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THE EXCRETION OF DEXAMPHETAMINE AND ITS DERIVATIVES

BY

A. M. ASATOOR, B. R. GALMAN, J. R. JOHNSON AND M. D. MILNE

*From the Department of Medicine, Westminster Medical School, London, S.W.*1

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The urinary excretion of amphetamine has been studied by Richter (1938), Jacobsen & Gad (1940), Beyer & Skinner (1940), Keller & Ellenbogen (1952) and Axelrod (1954). These investigators, however, have not examined the effect of acid-base changes on the excretion of the drug. Since amphetamine is a weak base with a high partition coefficient between lipoid solvents and water when in the un-ionized form, it was considered likely that its excretion would be influenced by changes in urinary *p*H (Milne, Scribner & Crawford, 1958). We have, therefore, examined the excretion and fate of dexamphetamine in man and in the rat when the urinary *p*H was varied.

METHODS

Rats. Albino rats (Wistar) weighing 250±15 g were used. In six rats, the urine was made acid by giving ammonium chloride (100 mg as a loading dose followed by 50 mg three-times daily). In six other rats, the urine was made alkaline by giving sodium bicarbonate (100 mg as a loading dose followed by 50 mg three-times daily). The ammonium chloride and sodium bicarbonate were administered in glucose solution by intragastric tube, beginning 24 hr before the test and continuing throughout the test. The rats were placed in metabolic cages and the urine was collected. After a 24-hr control period, 3.5 mg of dexamphetamine sulphate was administered to each rat by subcutaneous injection. The urine was collected for the subsequent 24 hr.

Humans. Dexamphetamine sulphate (10 mg) was given orally to three normal subjects. In each subject the urine was made acid by giving ammonium chloride (2 g four-times daily) on the previous day and throughout the days of the test. At a subsequent period the test was repeated on each subject, but on this occasion the urine was made alkaline by giving sodium bicarbonate (4 g as a loading dose followed by 2 g six-times daily). The amphetamine was given at 9 a.m. Urine was collected at 7, 9 and 11 a.m., 1, 3, 5, 7 and 9 p.m. on the first day, and at 9 a.m., 1, 5 and 9 p.m. on the second day.

Rabbit liver. Incubation with the supernatant fraction after centrifugation at 9,000 *g* of liver homogenates and the isolation of hydrazones were carried out by the method described by Axelrod (1955). Several tubes containing 0.6 μmoles of dexamphetamine and controls without addition of the drug were incubated and the carbon tetrachloride extracts of 2,4-dinitrophenylhydrazones derived from the incubation mixtures were pooled, concentrated to dryness under reduced pressure, and the residue was dissolved in ethanol. The hydrazones were separated by reversed-phase chromatography on paraffin-treated paper (Asatoor, 1962).

Human liver. A small quantity of human liver was obtained at laparotomy in a patient in whom a liver biopsy was necessary for clinical reasons. The biopsy was histologically normal, but the quantity of tissue obtained was insufficient to carry out a control incubation without added dexamphetamine. Three μmoles of dexamphetamine were incubated with the supernatant fluid derived from 1 g of human liver, and the hydrazones obtained were examined as described above.

Chemical methods

Amphetamine in urine was estimated by the method of Axelrod (1954). This is a modification of the dye-complexing method of Brodie, Udenfriend & Dill (1947) for the estimation of organic bases. Hydroxyamphetamine in urine was measured by the method of Axelrod (1954). This involves extraction of hydroxyamphetamine into diethyl ether under alkaline conditions. The compound is then returned to 0.1 N-hydrochloric acid, treated with 1-nitroso-2-naphthol reagent and assayed spectrophotometrically at 520 mμ.

Chromatography of the dinitrophenyl derivative of amphetamine in human urine was by the method of Asatoor (1960). Dinitrophenyl-amphetamine was prepared by the method described by Asatoor & Kerr (1961) modified as follows.

A volume of urine passed in 4 min was treated as described by Asatoor & Kerr (1961), omitting the preliminary treatment for the removal of ammonia. An aliquot (8 ml.) of the cyclohexane extract was evaporated to dryness and the residue was dissolved in a small volume of ethanol. Half the volume of the ethanolic solution was used for chromatography on paraffin-treated paper (Asatoor, 1960).

For direct chromatography of amphetamine and hydroxyamphetamine from rat and human urine both untreated and hydrolysed specimens were examined. Urine was hydrolysed by heating under reflux in a boiling-water bath for 1 hr with 2 N-hydrochloric acid. Samples were extracted with diethyl ether under alkaline conditions. The ether extract was evaporated to dryness and the residue was dissolved in a small volume of ethanol. This was then examined by two-dimensional chromatography on Whatman No. 1 paper, using mixtures of isopropyl alcohol, aqueous ammonia and water (8 : 1 : 1), and butanol, acetic acid and water (12 : 3 : 5) as the solvents. The chromatograms were sprayed with diazotized *p*-nitroaniline which gives a dull red colour with amphetamine and a brown colour with hydroxyamphetamine.

Chromatography of phenolic acids in urine was by the method of Armstrong, Shaw & Wall (1956).

RESULTS

Table 1 gives the amount of amphetamine and total (free plus conjugated) hydroxyamphetamine excreted in the urine of the rats during the first 24 hr after injection of dexamphetamine. The rats with acid urine excreted more than four-times as much of the unchanged drugs compared to the output in the animals with alkaline urine, a highly significant difference. By contrast there was no difference in the amount of hydroxyamphetamine excreted in the two groups.

TABLE 1

PARTITION OF URINARY EXCRETION PRODUCTS IN RATS DURING THE FIRST 24 HR AFTER INJECTION OF 3·5 MG OF DEXAMPHETAMINE SULPHATE

Values for output are percentages of the dose given, and are means and standard deviations

*p*H of urine	No. of rats	Unchanged drug			Total hydroxyamphetamine		
		Output (%)	*t*	*P*	Output (%)	*t*	*P*
Acid (5·6–5·8)	6	20·3±4·9			19·2±8·1		
Alkaline (8·5–9·1)	6	4·9±3·0	6·0	<0·001	20·2±6·7	0·23	0·8

Fig. 1 gives the mean rates of excretion of amphetamine in the human subjects after a single oral dose of 10 mg of dexamphetamine sulphate. Amphetamine output was highest in an acid urine 2 to 4 hr after ingestion of the drug and thereafter fell exponentially. The highest rates of excretion occurred during the first 12 hr, but even after 48 hr a small amount of the drug could still be detected in the urine by the dye-complexing method. When the urine was alkaline, however, the same subjects excreted only small amounts of amphetamine. Again, there was still some excretion of the drug up to 48 hr after administration.

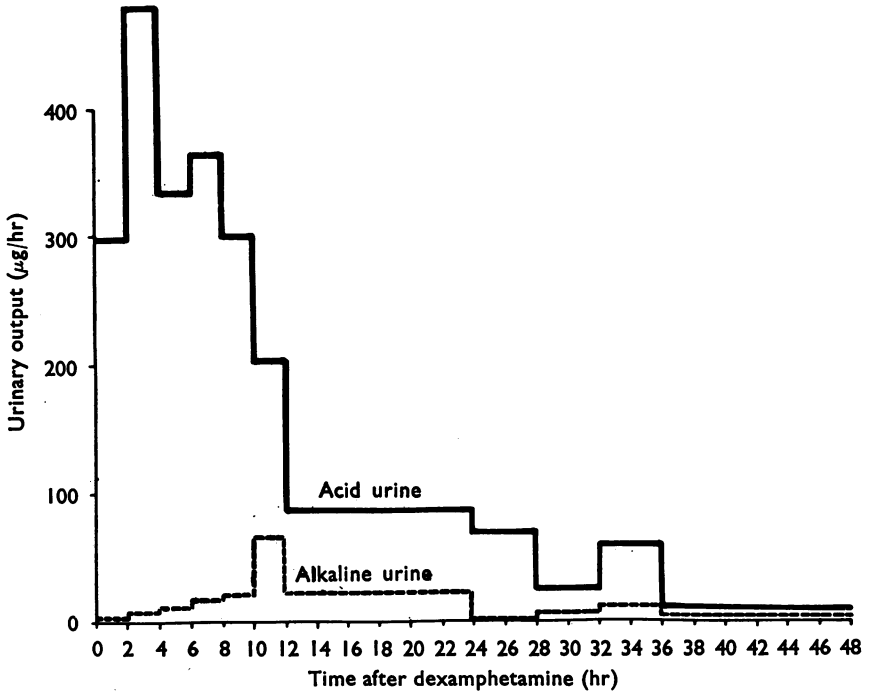

Fig. 1. Mean excretion of amphetamine by three normal subjects after ingestion of 10 mg of dexamphetamine sulphate. *p*H range of acid urine, 4.5 to 5.6; *p*H range of alkaline urine, 7.1 to 8.0.

Fig. 2 gives the corresponding cumulative outputs of amphetamine both in acid and in alkaline urine. When the urine was acid the mean output of unchanged drug after 48 hr was 57% of the administered dose. By contrast, in alkaline urine the mean cumulative excretion of amphetamine amounted to only 7%. This difference in excretion rates in acid and alkaline urine is statistically significant ($t=7$, $P<0.02$).

The marked difference between the excretion rates of amphetamine in acid and alkaline urines was confirmed by the more specific method of reversed-phase chromatography of the dinitrophenol derivative of the drug. These results are almost identical with those previously published in detail for the excretion of norpethidine after ingestion of pethidine (Asatoor, London, Milne & Simenhoff, 1963). The dinitrophenyl derivative of amphetamine migrates in the chromatographic system used to a distance of 0.18 of that of dinitrophenylmethylamine, whereas the corresponding value for dinitrophenylnorpethidine is 0.13. The two R_F values are sufficiently low to allow adequate separation from all naturally occurring urinary primary and secondary amines.

Both hydrolysed and untreated human urine specimens were examined for hydroxyamphetamine by the chemical method of Axelrod (1954), and by the two-dimensional chromatographic method, but none could be detected either in acidic or alkaline urine.

In order to investigate other metabolic pathways for amphetamine in the human, the urines during the first 24 hr after ingestion of dexamphetamine were examined for phenolic acids by the two-dimensional chromatographic technique of Armstrong *et al.* (1956). No

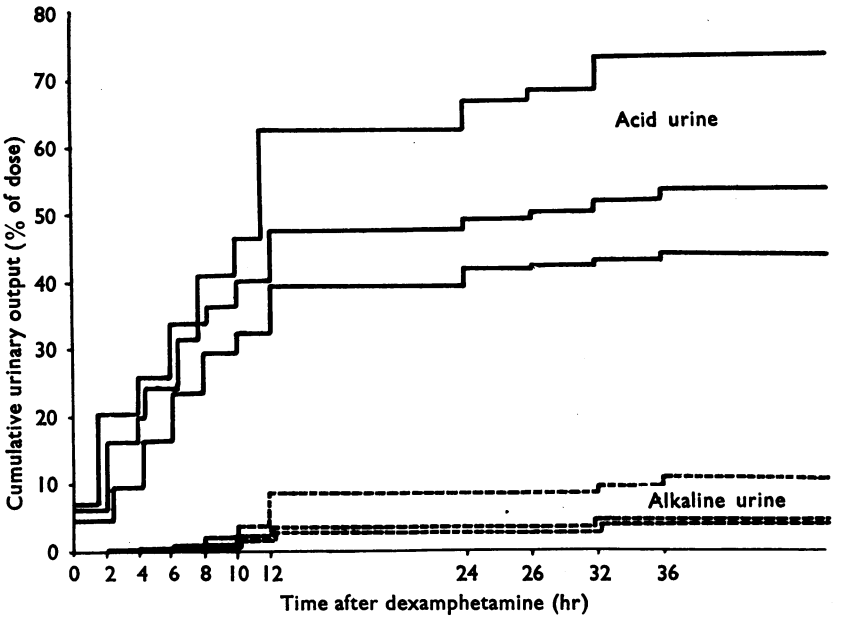

Fig. 2. Cumulative excretion of amphetamine in three normal subjects with acid urine and with alkaline urine during a 48-hr period after ingestion of 10 mg of dexamphetamine sulphate. The *p*H of the urine remained within the range 4.5 to 5.6 in the acid urines, and 7.1 to 8.0 in the alkaline urines.

abnormal phenolic acids were detected. Phenylacetone has been suggested as a probable amphetamine metabolite, both *in vitro* (Beyer, 1941; 1942; Axelrod, 1955) and, less certainly, *in vivo* in dogs (Beyer, 1941; 1942). Pooled human urine from both acid and alkaline groups was examined for phenylacetone and its metabolite benzylmethylcarbinol (probably excreted as a glucuronide conjugate) by the method of El Masry, Smith & Williams (1956). The precipitated dinitrophenylhydrazone was examined chromatographically using the reversed-phase system of Asatoor (1962). No phenylacetone was detected either in the untreated urine or in specimens following hydrolysis with 6 N-hydrochloric acid and oxidation by chromium trioxide (El Masry *et al.*, 1956).

Fig. 3 shows the results of incubation of liver homogenates with dexamphetamine. Phenylacetone was formed both by rabbit and human liver. Acetone itself was present both in the control incubation and those with added dexamphetamine. In the chromatogram from human liver unchanged dinitrophenylhydrazine is also present (R_F value higher than that of conjugates with aldehydes and ketones).

DISCUSSION

The possible and known metabolic pathways of amphetamine are shown in Fig. 4. The drug may be oxidized to hydroxyamphetamine, an important route of metabolism in the rat and the dog. In the present experiments the ratio of urinary hydroxyamphetamine to the unchanged drug in rats was 1 in acid urine, and 4 in alkaline urine. The difference is entirely due to the depression of the excretion rate of amphetamine in alkaline urine. Amphetamine is a weak base with a pK_a which has been quoted as 9.77 (Leffler, Spencer &

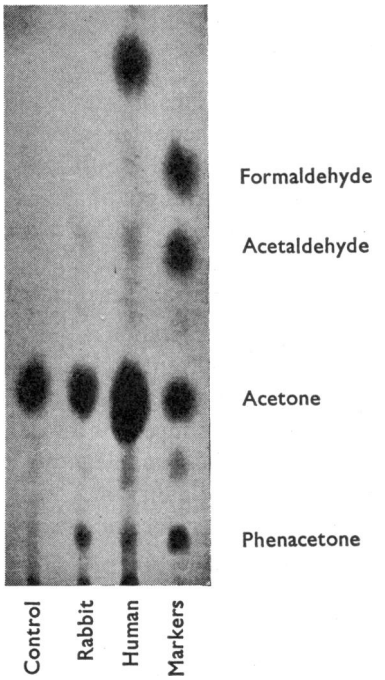

Fig. 3. Chromatograms of dinitrophenylhydrazones of ketones in incubates of dexamphetamine sulphate with homogenates of rabbit and human liver. Control specimen is a homogenate of rabbit liver without added dexamphetamine. Reversed-phase chromatography. Dinitrophenylhydrazones photographed in ultraviolet light. Markers are dinitrophenylhydrazones of phenylacetone, acetone, acetaldehyde, and formaldehyde. The spot of high R_F value in the incubate with human liver is unchanged dinitrophenylhydrazine. Figures under the chromatograms refer to times in hours.

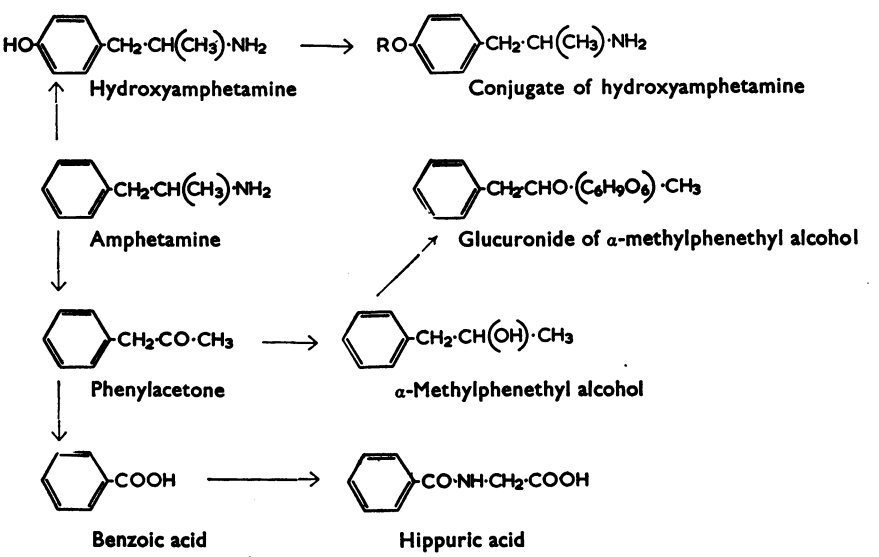

Fig. 4. Possible metabolic pathways of amphetamine. Formation of hydroxyamphetamine occurs in rats and dogs, whilst oxidative deamination to phenylacetone is probably the main route of metabolism in rabbits and man. It is not known whether the conjugate of hydroxyamphetamine is a glucuronide, a sulphate or both.

Burger, 1951) and as 9.93 (Lewis, 1954). This is within the optimum range of pK_a from 6.5 to 10.0 for weak bases to show the phenomenon of *p*H-dependent excretion (Milne *et al.*, 1958). The addition of a polar hydroxyl group reduces the lipoid solubility of the un-ionized fraction, and also increases the strength of the base, the pK_a of hydroxyamphetamine being approximately 10.7 (Lewis, 1954). Both these changes reduce the chances of *p*H-dependent excretion and, therefore, whilst the output of amphetamine is greatly depressed in alkaline urine, that of the metabolite is unchanged.

The effect of species differences in the metabolism of amphetamine was reported by Axelrod (1954). Whilst hydroxylation was found to be important in the rat and the dog, this did not occur in either the rabbit or the guinea-pig. Axelrod was unable to detect hydroxyamphetamine in human urine after ingestion of dexamphetamine, but postulated that hydroxylation might occur. We were also unable to detect the presence of the hydroxy-compound in human urine either by colorimetry or chromatography, and consider that hydroxylation of amphetamine in man either does not occur or is of negligible importance.

Axelrod (1955) found that rabbit liver homogenate deaminated amphetamine to phenylacetone and ammonia:

$$2C_6H_5.CH_2.CH(CH_3)NH_2+O_2\rightarrow 2C_6H_5.CH_2.CO.CH_3+2NH_3$$

triphosphopyridine nucleotide acting as a hydrogen acceptor. The enzyme system involved was located in the microsomal fraction. In the dog, rat and guinea-pig, he found that there was an inhibiting factor preventing the oxidative deamination of the drug. We have shown that human liver homogenate is as potent as rabbit liver in conversion of amphetamine to phenylacetone and suggest that this may be an important route of metabolism in man. This view is, however, difficult to prove with absolute certainty and we were unable to detect phenylacetone or likely metabolites of this ketone in human urine after small doses of dexamphetamine. Obviously, it would be useful to study amphetamine addicts in whom the dose might be considerably higher.

The fate of phenylacetone itself is unknown in man, but its metabolism has been studied in detail in the rabbit (Smith, Smithies & Williams, 1954; El Masry *et al.*, 1956). In this species it is reduced to the corresponding secondary alcohol, α-methylphenethyl alcohol. After administration of the latter compound to rabbits (Smith *et al.*, 1954; El Masry *et al.*, 1956) an average of 43% was excreted as the glucuronide and 19% as hippuric acid, presumably formed by glycine conjugation of benzoic acid. If the metabolic pathways are similar in man it would obviously be impossible to detect the small amount of hippuric acid formed, as there is a normal average daily output of over 1 g of the acid. The glucuronide of α-methylphenethyl alcohol might possibly be found after higher doses of dexamphetamine. Richter (1938) recovered 73% of ingested amphetamine from human urine and therefore thought that the drug was excreted unchanged. The low recoveries from highly alkaline urine (4 to 11%) prove that this is incorrect and that there must be appreciable metabolism of the drug in man. The results suggest that degradation via phenylacetone to conjugates of α-methylphenethyl alcohol is a possible metabolic fate.

The results are of practical importance in the treatment of amphetamine poisoning and in the diagnosis of addiction to the drug. Symptoms of headache, euphoria and excitement were noticeably shorter in the human experiments when the urine was highly acid and, similarly, hypermotility in the rats was also less prolonged. The drug is more rapidly

eliminated from the body when the high excretion rate with a highly acidic urine potentiates conversion to less-toxic metabolites. The widespread and increased use of amphetamine, often without medical supervision, has become an important social problem (Flemming, 1960). Ten cases of fatal amphetamine poisoning have been reported to date (Zalis & Parmley, 1963), and many more will have occurred. Prompt acidification of the urine either by ammonium chloride or better by infusion of 10 g of arginine hydrochloride is indicated in treatment, and would greatly increase the chance of detection of unchanged amphetamine in the urine of an addict. The method of reversed-phase chromatography of the dinitrophenyl derivative of amphetamine is a useful addition to previously described methods of chromatography of the unchanged drug (Axelrod, 1954).

SUMMARY

1. The excretion of amphetamine is increased in acid urine and decreased in alkaline urine both in man and the rat. Output of hydroxyamphetamine, the main metabolic derivative in the rat, is unaffected by variation in urinary *p*H.

2. Urinary excretion of unchanged amphetamine is the major route of elimination of the drug in man if the urine is acid. When the urine is alkaline, the mean output is only 7% of the ingested dose.

3. Evidence is given that hydroxylation of amphetamine in man either does not occur or is of negligible importance. An homogenate of human liver was shown to be as potent as rabbit liver in the oxidative deamination of amphetamine to phenylacetone, suggesting that this may be an important route of metabolism of the drug in man.

4. Acidification of the urine is indicated in the treatment of cases of amphetamine poisoning, and would increase the chance of analytical detection of the drug in the diagnosis of amphetamine addiction. Reversed-phase chromatography of the dinitrophenyl derivative of amphetamine is shown to be a useful addition to previously described analytical methods.

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