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Urinary excretion kinetics of amphetamine in man

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The urinary excretion of amphetamine has been examined in 11 male subjects after oral administration of (+)- and (−)- amphetamine sulphate. The excretion of unchanged drug was shown to be dependent upon urinary pH. Excretion of amphetamine, when the urine was maintained alkaline or acid, was measured in seven subjects. The implications of these results, especially in the evaluation of dosage forms, are discussed.

AMPHETAMINE is excreted in the urine of man substantially unchanged, the values of recovered drug varying from 12–100% (Richter, 1938; Beyer & Skinner, 1940; Jacobsen & Gad, 1940; Keller & Ellenbogen, 1952; Chapman, Shenoy & Campbell, 1959; Alles & Wisegarver, 1961; Cartoni & de Stefano, 1963). The various analytical techniques used may account for the spread of the recoveries although the changes in the urinary excretion of amphetamine with pH of the urine in the various trials may have been a contributing factor. Repeated use of amphetamine seemed to have little effect on amount of the dose recovered in the urine (Jacobsen & Gad, 1940; Harris, Searle & Ivy, 1947).

The present paper extends a preliminary communication (Beckett & Rowland, 1964a) which examined the influence of urinary pH on the excretion of unchanged amphetamine in man and also considered the kinetic significance of the results. Some of the clinical aspects have been discussed by Beckett, Rowland & Turner (1965).

Experimental

ORAL ADMINISTRATION OF AMPHETAMINE AND COLLECTION OF URINE

General method. Male subjects, 23–33 years, were used; no other drug (including alcohol) was taken for a day before and during the trials. Breakfast of tea or coffee and toast was taken at 8.0 a.m., urine voided just before 8.30 a.m. and the stated dose of (+)- or (−)-amphetamine sulphate then given orally in aqueous solution (50–100 ml). The urine was collected and measured every 2 hr for 16 hr and then at 24, 28, 32, 36, 40 and 48 hr. In most cases the pH was determined immediately, but never later than 12 hr after collection. If urination was not at the above times, the exact time was noted. In some trials, hourly samples were collected during the first day of the trial. The drug was occasionally administered in the morning other than at 8.30 a.m., but always 30 min after breakfast. The urine of seven subjects, who were not given the drug, was also examined.

Alkaline urine trials. The general method was used but an alkaline urine was induced and maintained alkaline by sodium bicarbonate (1 g/20 ml water orally). A typical regimen was a 3 g sodium bicarbonate dose 1 hr

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before drug administration, 2·5 g at the time of taking the drug and then every 2 hr for 14 hr. Urine was collected every 2hr for 16 hr after the drug had been taken. Trials were not begun until the urine was alkaline. A 40–48 hr sample of urine was collected in some instances.

Acid urine trials. The general method was used but an acid urine was induced and maintained acidic by ammonium chloride (0·5 g enteric coated tablets*). A typical regimen was 4 g ammonium chloride taken 2 hr before and 3 g taken 1 hr before the amphetamine and then 1 g hourly throughout the trial. Generally amphetamine was not taken until the pH was 5·3 or less. After amphetamine administration, urine was collected hourly for 16 hr and occasionally a 24 hr sample was taken. In several subjects urine was collected every 15 min for the first $2\frac{1}{2}$ hr.

INTRAVENOUS INJECTION OF AMPHETAMINE

Collection of urine. Approximately 13 mg (+)-amphetamine sulphate was given intravenously to three subjects with ammonium chloride-induced acid urine. After administration of the drug, urine was collected every 15 min for $2\frac{1}{2}$ hr and then hourly for 14 hr.

AMPHETAMINE IN OTHER BIOLOGICAL FLUIDS

Bile. (+)-Amphetamine sulphate, 10 mg, was given orally to a cholecystectomy patient with a bile duct fistula. The bile was collected 2, 4, 8 and 24 hr after drug administration.

Gastric contents. After an intravenous injection of approximately 10 mg (+)-amphetamine sulphate to two subjects, gastric contents were withdrawn continually by Ryle's tube, starting 10 min before and stopping 40 min after the injection. Volumes and pH values of the samples were noted.

Plasma amphetamine. Blood was collected from a subject (M.D.) taking 30 mg (+)-amphetamine sulphate daily. A narcoleptic patient (E.H.) taking 70 mg (+)-amphetamine sulphate daily was given an intravenous injection of 15 mg (+)-amphetamine sulphate and blood collected $\frac{1}{2}$ and $2\frac{1}{2}$ min after injection.

Clinical effects. Any subjective effects experienced after administration of the drug were noted.

DETERMINATION OF AMPHETAMINE

Amphetamine, in urine, was determined by the gas-liquid chromatographic method described by Beckett & Rowland (1965a). Some of the results were obtained by the method of Beckett & Rowland (1964b). In some instances amphetamine was further identified as its acetone derivative (Beckett & Rowland, 1965a). Amphetamine (2 μg/ml) was added to acid and alkaline urine, stored at 4°, and the amphetamine content determined daily for 4 days.

Amphetamine in gastric juice, plasma and bile was determined using the method of Beckett & Rowland (1965a). Known amounts of drug

* The ammonium chloride tablets caused acute diarrhoea on some occasions.

were also added to samples of the three fluids to check recoveries. These fluids, to which no drug had been added, were also analysed.

CALCULATION OF TOTAL AMPHETAMINE EXCRETED

The amount of amphetamine excreted at infinite time was calculated to determine the fraction of the dose which would eventually be excreted unchanged in the urine, assuming that the biological half-life remained constant throughout the elimination of the drug. From urinary excretion data (see Fig. 2), it appears that absorption of the drug is complete within the first 4 hr after administration; the amount of amphetamine excreted between 4 hr and infinity $\left(\overset{A_e}{4 \rightarrow \infty}\right)$ was therefore calculated using equation (1)

$$\overset{A_e}{4 \rightarrow \infty} = \frac{\overset{A_e}{4 \rightarrow t}}{1 - \exp\text{-}[K_d(t-4)]} \quad .. \quad .. \quad (1)$$

where $\overset{A_e}{4 \rightarrow t}$ is the amount of amphetamine excreted between 4 and t hr after administration of the drug ($t > 4$), and K_d is the first order elimination constant ($0{\cdot}693/t_{\frac{1}{2}}$). Generally t was taken as 16 hr and the amount of amphetamine excreted between 4 and 16 hr determined from the graph of the cumulative excretion of amphetamine. The biological half-life ($t_{\frac{1}{2}}$) was calculated from the slope of the log-urinary excretion rate graph by applying the method of the least sum of squares. The total drug excreted unchanged A_{e_∞} was then given by

$$A_{e_\infty} = \overset{A_e}{0 \rightarrow 4} + \overset{A_e}{4 \rightarrow \infty} \quad .. \quad .. \quad .. \quad (2)$$

where $\left(\overset{A_e}{0 \rightarrow 4}\right)$ is the amount of amphetamine excreted in the first 4 hr following the dose.

CALCULATION OF ABSORPTION RATES

The percentage of the dose absorbed at various times was calculated using equation (3) (Wagner & Nelson, 1964).

$$\% \text{ absorbed} = \frac{A_t}{A_\infty} \times 100 = \frac{\left[\frac{1}{K_d}\left(\frac{dA_e}{dt}\right) + A_e\right]}{A_{e_\infty}} \times 100 \quad .. \quad (3)$$

where A_t is cumulative amount of drug absorbed at time t, $A\infty$ is the total amount of drug finally absorbed, and A_e is the cumulative amount of drug excreted in the urine at time t. The corresponding values of the excretion rate (dAe/dt) and Ae were determined from cumulative urinary excretion plots as described by Wagner & Nelson (1964).

Results

Amphetamine was stable in both acid and alkaline urine for at least 4 days when stored at 4° and was recovered quantitatively from gastric juice, plasma and bile; the contents of these fluids did not interfere with the determination of the drug.

URINARY EXCRETION TRIALS

pH not controlled. Table 1 shows the amount of unchanged amphetamine excreted in 48 hr after the oral administration of 5–15 mg (+)- and 10–15 mg (−)-amphetamine sulphate. The excretion rate showed fluctuations throughout the day (e.g. see Fig. 1); these occurred in all subjects and appeared to parallel changes in urinary pH. An acid urine effected

TABLE 1. URINARY EXCRETION OF AMPHETAMINE 48 HR AFTER ORAL ADMINISTRATION OF (+)- AND (−)-AMPHETAMINE SULPHATE

Subject	Dose (mg sulphate)	Unchanged amphetamine excreted (as % of dose administered)	
		(+)-Amphetamine	(−)-Amphetamine
G.W.	5	18·0	—
A.S.	5	28·0	—
A.S.	10	40·9	57·3
A.T.	10	38·2	66·0
M.B.	10	18·6	51·6
J.W.	10	27·9	29·2
J.W.	10	12·0	—
G.W.	10	31·4	39·4
G.W.	10	35·1	—
G.W.	10	32·7	—
P.T.	10	20·1	—
P.W.	10	36·3	—
C.M.L.	10	32·7	—
M.R.	10	—	41·3
M.B.	15	36·0	—
A.T.	15	34·2	—
M.R.	15	16·0	48·7
E.J.T.	15	44·0	70·7
N.B.	15	27·0	38·3
Mean.. ..		32·9	49·2

a high excretion rate of amphetamine whereas a low excretion rate was obtained when the urine was alkaline. A urine volume effect was sometimes noticeable at urinary pH values of about 7, a high urine flow rate resulting in an increased excretion of amphetamine. Also, during both amphetamine excretion trials, and when no drug was taken, fluctuations in urinary pH occurred (range pH 4·9–8·3). A pH rhythm was generally apparent with high pH values between 8·0 p.m. and midnight and a fall overnight. The excretion rate of amphetamine decreased between 8.0 p.m. and midnight and rose again during the night. Several subjects exhibited an alkaline urine (pH 6·8–7·6) throughout most of the morning and afternoon.

Alkaline urine trials. The mean 16 hr excretion of amphetamine in alkaline urine was 2·6% and 2·2% of the dose after ingesting (+)- and (−)- amphetamine sulphate respectively (Table 2). Significant quantities of amphetamine (2·7–3·6%) were still excreted 40–48 hr after the administration of drug, when the urine had become more acidic (pH 6·3–6·7).

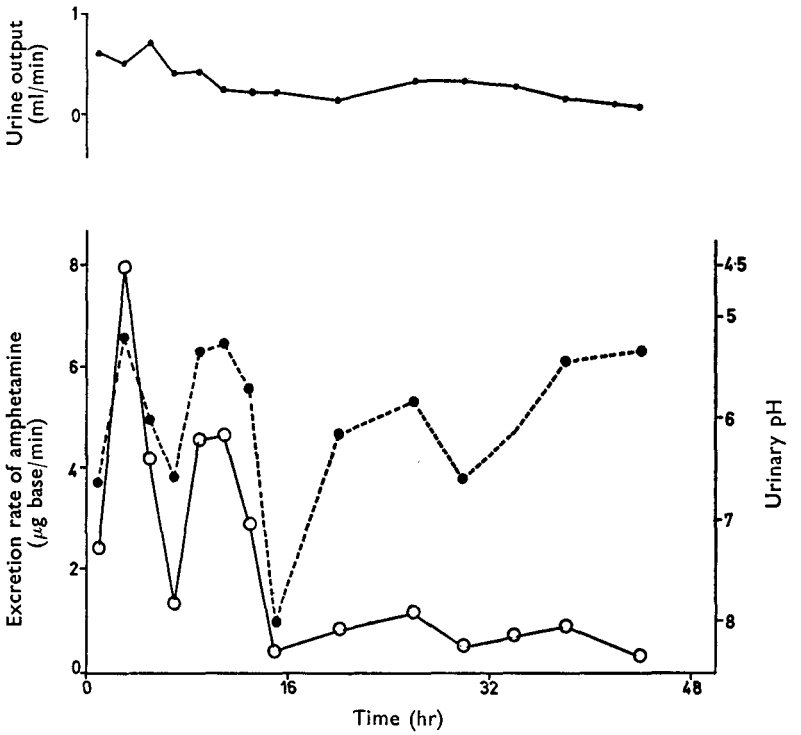

FIG. 1. Influence of urinary pH and urine output on the urinary excretion of amphetamine in man, after oral administration of 15 mg (+)-amphetamine sulphate. Subject E. J. T. (A similar pattern was observed with other subjects) —○— Amphetamine excretion rate. – – –●– – – Urinary pH.

Acidic urine trials. Table 3 gives the 16 hr urinary excretion (mean values 57 and 66%), of unchanged amphetamine after oral administration of (+)- and (−)-amphetamine sulphate to subjects whose urine was

TABLE 2. URINARY EXCRETION OF AMPHETAMINE 16 HR AFTER ORAL ADMINISTRATION OF (+)- AND (−)-AMPHETAMINE SULPHATE AND ALKALINE URINE CONTROL

Subject	Dose (mg sulphate)	Isomer	Amphetamine excreted (% of dose)	Urinary pH
P.T.	10	(+)	2·4	7·8 ± 0·2
P.W.	10	(+)	2·5	7·85 ± 0·2
G.R.W. ..	10	(+)	2·6	8·0 ± 0·2
C.M.L.	10	(+)	3·3	8·1 ± 0·2
M.R.	15	(+)	3·0	7·95 ± 0·25
	15	(−)	1·2	7·80 ± 0·20
E.J.T.	15	(+)	2·2	7·95 ± 0·25
	15	(−)	0·9	8·1 ± 0·2
N.B.	15	(+)	4·2	7·8 ± 0·25
	15	(−)	4·6	7·90 ± 0·25

TABLE 3. TABLE SHOWING AMPHETAMINE EXCRETION AND BIOLOGICAL HALF-LIFE VALUES AFTER ORAL ADMINISTRATION OF (+)- AND (−)-AMPHETAMINE SULPHATE AND INTRAVENOUS INJECTION OF (+)-AMPHETAMINE SULPHATE, URINE BEING MAINTAINED ACID

Subject	Dose (mg sulphate)	Route	Isomer	$t_{\frac{1}{2}}$ (hr)	Amphetamine excreted (as % dose administered)		Urinary pH
					16 hr	Total	
N.B. ..	15	oral	(+)	4·75	55·4	61·5	4·95 ± 0·2
	—	i.v.	(+)	4·50	—	—	5·15 ± 0·2
	15	oral	(−)	5·94	62·7	76·2	5·10 ± 0·2
M.R. ..	15	oral	(+)	5·02	56·5	63·6	4·80 ± 0·2
	—	i.v.	(+)	4·52	—	—	4·90 ± 0·2
	15	oral	(−)	5·94	62·5	74·8	4·95 ± 0·25
E.J.T. ..	15	oral	(+)	4·93	73·5	83·5	4·90 ± 0·25
	—	i.v.	(+)	4·60	—	—	5·05 ± 0·2
	15	oral	(−)	4·82	71·8*	82·3	4·80 ± 0·2
G.W. ..	10	oral	(+)	6·80	48·0	61·2	4·90 ± 0·2
C.M.L. ..	10	oral	(+)	4·21	54·4	59·0	5·10 ± 0·2

* 15 hr only

maintained at a relatively constant acid pH. The fluctuations in the excretion of amphetamine, observed with no pH control, were abolished. Amphetamine excretion rate reached a maximum about 2 hr after

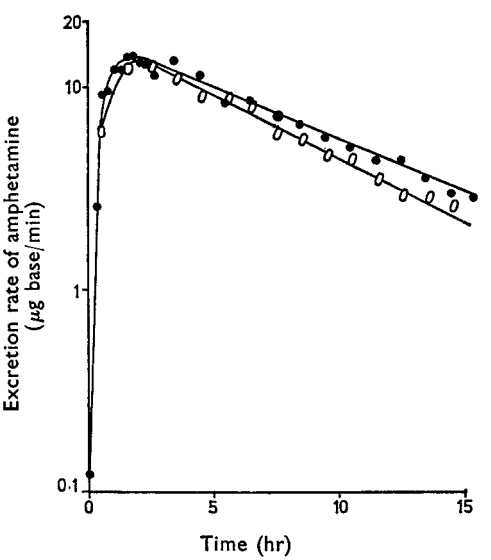

FIG. 2. Urinary excretion of amphetamine after oral administration of 15 mg (+)- and (−)-amphetamine sulphate under acidic urine conditions. Subject M.R. —●— (−)-Amphetamine. —○—(+)-Amphetamine.

administration and, thereafter, fell exponentially (e.g., Fig. 2). Urine flow rate appeared to have little influence on the excretion rate of amphetamine.

INTRAVENOUS INJECTION OF AMPHETAMINE

Acid urine. Fluctuations in the excretion rate of amphetamine occurred during the first 3 hr (e.g. Fig. 3). The amount of amphetamine excreted in the first 15 min urine sample was lower than that in the subsequent

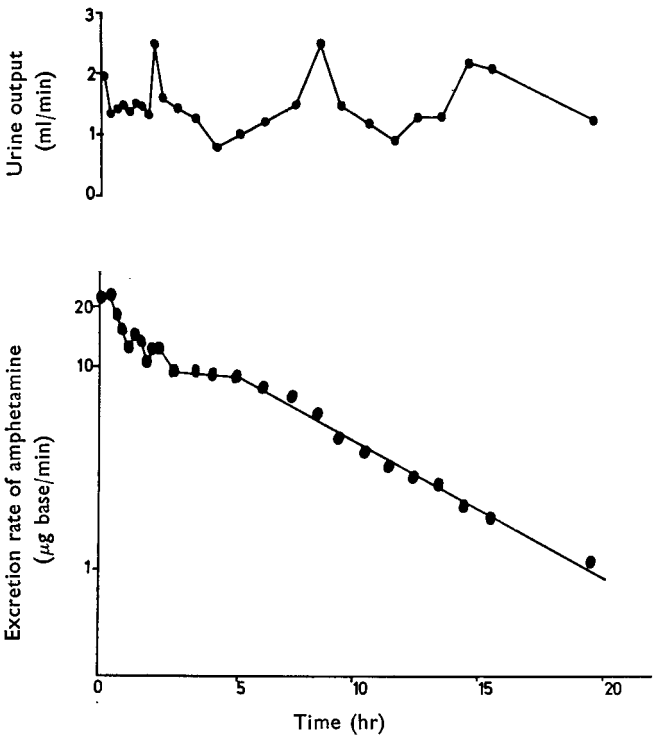

FIG. 3. Urinary excretion of amphetamine and urine output after intravenous administration of 15 mg (+)-amphetamine sulphate under acid urine conditions. Subject N.B.

sample. Also, one individual (see Fig. 3) exhibited a constant excretion rate before a logarithmic fall was observed.

AMPHETAMINE IN BIOLOGICAL FLUIDS

Bile. No amphetamine was found in the bile, before or after digestion of the bile with 2N hydrochloric acid for 1 hr.

Gastric contents. 0·5–1% of the dose administered was found in the stomach contents after 40 min, the contents being acid throughout the trial (pH 1·7–4·5).

Plasma. No amphetamine could be demonstrated in the plasma of the subject taking 30 mg (+)-amphetamine sulphate daily. Amphetamine plasma levels of 0·17 and 0·12 μg base/ml were found ½ and 2½ min after an intravenous injection into the narcoleptic patient.

Clinical effects. Central nervous stimulation and dryness of the mouth were the most common effects experienced when taking (+)-amphetamine

sulphate. These effects were more pronounced under alkaline than normal urine conditions and sometimes resulted in insomnia that night, even though the drug was taken at 8.30 a.m. No effects were observed when the (−)-isomer was administered.

Discussion

The 48 hr urinary excretion of amphetamine after oral administration of (+)- and (−)-amphetamine sulphate (Table 1) is within the range reported by previous workers. The present results with amphetamine (pK_a 9·77, Leffler, Spenser & Burger, 1951; 9·93, Lewis, 1954) may be explained by the passive reabsorption of unionised drug from the kidney, the process being pH and volume dependent (Milne, Scribner & Crawford, 1958; Weiner & Mudge, 1964). The more alkaline the urine the higher the percentage of unionised drug and hence a greater reabsorption of amphetamine with a subsequent decrease in the excretion rate. The increase in duration of pharmacological effects, observed when the urine is maintained alkaline, is probably due to retention of amphetamine in the body.

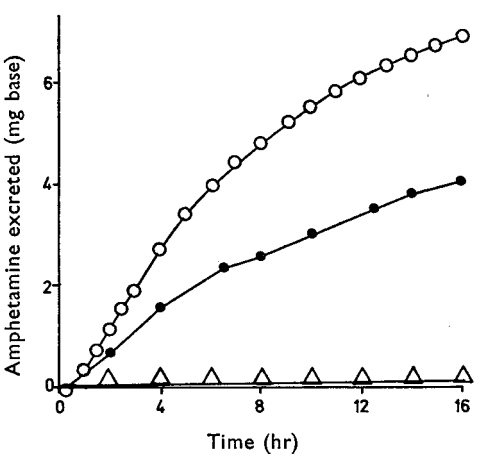

FIG. 4. Cumulative urinary excretion of amphetamine under normal —●—, alkaline —△— and acidic —○— urine conditions after oral administration of 15 mg (−)-amphetamine. Subject M.R.

The difference in excretion of amphetamine in alkaline (Table 2) and acid urine (Table 3) cannot be attributed to changes in urine output since the mean 16 hr urine output was 1,250 and 1,640 ml respectively. These results contrast with those for the excretion of amphetamine under normal urinary pH conditions (e.g. see Fig. 4). Similar results in man and rat have been reported by Asatoor, Galman, Johnson & Milne (1965).

The influence of changes in urine output on the excretion rate of amphetamine at pH 7·0 is probably due to dilution effects of amphetamine in the kidney tubules which alters the rate of reabsorption of the drug.

The observed lack of influence of urine output on the excretion of amphetamine, when urine is maintained acid or alkaline, suggests that reabsorption is respectively negligible and almost maximal under these conditions.

Since diurnal variations in urinary pH under normal conditions are well known (Brunton, 1933; Kenyon, Wilson & Macy, 1934; Bridges & Mattice, 1940; Elliott, Sharp & Lewis, 1959), it is probable that the rhythmic pattern of amphetamine excretion is caused by this urinary pH

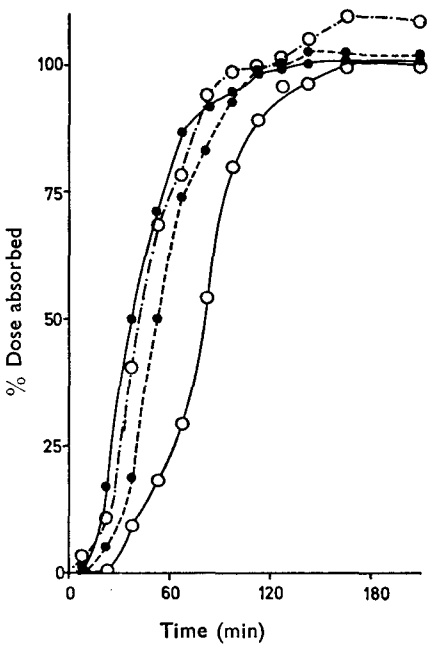

FIG. 5. Amphetamine absorbed (%) as a function of time after an oral dose of amphetamine to 4 subjects. N.B. (−)-isomer — - —○— - —. E.J.T. (−)-isomer - - -●- - -. M.R. (−)-isomer ——●——. G.R.W. (+)-isomer ——○——.

rhythm. These fluctuations in the excretion rate cannot be explained by transfer of drug from plasma to stomach contents with subsequent reabsorption in the gut, as only small amounts appeared in the stomach contents after an intravenous dose of amphetamine, a similar result to that of Jacobsen & Gad (1940). Enterohepatic recycling can also be excluded as no amphetamine was found in the bile either free or conjugated.

KINETIC STUDIES IN ACID URINE

When the urine is maintained acid, urinary excretion is the major route of elimination of amphetamine from the body (Table 3). The maximal subjective effects, which were noted to be 1½–3 hr after ingestion of (+)-amphetamine sulphate, are in accord with the maximum excretion rate of amphetamine occurring at 2 hr (e.g. Fig. 2). Since the excretion rate falls exponentially during the period of observation, K_d is constant

during this time and therefore k_e, k_m ($K_d = k_e + k_m$), the rate constants for excretion and metabolism, are constant. In addition, assuming that K_d remains constant beyond the period of observation, the anticipated total excretion of amphetamine (Table 3) shows that excretion is largely complete within the first 16 hr.

Transposition of urine excretion data to absorption rate data (Fig. 5) shows that absorption of amphetamine was complete within $2\frac{1}{2}$ hr of an oral dose of drug, so that the determination of $t_{\frac{1}{2}}$ values from data obtained after 4 hr was valid; furthermore the absorption was not described by a single first order process. The S-shaped absorption curves (Fig. 5) might be due to the effect of stomach emptying, so that the amount of amphetamine in the gut, where absorption takes place, is not maximal at zero time but at some later time dependent on the rate constants for stomach emptying and absorption of the drug. However, the slow initial rise in the absorption of amphetamine is exaggerated owing to the time necessary for drug to pass through the kidney into the urine, and also to incomplete emptying of the bladder so that, in the first 15 min sample, the measured excretion rate is lower than the true value. The low initial excretion rate observed after an intravenous injection of (+)-amphetamine sulphate may be explained similarly.

The observed fluctuations in the excretion rate during the first 3 hr after intravenous injection of (+)-amphetamine sulphate (e.g., Fig. 3) are at present inexplicable. The fluctuations at constant urinary pH cannot be generally correlated with changes in urine output or explained by the passage of drug from the plasma to stomach or by enterohepatic recycling of the drug. When the excretion rates of the drug had become exponential, $t_{\frac{1}{2}}$ values were then the same as those of the (+)-isomer given orally (Table 3) suggesting that, after equilibrium had been established, the route of administration did not influence the kinetics of elimination of amphetamine from the body. Since variations occurred in the volume injected when 1 ml containing 15 mg (+)-amphetamine sulphate was used, quantitative treatment of the intravenous results has not been attempted.

FINDINGS WITH OPTICAL ISOMERS

In acid urine trials the biological half-life for (+)-amphetamine was slightly lower than that of the (−)-isomer for two subjects (Table 3) whilst in another subject (E.J.T.), who excreted larger amounts of unchanged drug, no difference in the biological life of the two isomers could be seen. Assuming excretion is the same for both isomers, the results suggest that the metabolism of (−)-amphetamine was less extensive than the (+)-isomer, as was also found by Alles & Wisegarver (1961). Under acid conditions, i.e., when excretion of amphetamine is high and metabolism low, any differences in the metabolism of the isomers tends to be obscured. (Further work is in progress to investigate the influence of stereochemistry on the metabolism and excretion of amphetamine and other amines).

Urinary excretion kinetic studies are only valid if excretion rates are indicative of blood concentrations of the drug. This relationship does not appear to be the case with either amphetamine or methylamphetamine (Beckett & Rowland, 1965b) under normal conditions. Cavallito & others (1963) found no relationship between blood levels and excretion rates of tritiated phenylephrine, a result which might in part be due to excretion being pH dependent, as found with the related amine, adrenaline

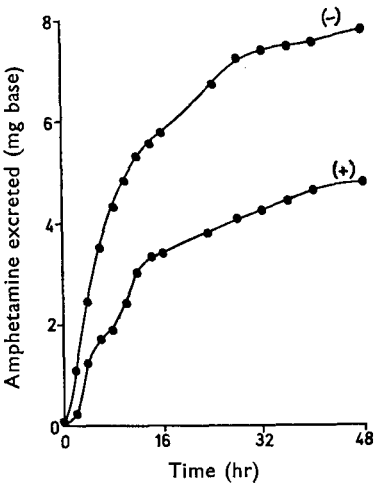

FIG. 6. Cumulative urinary amphetamine excretion after oral administration of 15 mg amphetamine sulphate. Subject E.J.T.

(Braun, 1964). Thus, since the urinary excretion of many drugs is pH dependent and in some cases urine-volume dependent (Milne & others, 1958; Peters, 1960; Weiner & Mudge, 1964; Braun, 1963), and since pH and urine output fluctuate throughout the day, urinary excretion data should be interpreted with caution. Important information may also be obscured by the incorrect times of sampling of urine and by the pooling of data. Cumulative excretion graphs tend to obscure any fluctuations in the excretion rate (e.g., cf. Fig. 6 and Fig. 1).

With drugs like amphetamine, in which there is marked extravascular concentration, as shown by the extremely low plasma levels even when large doses of amphetamine were ingested, urinary excretion studies may be the only practical method of examining the *in vivo* release of drugs from their preparations. If, as with amphetamine, urinary excretion is pH dependent, then a practical solution would be to render the urine acidic with ammonium chloride and thus give high but constant K_d values. Under these circumstances, urinary excretion rates reflect drug levels in the plasma and so would enable the absorption profiles of drugs from conventional and prolonged-release dosage forms to be examined, provided that ammonium chloride does not interfere directly with drug release.

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