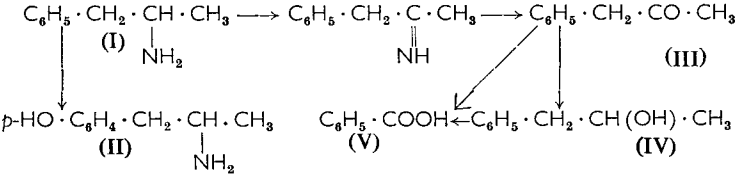
The fate of amphetamine in man and other mammals

SIR,—Axelrod (1954a,b; 1955) showed that amphetamine (I) could be metabolised along two routes; by aromatic hydroxylation to *p*-hydroxyamphetamine (II) and by deamination to benzyl methyl ketone (III). This ketone could then yield 1-phenylpropan-2-ol (IV) *in vivo*, and it has been shown in this laboratory (Smith, Smithies & Williams, 1954; El Masry, Smith & Williams, 1956) that it is, in fact, metabolised to (+)-1-phenylpropan-2-ol and benzoic acid, whilst the alcohol is partly oxidised to benzoic acid (V) and partly conjugated with glucuronic acid. The main pathways of amphetamine metabolism (excluding conjugation) could be expressed in this scheme.



p-Hydroxyamphetamine and benzoic acid have been shown to be urinary metabolites of amphetamine (Axelrod, 1954b; Alleva, 1963) but benzyl methyl ketone and alcohol have not. If one allows for deamination and hydroxylation occurring in the same molecule and for stepwise oxidation of the side-chain, then theoretically, amphetamine could give rise *in vivo* to 25 or more metabolites.

We are examining the urinary metabolites of ^{14}C-amphetamine (α-methyl-[β-^{14}C]-phenethylamine) in detail in man, the rat, rabbit and greyhound, and our preliminary results are reported here. ^{14}C-labelled (+)-, (−)- or (±)-amphetamine sulphate in aqueous solution was administered orally or by intraperitoneal injection. ^{14}C excretion was estimated by end-window and scintillation counting and the excretion of metabolites by paper chromatography and reverse isotope dilution.

Table 1 shows that most of the ^{14}C is eliminated in the urine mainly in the first 24 hr after dosing, and after 3 days nearly all the ^{14}C has been excreted.

In the rat, the figures in Table 1 suggest that the ^{14}C from the (−)-isomer may be excreted a little slower than from the other isomers, but the difference does not appear to be significant.

Table 2 shows that aromatic hydroxylation is the major metabolic reaction of amphetamine in the rat whereas deamination is the major reaction in man, rabbit and dog. Amphetamine itself is a major excretory product in man. The urinary outputs of the drug in the three subjects examined were 23, 33 and 35% of the dose, or an average of about 45% of the ^{14}C excreted in 24 hr. Unchanged amphetamine is also a major excretory product in the one dog examined, the amount being 38% of the dose or about 43% of the 24-hr excretion of ^{14}C. In the rabbit, the excretion of unchanged amphetamine is low (4% of the dose in 24 hr), but in the rat there is a moderate excretion of about 15% of the dose in 48 hr. It has been shown by others (Asatoor, Galman, Johnson & Milne, 1965; Beckett, Rowland & Turner, 1965) that the amount of amphetamine excreted unchanged by man and the rat depends on the pH of the urine, more being excreted in an acid than an alkaline urine. In our experiments, the human urines had pH values of 6·2–6·8. The urines from the rats, rabbits and the dog were collected during 24 hr after dosing, and after this time the pH was about 7·5 for the rats and the dog and about 8 for the rabbits. Freshly collected urines from rabbits had values of about pH 6, which rose on standing at room temperature. The output of unchanged amphetamine was most in man and

least in rabbits. The dose of amphetamine given to the human subjects was less than 1/100 of that given to the rats and rabbits.

p-Hydroxyamphetamine is a major metabolite in rats but not in man, rabbit and the dog; it was excreted in a conjugated form which was hydrolysed by heating the urine with an equal volume of 10N hydrochloric acid at 100° for 2 hr.

Benzyl methyl ketone was not found as such in any of the urines examined, but when human, rabbit or dog urine was heated as above with 10N hydrochloric acid, benzyl methyl ketone was produced. None was found in rat urine under the same conditions. A total of 2·4 g of (±)-amphetamine sulphate was fed during 3 days to 8 rabbits at the rate of 100 mg/rabbit/day. The urine from these rabbits was hydrolysed with 10N hydrochloric acid as above and then steam distilled. The cloudy steam-distillate was treated with 2,4-dinitrophenylhydrazine hydrochloride in ethanol (Brady's reagent) and the 2,4-dinitrophenylhydrazone of benzyl methyl ketone (m.p. and mixed m.p. 151°) was isolated (60 mg) and characterised. For quantitative estimations by isotope

TABLE 1. THE ELIMINATION OF ^{14}C AFTER ^{14}C-AMPHETAMINE SULPHATE IN VARIOUS MAMMALS

	Rat			Rabbit	Man	Dog
	(+)**	(−)	(±)	(±)	(±)	(±)
Dose (oral) of drug, mg/kg	10	10	10	10	0·07	5 (i.p.)*
Dose of ^{14}C, μC	2	10	10	15	7	20
No. of animals	3	3	2	2	3	1
^{14}C output	% of dose†					
In urine on Day 1	79	68	81	81	66	89
2	6	11	4	6	19	2
3	3	2	1	5	6	0·5
Total	88	81	86	92	91	91·5
In faeces on Day 1	2	3	3	5	—	—
2	1	1·5	2	1	—	—
3	0·25	0·1	0·5	1	—	—
Total	3·25	4·6	5·5	7	—	—
Total excretion	91	86	91	99	91	91·5

* The drug was administered intraperitoneally to this greyhound.
** Optical form of drug
† Average values, to nearest whole number in most experiments.

TABLE 2. THE URINARY EXCRETION OF VARIOUS METABOLITES OF AMPHETAMINE SULPHATE IN MAN AND OTHER MAMMALS
Dose of drug and ^{14}C and number of animals as in Table 1

	Rat			Rabbit	Man	Dog
	(+)*§	(−)	(±)	(±)	(±)	(±)
Metabolites found in urine in % of dose**	Days after dosing†					
	2	2	2	1	1	1
Amphetamine	12	17	13	4	30	38
p-Hydroxyamphetamine	48	63	60	7	3	7
Benzyl methyl ketone	0	0	0	22	3	2·5
1-Phenylpropan-2-ol	0	0	0	8	0	2
Benzoic acid	2	2	3	27	20	32
Total of above metabolites	62	82	76	68	56	82
^{14}C in urine‡	85	80	84	81	66	89

* Optical form of drug. ** Average values to nearest whole number.

† Isotope dilution was carried out on urine collected for 2 days after dosing in the rat, and one day for the other species.

‡ The urinary pH was about 7·5 in rats and the dog, about 8 in the rabbits and 6·5 in man.

§ We are grateful to Dr. S. Kaplan for the data on the (+)-isomer.

dilution, the ketone was counted as the semicarbazone (m.p. 197–198°). The amount of the ketone obtained in this way was high in the two rabbits examined (18 and 25% of the dose) but low in the three human subjects (1·3, 2·0 and 6·8%), and the dog (2·5%). The nature of the precursor of the ketone in the urine has not yet been elucidated but it does not appear to be the corresponding imine, benzyl methyl ketimine. A conjugated form of 1-phenylpropan-2-ol (shown to be a glucuronide with β-glucuronidase) was also found in rabbit and dog urine (Table 2) and there appeared to be traces in human urine, but none in rat urine. The alcohol was measured by reverse isotope dilution after acid hydrolysis of the urine and counted as the phenylurethane (m.p. 92°).

The total ^{14}C-benzoic acid in the urine of rabbit, man and dog after ^{14}C-amphetamine amounted to about 20–30% of the dose (Table 2) in 24 hr. This benzoic acid occurred mainly as hippuric acid (about 80% of the total) in man, rabbit and dog urine. Paper chromatography of the radioactive urine from man suggested that the other 20% of the benzoic acid occurred as benzoyl-glucuronide. No spot was found corresponding to free benzoic acid. A small amount (2–3% of the dose) of labelled benzoic acid occurred in rat urine, mainly as hippuric acid.

Table 2 shows that there may be a difference in metabolism between (+)- and (−)-amphetamine in the rat. Less *p*-hydroxyamphetamine is excreted in 48 hours after administration of the (+)-than after the (−)-isomer. In the individual animals the output of *p*-hydroxyamphetamine after the (+)-isomer was 44, 49 and 50% of the dose, after the (−)-isomer, 58, 65 and 67%, and after the (±)-isomer, 58 and 62%. This could suggest that either the (+)- is less readily hydroxylated than the (−)-isomer or that (+)-*p*-hydroxyamphetamine is metabolised rather more readily than the (−)-isomer. In the 48-hr urines from rats, Table 2 shows that there is more urinary ^{14}C unaccounted for in (+)-amphetamine urine than in (−)-amphetamine urine. This could suggest that there are other minor metabolites which have not yet been identified. Preliminary isotope dilution studies have been made on rabbit urine for *p*-hydroxybenzyl methyl ketone, phenylpropionic acid, phenylpyruvic acid, phenyl-lactic acid, cinnamic acid, 2-amino-1-phenylpropan-1-ol (β-hydroxy-amphetamine), phenylacetic acid, mandelic acid and *p*-hydroxybenzoic acid, but the results have been negative. Rat urine was similarly tested for phenyl-alanine, β-hydroxyamphetamine, phenylacetic acid and *p*-hydroxybenzoic acid with negative results. *N*-Methylamphetamine (methamphetamine) was looked for in human urine, again with negative results.

Acknowledgements. This work was supported by a grant from Shell Research Ltd. We are grateful to Smith, Kline & French Laboratories, Philadelphia, for samples of the labelled compounds.

Department of Biochemistry,
St. Mary's Hospital Medical School,
London, W.2.

L. G. DRING
R. L. SMITH
R. T. WILLIAMS

April 6, 1966

References

Alleva, J. J. (1963). *J. med. Chem.*, **6**, 621–624.
Asatoor, A. M., Galman, B. R., Johnson, J. R. & Milne, M. D. (1965). *Br. J. Pharmac. Chemother.*, **24**, 293–300.
Axelrod, J. (1954a). *J. Pharmac. exp. Ther.*, **110**, 2.
Axelrod, J. (1954b). *Ibid.*, **110**, 315–326.

Axelrod, J. (1955). *J. biol. Chem.*, **214**, 753–763.
Beckett, A. H., Rowland, M. & Turner, P. (1965). *Lancet*, **1**, 303.
El Masry, A. M., Smith, J. N. & Williams, R. T. (1956). *Biochem. J.*, **64**, 50–56.
Smith, J. N., Smithies, R. H. & Williams, R. T. (1954). *Ibid.*, **57**, 74–76.

The effects of hypotonicity on the degranulating action of Compound 48/80 on mast cells

SIR,—The range of compounds releasing histamine and producing degranulation of mast cells is so wide and chemically diverse that it has proved difficult to find a unified concept which will explain their mechanism of action. Uvnäs & Antonsson (1963) have suggested that the degranulation process may be initiated by different chemical reactions and that the main differences in action of the compounds lie in their activity in this initial "triggering process." The nature of this process and of the final common pathway for these reactions remains in doubt.

As a result of a study of the action of Compound 48/80 in hypotonic solutions on rat mesenteric mast cells, Norton (1954) has postulated that Compound 48/80 produces degranulation of mast cells by increasing the permeability of the outer cell membrane to extracellular ions and that the concentration of ions within the cell leads to osmotic rupture and the release of granules. Furthermore, Asboe-Hansen (1964) has suggested that one function of the mast cell mucopolysaccharides is to absorb an excess of tissue water and that degranulation, with the release of these mucopolysaccharides, occurs in response to such an excess. Against the view that degranulation is produced by osmotic rupture of the cell, it has been shown by cinephotomicrography and electron microscopy (Horsfield, 1965a,b) that degranulation is an active process and does not involve dissolution of the external cell membrane. In addition, the degranulation produced by the application of chemical reagents takes up to 20 min before completion whereas osmotic rupture following the application of distilled water is complete within a few seconds. In view of these various findings the effects of hypotonic solutions on the degranulating action of Compound 48/80 have been re-examined.

In these *in vitro* experiments biopsies of rat mesentery were taken using a metal spring clip with opposing loops at one end. The biopsies, still in the clips, were placed in test solutions for 20 min at room temperature. The specimens were then fixed in methanol for 30 min and the discs of mesentery put on slides and a few drops of 0·1% toluidine blue in 50% methanol applied. The preparations were ringed with petroleum jelly and cover slips applied. 300 mast cells were counted in each preparation and the % degranulated cells recorded. Experiments were made in duplicate and average values plotted.

In the control series the biopsies were placed in various concentrations of saline and in the test series 0·1 μg/ml of Compound 48/80 was added to each solution. Since Högberg & Uvnäs (1960) have shown that calcium ions are necessary for degranulation with Compound 48/80, 1 mmol/100 ml of calcium chloride was added to the saline.

The results for the control series of experiments are shown in Fig. 1A. It is evident that significant degranulation does not occur until the tonicity of the test solution falls below one half that of an isotonic solution. Therefore above this level no correction need be applied in this system for degranulation due to the hypotonicity of the test solution. The results of the test series of experiments are shown in Fig. 1B. It can be seen that when Compound 48/80 is present