order processes, with the elimination consisting of two simultaneous first-order reactions in essentially the same ratio as found after the oral administration of amphetamine itself. In the two subjects studied, the overall elimination rate constant, ky, was larger than the same value determined for (+)-amphetamine after oral administration, the increase being 75–80% (cf. Tables 2 and 4). Wilkinson (1966) reports the same effect from a similar computer analysis of urinary excretion data for ephedrine and its metabolite, norephedrine. The significance of this effect remains to be clarified.

"CAPSULE" AND "PROLONGED-RELEASE" DATA

The release, absorption, and elimination of amphetamine and methylamphetamine, after administration of the various dosage forms, was well described by pharmacokinetic models (III) and (IV), respectively. Furthermore, simulations of the experimental data were possible using parameters for excretion and metabolism essentially consistent with corresponding values obtained with the forms from which drug was rapidly available for absorption (see Tables 2 and 4). In particular, comparison of the "capsule" data in Table 2 with the data for "solution" and "free pellet" forms confirms the absence of marked inter- and intra-subject variation in the elimination of amphetamine under constant acidic urine conditions.

The present results also illustrate the dramatic effect which formulation in hard gelatin capsules can have on the rate at which drug becomes available for absorption (see kr_1 and kr_2 values in Table 2). Although effects due to variation between capsule batches, age, size, or manufacturing procedures were not systematically investigated, the results are supported by the work of Wood (1965) who has also shown, using the onset of serum salicylate levels in man, an appreciable delay (approx. 15 min) in release from hard gelatin capsules, relative to fast disintegrating, rapidly dissolving tablets.

A combination of possibilities could explain the observed "capsule-effect": thus, the gelatin may be slowly and incompletely dissolved forming an adhesive mass, around the drug-coated pellets, from which the drug is slowly released for absorption. Slow dissolution of the gelatin shells observed in the B.P. capsule disintegration test; and reports that agitation within the stomach is relatively mild (Levy, 1963; Steinberg, Frey & others, 1965) suggest this possibility. Alternatively, by preventing an initial dispersion of pellets throughout the stomach contents, the capsule may merely delay passage of the drug through to absorption sites in the small intestine.

Although the explanation of the "capsule-effect" is uncertain, its significance in terms of the pharmacological and clinical evaluation of drugs is immediately apparent. The administration of drugs in hard gelatin capsules is common practice. Consequently, in the determination of relative response times or of time of onset of a given response, the release pattern from the dosage form can be of considerable importance (Wood, 1965).

The curves in Fig. 9 indicate an excellent correlation between *in vivo* and *in vitro* drug release rate for preparation D and a fair correlation for preparation B. *In vivo* release of amphetamine from the latter preparation appears to be slower than *in vitro* release in the earlier time intervals. The reason for the sudden increase in *in vivo* release after the loss of about 10% of the dose is obscure, but does not appear to be related to a "capsule-effect" (cf. subject 4 and preparation B pellet data and also preparation B capsule data, in Table 2) or to delayed stomach emptying (cf. data in Table 2). The significance of these *in vivo/in vitro* correlations must be qualified by the following considerations.

In model (III), "lag time" is a composite of an absorption lag time and a lag time assumed in the release of drug from the formulation (cf. model (IV), where lag time refers to absorption only; the drug is assumed to release from the maintenance form immediately after administration). However, since lag times are relatively short, this factor would not appreciably influence the results.

Since prolonged-release preparations did not produce the high peak excretion rate levels, and therefore presumably lower peak blood drug levels, than solution forms, and results with divided 15 mg doses of amphetamine and doses less than 15 mg have indicated that there may be a "dose-effect" in the distribution or elimination of the drug, or both, the assumption that distribution and elimination processes are completely independent of dosage form may not be valid. Whether such a possibility has any great significance in relation to the reported correlations is doubtful.

Although good computer simulations were obtained for the complete excretion profiles after administration of preparation B to subject 4, simulations of "free" form data in the same subject were not entirely satisfactory over the short period when the excretion rate was at its peak. Therefore, in the determination of *in vivo* release of amphetamine from preparation B, in this subject, errors may have been introduced as a result of considering his "body" as a single homogeneous compartment.

It is impossible to design a laboratory device which will not contribute, by its very design, something to an *in vitro* release rate, but it is essential that its design be such that the contribution of the apparatus is minimal if *in vivo/in vitro* correlations are to be meaningful. Accordingly, release of methylamphetamine from preparation D was found to be essentially the same using three types of apparatus (see Fig. 4), and the release of amphetamine from preparation B was determined under conditions such that the observed release rate was due to the permeability of the membranes and not significantly to the conditions of the test

(Dr. R. Goldman, personal communication). The degree of agitation to be used in any *in vitro* test is usually one of the considerations of greatest concern. However, when diffusion from within a particle or matrix (as in preparations B and D respectively), assumes the controlling step in drug release, rather than a dissolution rate, then a lack of marked dependence on agitation intensity might reasonably be expected (see Wood, 1967). It is significant that other studies using matrix-type products have also demonstrated good *in vivo/in vitro* correlations (Wiegand & Taylor, 1960; Sjögren & Ostholm, 1961; Brändström & Sjögren, 1967).

The process of curve-fitting using the analogue computer has an element of subjectivity. Nevertheless, considering the simplicity of the pharmacokinetic models which were applicable this method of data analysis is adequate to give meaningful results consistent with the precision of the analytical data.

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APPENDIX 1

The terms used in the equations are:

t,	time in hr after ingestion of the dose.
Lag time,	the time interval between ingestion of the dose and zero time.
Zero time,	the time at which loss of drug from the gastrointestinal tract may be described as a first-order process.
Break time,	the time after dosage at which kr_1 is changed to kr_2.
A,	the amount of drug (amphetamine or methylamphetamine) present in the gastrointestinal tract.
B,	the amount of methylamphetamine in the body.
M_1,	the amount of amphetamine in the body.
M_2,	the amount of metabolite(s) of methylamphetamine, other than amphetamine, in the body.
M_3,	the amount of metabolite(s) of amphetamine in the body.
E,	the amount of methylamphetamine in the urine.
U,	the amount of amphetamine in the urine.
D_m,	the amount of drug (amphetamine or methylamphetamine) in "maintenance" dosage form.
f_1,	the fraction of dose in "free" form.
f_m,	the fraction of dose in "maintenance" form.
ka,	the rate constant for the absorption of drug (amphetamine or methylamphetamine) from the gastrointestinal tract into the body.
ke,	the rate constant for the excretion of methylamphetamine from the body into the urine.
km_1,	the rate constant for the formation of amphetamine from methylamphetamine.
km_2,	the rate constant for the formation of metabolite(s) of methylamphetamine, other than amphetamine.
kd,	the rate constant for the elimination of methylamphetamine from the body by all processes, i.e. $kd = ke + km_1 + km_2$.
ku,	the rate constant for the excretion of amphetamine from the body into the urine.
km_3,	the rate constant for the formation of metabolite(s) of amphetamine.
ky,	the rate constant for the elimination of amphetamine from the body by all processes, i.e. $ky = ku + km_3$.

k_r, the rate constant for the release of drug (amphetamine or methylamphetamine), from "maintenance" dosage form into the gastrointestinal tract.

The term "maintenance" form refers to dosage forms from which the drug is not immediately available for absorption, i.e. formulated fractions of prolonged-release preparations *and* capsule forms.

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