Biochem. J. (1970) **116**, 425–435
Printed in Great Britain

The Metabolic Fate of Amphetamine in Man and other Species

By L. G. DRING, R. L. SMITH and R. T. WILLIAMS
Department of Biochemistry, St Mary's Hospital Medical School, London W.2., U.K.

(*Received* 16 *September* 1969)

1. The fate of [^{14}C]amphetamine in man, rhesus monkey, greyhound, rat, rabbit, mouse and guinea pig has been studied. 2. In three men receiving orally 5mg each (about 0.07mg/kg), about 90% of the ^{14}C was excreted in the urine in 3–4 days. About 60–65% of the ^{14}C was excreted in 1 day, 30% as unchanged drug, 21% as total benzoic acid and 3% as 4-hydroxyamphetamine. 3. In two rhesus monkeys (dose 0.66mg/kg), the metabolites excreted in 24h were similar to those in man except that there was little 4-hydroxyamphetamine. 4. In greyhounds receiving 5mg/kg intraperitoneally the metabolites were similar in amount to those in man. 5. Rabbits receiving 10mg/kg orally differed from all other species. They excreted little unchanged amphetamine (4% of dose) and 4-hydroxyamphetamine (6%). They excreted in 24h mainly benzoic acid (total 25%), an acid-labile precursor of 1-phenylpropan-2-one (benzyl methyl ketone) (22%) and conjugated 1-phenylpropan-2-ol (benzylmethylcarbinol) (7%). 6. Rats receiving 10mg/kg orally also differed from other species. The main metabolite (60% of dose) was conjugated 4-hydroxyamphetamine. Minor metabolites were amphetamine (13%), *N*-acetylamphetamine (2%), norephedrine (0.3%) and 4-hydroxynorephedrine (0.3%). 7. The guinea pig receiving 5mg/kg excreted only benzoic acid and its conjugates (62%) and amphetamine (22%). 8. The mouse receiving 10mg/kg excreted amphetamine (33%), 4-hydroxyamphetamine (14%) and benzoic acid and its conjugates (31%). 9. Experiments on the precursor of 1-phenylpropan-2-one occurring in rabbit urine suggest that it might be the enol sulphate of the ketone. A very small amount of the ketone (1–3%) was also found in human and greyhound urine after acid hydrolysis.

Amphetamine (2-amino-1-phenylpropane) could be metabolized along two pathways, either by hydroxylation of the aromatic ring to *p*-hydroxyamphetamine [4-(2′-aminopropyl)phenol] or by deamination of the side chain to 1-phenylpropan-2-one (benzyl methyl ketone), which could then be degraded to benzoic acid. These two pathways have been shown to occur and their relative extent appears to vary with species (Axelrod, 1954*a*,*b*, 1955; Alleva, 1963; Ellison, Gutzait & Van Loon, 1966). The known metabolites of amphetamine in urine, apart from the unchanged drug itself, are *p*-hydroxyamphetamine and benzoic acid and their conjugates (Axelrod, 1954*b*; Alleva, 1963). The extent to which amphetamine is excreted unchanged depends on urinary pH (Asatoor, Galman, Johnson & Milne, 1965; Beckett & Rowland, 1965) and it appears that one optical isomer may be metabolized more readily than the other (Gunne, 1967; Gunne & Galland, 1967).

The present paper describes a detailed investigation of the fate of ^{14}C-labelled (±)-, (−)- and (+)-amphetamine in man and six other species, namely the greyhound, guinea pig, mouse, rabbit, rat and rhesus monkey. Wide species variations and some new metabolites have been found. Preliminary accounts of this work have been presented (Dring, Smith & Williams, 1966, 1968).

MATERIALS AND METHODS

Compounds

Amphetamine [(±)-2-amino-1-phenylpropane] sulphate, m.p. 302°C (decomp.), and its (+)- and (−)- forms, m.p. 308°C (decomp.) and 300°C (decomp.) respectively, Paredrine [(±)-2-amino-1-(4′-hydroxyphenyl)propane] hydrobromide, m.p. 190–192°C, methamphetamine [(±)-2-methylamino-1-phenylpropane] hydrochloride, m.p. 132–134°C, and norephedrine [(±)-2-amino-1-phenylpropanol] hydrochloride, m.p. 190–194°C, were gifts from Smith, Kline and French Laboratories, Philadelphia, Pa., U.S.A. Cobefrine [(±)-2-amino-1-(3′,4′-dihydroxyphenyl)propan-1-ol] hydrochloride, m.p. 176–178°C, was a gift from Bayer Products Ltd., Surbiton, Surrey, U.K. (±)-1-Phenylpropan-2-ol (benzylmethylcarbinol), b.p. 105°C/20mmHg (Smith, Smithies & Williams, 1954),

(±)-2-amino-1-(3',4'-dihydroxyphenyl)propane hydrochloride, m.p. 190°C (Mannich & Jacobsohn, 1910), (±)-2-amino-1-(4'-methoxyphenyl)propane hydrochloride, m.p. 207°C (Hoover & Hass, 1947), (±)-*N*-acetylamphetamine, m.p. 66–67°C (Hey, 1930), 1-(4'-methoxyphenyl)propan-2-one (4-methoxybenzyl methyl ketone), b.p. 140°C/15mmHg (Kratzl & Schweers, 1956), and 1-(4'-hydroxyphenyl)propan-2-one (4-hydroxybenzyl methyl ketone), b.p. 147°C/5mmHg (Le Brazidec, 1922), were prepared as described in the literature. Other known compounds used were purchased and purified. (±)-, (+)- and (−)-[1-^{14}C]Amphetamine sulphate (see Blackburn & Burghard, 1965) of specific radioactivity 16.4, 2.92 and 6.0 μCi/mg respectively were gifts from Smith, Kline and French Laboratories.

(±)-2-*Amino*-1-(4'-*hydroxy*-3'-*methoxyphenyl*)*propane*. Vanillin benzyl ether (60g) (Späth, Orechoff & Kuffner, 1934), nitroethane (25g), piperidine (1.9ml) and butylamine (1.25ml) in benzene (125ml) were refluxed for 40h, a Dean–Stark trap being used to remove the water formed. The mixture was washed with M-HCl (2×200ml) and then water (3×200ml). The benzene layer was dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure. Recrystallization of the residue from ether–benzene (8:1, v/v) (450ml) gave yellow crystals (33g) of 1-(4'-benzyloxy-3'-methoxyphenyl)-2-nitroprop-1-ene, m.p. 91°C [Fodor (1943) gives m.p. 92°C]. This (29.9g) was reduced with $LiAlH_4$ (11.4g) in ether (500ml) in a Soxhlet apparatus. When the mixture became colourless (4h), it was cooled and treated with water. 2M-NaOH was added to dissolve insoluble material and the ether layer was separated and evaporated. The residue was taken up in 2M-HCl (200ml), made alkaline with 2.5M-NaOH and extracted with ether (200ml). The extract was evaporated to an oil, which solidified overnight at 0°C. This solid (15g) in acetic acid (225ml) was treated for 10min with 10M-HCl (52ml) and the whole heated for 1h at 100°C. It was then evaporated to dryness *in vacuo* and the solid recrystallized from aq. ethanol to give (±)-2-amino-1-(4'-hydroxy-3'-methoxyphenyl)propane hydrochloride [3.7g; m.p. 254–255°C (decomp.)] as white needles [Robinson, Lowe & Imperial Chemical Industries Ltd. (1940) give m.p. 254–255°C (decomp.)].

(±)-2-*Amino*-1-(3',4'-*dimethoxyphenyl*)*propane*. The hydrochloride of this compound was prepared as above starting with veratraldehyde (42g), nitroethane (24ml), piperidine (1.9ml) and butylamine (1.25ml). The yield was 3.8g of hydrochloride, m.p. 144°C (cf. Mannich & Jacobsohn, 1910).

(±)-2-*Amino*-1-(4'-*hydroxyphenyl*)*propan*-1-*ol*. To a solution of *p*-benzyloxypropiophenone (48g; Späth *et al.* 1934) in methylene dichloride (250ml), solid $CaCO_3$ (25g) was added. The mixture was heated under reflux with stirring and bromine (10.25ml) was added dropwise over 1.5h to keep the mixture a pale yellow. After cooling, 2M-HCl was added to dissolve insoluble material, and the methylene dichloride layer was separated, washed with $NaHCO_3$ (5%; 125ml) and then water (2×100ml) and dried over anhydrous Na_2SO_4. Evaporation of the solvent *in vacuo* gave an oil, which crystallized at 0°C. Recrystallization from ethanol gave white platelets of (±)-1-(4'-*benzyloxyphenyl*)-2-*bromopropan*-1-*one* (24g) m.p. 77–78°C (Found; C, 60.2; H, 4.7; Br, 25.3; $C_{16}H_{15}BrO_2$ requires C, 60.2; H, 4.7; Br, 25.0%).

To a solution of the above ketone (16g) in warm ethanol (100ml), dibenzylamine (19.9g) was added during 10min and the solution refluxed with stirring for 4.5h. After keeping at room temperature overnight, excess of dibenzylamine hydrochloride was filtered off, washed with ether and the washings were added to the filtrate. The latter was evaporated *in vacuo* to a yellow solid, which was extracted with ether. The ethereal solution was washed with water, dried over anhydrous Na_2SO_4 and evaporated to dryness. The yellow solid was recrystallized from methanol (300ml) to give white rhombic plates (23g) of 1-(4'-benzyloxyphenyl)-2-(*NN*-dibenzylamino)propan-1-one, m.p. 94–95.5°C (Found: C, 82.4; H, 6.7; N, 3.35%; $C_{30}H_{29}NO_2$ requires C, 82.7; H, 6.7; N, 3.2%).

The above compound (8g) in warm ethanol (50ml) was neutralized with dry ethanolic HCl. Palladium–charcoal (10%; 3g) was added and the mixture hydrogenated at 60lb/in^2 for 13h. After filtration, the solution was evaporated *in vacuo* and the resulting oil taken up in acetone and neutralized with ethanolic HCl. The solution was evaporated and the residue partially crystallized on keeping overnight in a vacuum desiccator. It further crystallized on trituration with acetone. On keeping, white crystals of 4-hydroxynorephedrine [(±)-2-amino-1-(4'-hydroxyphenyl)propan-1-ol] hydrochloride (2.4g) separated with m.p. 190–192°C from ethanol–ether [Van Dijk & Moed (1959) give m.p. 193–194°C].

1-*Phenyl*[1-^{14}C]*propan*-2-*one*. A rabbit (3kg) was given orally (±)-[^{14}C]amphetamine (25μCi; 30mg) in water. The 24h urine (100ml) was mixed with an equal volume of 10M-HCl and heated under reflux for 2h. 1-Phenylpropan-2-one (3ml) was added to the cooled solution and the whole extracted with ether (3×50ml). The extract was washed with 2% $NaHCO_3$ solution (2×50ml) followed by water (2×50ml) and then evaporated at 20°C. The residue was treated with excess of sodium bisulphite solution (4.5g in 17ml of aq. 30% ethanol). After 30min the bisulphite compound of 1-phenylpropan-2-one (2.6g) that had separated was filtered, washed with a little water followed by ethanol and ether and sucked dry. It was then stirred into 2M-NaOH (20ml), and the ketone that separated was taken up in ether (50ml). The ether solution was washed with water, dried over anhydrous Na_2SO_4 and evaporated to a pale-yellow liquid (0.76ml). The 1-phenyl[1-^{14}C]propan-2-one had a specific radioactivity of 2μCi/g.

Dibenzoyl derivative of 4-*hydroxyamphetamine*. (±)-4-Hydroxyamphetamine was benzoylated by standard methods with benzoyl chloride and NaOH.

(±)-2-*Benzamido*-1-(4'-*benzoyloxyphenyl*)*propane*. This compound formed fine white needles, m.p. 143°C, from aq. ethanol (Found: C, 77.1; H, 5.8; N, 4.0; $C_{23}H_{21}NO_3$ requires C, 76.9; H, 5.9; N, 3.9%).

Di- and tri-benzoyl derivatives of 4-*hydroxynorephedrine*. (±)-2-Amino-1-(4'-hydroxyphenyl)propan-1-ol hydrochloride (0.3g) in 2.5M-NaOH (5ml) was treated with benzoyl chloride (1.5ml) at room temperature. After shaking for 5min, the precipitate that formed was collected and washed with water. The solid was recrystallized from the minimum volume of aq. 80% ethanol to give 2-*benzamido*-1-(4'-*benzoyloxyphenyl*)-1-*benzoyloxypropane* as white needles, m.p. 181–182°C (Found: C, 75.3; H, 5.5; N, 3.0; $C_{30}H_{25}NO_5$ requires C, 75.1; H, 5.2; N, 2.9%). The tribenzoyl compound was the main product and was

used in the isotope-dilution procedures given below. Ethanol was removed from the mother liquor *in vacuo*, and a small amount of solid that separated was recrystallized from the minimum of aq. 50% ethanol. This was found to be a *dibenzoyl derivative* of 4-hydroxynorephedrine and formed white needles, m.p. 162–163°C, from aq. 50% ethanol (Found: C, 73.9; H, 5.9; N, 3.7; $C_{23}H_{21}NO_4$ requires C, 73.6; H, 5.6; N, 3.7%). This compound is probably either the 4′-benzoyloxy or the 1-benzoyloxy derivative of 2-benzamido-1-hydroxy-1-(4′-hydroxyphenyl)propane.

Animals

Female Wistar albino rats (200±20g body wt.), English guinea pigs (700±100g), New Zealand White rabbits (3.5±0.5kg), greyhounds (23±2kg), Rhesus monkeys (4±0.1kg) and S.A.S./I.C.I. mice (22±2g) were used. Amphetamine sulphate dissolved in water was administered orally by stomach tube or by intraperitoneal injection, except for the greyhounds, when it was injected in 0.9% NaCl, and the monkeys, when the solid was concealed in a banana. The animals were then placed in cages suitable for collection of urine and faeces for up to 5 days. They had free access to food and water, except for the rodents, when no food was allowed during the first 24h after dosing to avoid contamination of the urine. Human subjects took the compound dissolved in 50ml of water.

Spectra

The u.v. spectra were determined in a Unicam SP.600 spectrophotometer, and i.r. spectra in a Perkin–Elmer Infracord spectrometer. N.m.r. spectra were determined for us at the University of London Intercollegiate Nuclear Magnetic Resonance Service, with a 60MHz Perkin–Elmer R10 instrument. Mass spectra were determined by Dr B. Millard, Department of Chemistry, School of Pharmacy, University of London, on an A.E.I. MS902 mass spectrometer.

Radiochemical techniques

^{14}C in urine and faeces was determined with a Packard Tri-Carb scintillation spectrometer (model 3214) essentially as described by Bridges, Davies & Williams (1967). Amphetamine and its metabolites in urine were determined by the isotope-dilution procedures given below. In some experiments they were determined by scanning paper strip chromatograms of urine (0.05–0.15ml) in a Packard radiochromatogram scanner (model 7200), identification of the ^{14}C peaks being made by comparison of R_F values with authentic samples and in some cases by co-chromatography.

Isotope-dilution procedures. Unless otherwise stated, one-tenth of the volume of the urine collected for 24h after dosing was used.

Amphetamine. For free amphetamine, (±)-amphetamine sulphate (1.363g) was dissolved in the urine, which was then brought to pH12 with 10M-NaOH and then extracted with ether (3×100ml). The extract was evaporated and the residue treated with 5% NaOH (20ml) and benzoyl chloride (2ml). After shaking for 5min the *N*-benzoylamphetamine (m.p. 128°C) was filtered and recrystallized from aq. 50% ethanol to constant specific radioactivity. For total (free+conjugated) amphetamine, the urine was boiled for 2h with an equal volume of 10M-HCl. Carrier amphetamine sulphate was added and the benzoyl derivative prepared as above.

4-Hydroxyamphetamine. The carrier (±)-4-hydroxyamphetamine hydrobromide (1.536g) was added to the urine, which was brought to pH13 with 10M-NaOH and then extracted with ether (50ml). This extract, containing amphetamine, was discarded. The urine was now adjusted to pH9.5–10.0 with 10M-HCl and again extracted with ether (3×100ml). The extract was evaporated and the residue benzoylated with 5% NaOH (70ml) and benzoyl chloride (5ml). The *NO*-dibenzoyl derivative of *p*-hydroxyamphetamine was washed with 5% NaOH and water and dried. It was recrystallized (m.p. 143°C) from aq. 50% ethanol to constant specific radioactivity. Total *p*-hydroxyamphetamine was determined after acid hydrolysis as described above for amphetamine.

Benzoic acid. The carrier (1g) was dissolved in the urine, solution being assisted by addition of a little 40% NaOH. After acidification with 10M-HCl, the benzoic acid was extracted with ether (3×100ml), the extract evaporated and the benzoic acid (m.p. 122°C) recrystallized to constant specific radioactivity. For total benzoic acid, urine containing an equal volume of conc. HNO_3 was boiled under reflux for 2h. The carrier was added and extracted as before.

Hippuric acid. The carrier (1g) was dissolved in urine, solution being assisted by addition of a little 40% NaOH. The urine was adjusted to pH2 and extracted with ethyl acetate (3×100ml). The extract was evaporated and the residue of hippuric acid recrystallized (m.p. 187°C) from hot water to constant specific radioactivity. In calculation of the amount of hippuric acid derived from amphetamine, allowance was made for the endogenous acid in the urine, which was determined fluorimetrically (Ellman, Burkhalter & La Dou, 1961).

1-Phenylpropan-2-one. The ketone (1g) was added to urine or acid-hydrolysed urine (see as described above for amphetamine), solution being assisted by the addition of a little ethanol. The ketone was then extracted with ether (3×100ml) and the extract evaporated. The residue in ethanol (5ml) was heated on a water bath for 15min with a solution of semicarbazide hydrochloride (1g) and anhydrous sodium acetate (0.9g) in water (5ml). The semicarbazone of the ketone was filtered, washed with water and recrystallized (m.p. 194°C) from ethanol to constant specific radioactivity.

1-Phenylpropan-2-ol. The (±)-carbinol (1g) was added to untreated or acid- or enzyme-hydrolysed urine. To obtain the last-mentioned urine, a suitable volume of urine was brought to pH5 with acetic acid and then incubated for 24h with an equal volume of β-glucuronidase solution (Ketodase; Warner–Chilcott, Morris Plains, N.J., U.S.A.). The carbinol was extracted with ether (3×100ml) and the extract dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The residue in light petroleum (b.p. 100–120°C) (10ml) was treated with phenyl isocyanate (1.2ml) and the mixture was refluxed on a boiling-water bath for 2h and then filtered hot. The phenylurethane of the carbinol separated on cooling and was recrystallized (m.p. 89°C) from light petroleum (b.p. 100–120°C) to constant specific radioactivity.

2-Amino-1-phenylpropanol. (±)-Norephedrine hydrochloride (1.235g) was dissolved in urine, which was then adjusted to pH12 with 10M-NaOH and extracted with ether (3×100ml). The residue from evaporation of the extract was treated with 3M-NaOH (10ml) and acetic anhydride (3ml), and the mixture was shaken and kept at 0°C overnight. 2-Acetamido-1-phenylpropan-1-ol separated and was recrystallized (m.p.133°C) from an ethyl acetate–hexane mixture to constant specific radioactivity.

2-Amino-1-(4'-hydroxyphenyl)propan-1-ol. The urine was mixed with an equal volume of 10M-HCl and then heated at 100°C for 2h. The carrier [0.304g of (±)-4-hydroxynorephedrine hydrochloride] was added and the mixture made just alkaline with 10% NaOH. This solution (10–15ml) was now made alkaline with 10% NaOH (5ml), and benzoyl chloride (1.5ml) was added. The mixture was shaken for 5min, and the precipitate that formed was filtered and the 2-benzamido-1-benzoyloxy (4'-benzoyloxyphenyl)-1-propane, m.p.181–182°C, recrystallized to constant specific radioactivity from aq. 80% ethanol.

Isotope-dilution procedures were also carried out on the rat or rabbit urine with 2-methylamino-1-phenylpropane (hydrochloride, m.p.132°C, from ethanol–ether), (±)-phenylalanine (*N*-benzoyl, m.p.184°C, from aq. ethanol), phenylacetic acid (free acid, m.p.76°C, from water), (±)-mandelic acid (*S*-benzylthiouronium salt, m.p.166°C, from water), (±)-phenyl-lactic acid (free acid, m.p.94°C, from benzene), phenylpyruvic acid (2,4-dinitrophenylhydrazone, m.p.191°C, from aq. ethanol), phenylpropionic acid [free acid, m.p.47°C, from light petroleum (b.p.40–60°C)], cinnamic acid (free acid, m.p.132°C, from aq. ethanol), *p*-hydroxybenzoic acid (free acid, m.p. 213°C, from ethanol–xylene) and 1-(4'-hydroxyphenyl)-propan-2-one (semicarbazone, m.p.213°C, from ethanol). None of these compounds entrained radioactivity when added to the urine and reisolated.

Chromatography

The R_F values and colour reactions of the compounds relevant to this study are given in Table 1.

Precursor of 1-phenylpropan-2-one

1-Phenylpropan-2-one was found in the acid-hydrolysed urine of men, dogs and rabbits receiving amphetamine but not in the unhydrolysed urine. In rabbit urine the ketone appeared as an acid-labile precursor, X, showing R_F 0.60 in solvent *B* and R_F 0.33 in solvent *C* (Table 1). About 20% of the dose of [^{14}C]amphetamine appeared as compound X in rabbit urine (see Table 6) and about 1–7% probably as compound X in human and dog urine (see Tables 3 and 6). On chromatograms, compound X gave no positive colour reactions for glycine, glucuronic acid, acetylcysteine or glutathione (Knight & Young, 1958) conjugates, nor did it give the ninhydrin reaction. Suitable incubations of the urine, voided after dosing of rabbits with [^{14}C]amphetamine, with β-glucuronidase (Ketodase) or limpet arylsulphatase (sulphatase; Sigma Chemical Co., St Louis, Mo., U.S.A.) did not appear to cause hydrolysis of compound X. When the urine was kept overnight at 18°C with an equal volume of M-NaOH or M-HCl and then chromatographed and the strips examined in the radiochromatogram scanner, compound X was found to have disappeared and had not been replaced by a new radioactive peak. It thus appeared that compound X was converted by dilute acid or alkali into a product volatile under the conditions of the experiment.

Attempted isolation of compound X. Three rabbits (body wt. 3kg) were each given daily for 3 days 25mg of (±)-amphetamine/kg orally. For location of compound X by radiochromatogram scanning, their urine (900ml) was mixed with urine (100ml) from a rabbit that had received similarly 20μCi of [^{14}C]amphetamine. The mixed urine was filtered through glass wool and then freeze-dried. The residue was extracted with 95% ethanol (4×100ml), and the extract filtered and evaporated *in vacuo* to 20ml. The solids, mainly urea, were filtered off and the filtrate used for preparative t.l.c. Portions (0.5ml each) of the filtrate were applied to 20 alumina plates (aluminium oxide-G; E. Merck A.-G., Darmstadt, Germany; 20cm×20cm×1mm thick), which were developed with pyridine–pentan-1-ol–water (7:7:6, by vol). The position of compound X was determined by scanning a suitable plate (5cm×20cm by 1mm thick) developed at the same time. The band with R_F 0.7–0.8 was scraped off the plates and compound X was eluted with aq. 90% ethanol. The eluates were evaporated *in vacuo* to 11ml, which was applied to five t.l.c. plates (2ml each) prepared as above. The plates were developed with solvent *C* (Table 1), in which compound X was found to have R_F 0.2–0.3. The appropriate area was scraped from the plates and eluted with aq. 90% ethanol. The eluates were filtered and evaporated to 1ml, which was again chromatographed on a t.l.c. plate with the first solvent (pyridine–pentan-1-ol–water) as above. Compound X was again eluted with aq. 90% ethanol, and the eluate was evaporated to 1ml, which was then freeze-dried to a pale-yellow solid (about 20mg).

Hydrolysis of compound X. The solid (4mg) in 5M-HCl (3ml) was heated under reflux at 100°C for 2h and after cooling part of the hydrolysate (2.5ml) was warmed with 2,4-dinitrophenylhydrazine (5mg) in ethanol (1ml). The precipitate that formed on cooling was filtered, washed with water and recrystallized from ethanol to give deep-yellow platelets of the 2,4-dinitrophenylhydrazone of 1-phenylpropan-2-one, m.p. and mixed m.p.152–153°C. In ethanol, the crystals showed light-absorption at $\lambda_{max.}$ 360nm and ~260nm, the known values found for the authentic compound (El Masry, Smith & Williams, 1956). The rest of the hydrolysate (0.5ml) was diluted with ethanol and the solution showed light-absorption with $\lambda_{max.}$ 260nm and ~285nm. In ethanol the ketone shows $\lambda_{max.}$ 260nm ($\epsilon_{max.}$ 249) and ~285nm ($\epsilon_{max.}$ 156) (Kumler, Strait & Alpen, 1950).

Another sample was hydrolysed as above and the hydrolysate examined in the amino acid auto-analyser (Locarte). Traces of glycine, arginine and cysteic acid were found, but in amounts that suggested that they were impurities. A sodium fusion test on compound X showed the presence of sulphur but not of nitrogen. When a small sample of compound X was warmed with 5M-HCl followed by a few drops of $BaCl_2$ solution a white precipitate formed, suggesting the presence of sulphate. At this point it appeared that compound X contained sulphate

Table 1. *R_F values and colour reactions of amphetamine and related compounds*

Descending chromatography on Whatman no. 1 paper was used. The solvent systems employed were: *A*, butan-1-ol–aq. 2% (w/v) citric acid (1:1, v/v); in this case the paper had been previously dipped in aq. 5% sodium dihydrogen citrate and then dried (Curry & Powell, 1954); *B*, butan-1-ol saturated with 1.5M-ammonia–ammonium carbonate buffer (Fewster & Hall, 1951); *C*, 3-methylbutan-1-ol–2-methylbutan-2-ol–water–formic acid (5:5:10:2, by vol.) (Alleva, 1963); *D*, butan-1-ol–toluene–acetic acid–water (2:2:1:1, by vol.). Chromatograms were run until the front had travelled 35cm from the origin. The diazotized nitroaniline spray was used as described by Wichstrom & Salversen (1952). All compounds appeared as dark spots under u.v. light (254nm, Hanovia Chromatolite lamp). Benzoic acid and 4-hydroxybenzoic acid were detected with Bromophenol Blue (Opieńska-Blauth, Sakławska-Szymonowa & Kanski, 1951), benzoyl glucuronide with naphtharesorcinol (cf. ridges, Kibby & Williams, 1965) and hippuric acid with *p*-dimethylaminobenzaldehyde (Gaffney, Schreier, DiFerrante & Altman, 1954).

Compound	R_F value				Colour with diazotized nitroaniline
	Solvent *A*	Solvent *B*	Solvent *C*	Solvent *D*	
Amphetamine	0.60	0.88	0.51	0.57	Pale pink
N-Methylamphetamine	0.61	0.92	0.57	0.57	None
N-Acetylamphetamine	—	0.98	0.98	0.98	None
4-Hydroxyamphetamine	0.45	0.79	0.32	0.30	Purple
4-Methoxyamphetamine	0.58	0.94	0.50	0.52	Pale pink
Norephedrine	0.45	0.82	0.39	0.44	Pale pink
4-Hydroxynorephedrine	0.27	0.64	0.17	0.08	Purple
2-Amino-1-(3′,4′-dihydroxyphenyl)propane	0.22	—	—	0.08	Pale pink slowly
2-Amino-1-(4′-hydroxy-3′-methoxyphenyl)-propane	0.32	—	—	0.29	Purple
2-Amino-1-(3′,4′-dimethoxyphenyl)propane	0.42	—	—	0.42	Pale pink
2-Amino-1-(3′,4′-dihydroxyphenyl)propan-1-ol	0.16	—	—	0.04	Green
Benzoic acid	0.97	0.43	0.93	—	None
Hippuric acid	0.85	0.30	0.82	—	None
Benzoyl glucuronide	—	0–0.2	0.60	—	None
4-Hydroxybenzoic acid	—	0–0.1	0.63	—	—

associated in some way with 1-phenylpropan-2-one. Since 1-phenylpropan-2-one is reported to occur to the extent of about 2.9% in the enol form (Gero, 1954), i.e. 1-phenylprop-1-en-2-ol, it was possible that compound X might be 1-phenylprop-1-en-2-yl hydrogen sulphate.

Formation of compound X from 1-phenylpropan-2-one in rabbits. 1-Phenyl[1-^{14}C]propan-2-one (2g; 1.1μCi) was fed to a doe rabbit (3kg) and a 72h urine sample was collected. Of the administered ^{14}C, 91% was excreted in 24h and a further 3% during the next 2 days. Samples (0.1ml) of the 24h urine were chromatographed on Whatman no. 1 paper strips with solvent *B* or *C* (Table 1). The strips were cut into 1.5cm pieces and their radioactivity was determined in the scintillation spectrometer. Four radioactive peaks were found as follows (the values in parentheses being the percentages of the ^{14}C on the paper): R_F in solvent *B*: 0.21 (12.4), 0.37 (37.6), 0.42 (30.5), 0.58 (10.5); R_F in solvent *C*: 0.48 (13.6), 0.63 (41.4), 0.79 (30), 0.26 (6). The first two peaks gave a positive naphtharesorcinol test for glucuronides. The first peak after acid hydrolysis (5M-HCl) also gave a positive ferric chloride test for a phenol. The first peak could thus be a glucuronide of a phenol derived from 1-phenylpropan-2-one, and the second peak a glucuronide of 1-phenylpropan-2-ol; such a glucuronide is a known metabolite of the ketone (El Masry *et al.* 1956). The third peak was identified by colour reactions as hippuric acid, also a known metabolite of the ketone. The fourth peak (R_F 0.58 in solvent *B* and R_F 0.26 in solvent *C*) corresponded to metabolite X of amphetamine urine.

An isotope dilution for 1-phenylpropan-2-one on a sample of the urine showed that none of the original ketone was present. However, after acid hydrolysis (with an equal volume of 10M-HCl for 2h at 100°C under reflux), isotope dilution showed that 14.9% of the dose was present as the ketone.

Injection of inorganic [^{35}S]sulphate. A rabbit was injected intraperitoneally with 250μCi of $Na_2{}^{35}SO_4$ (The Radiochemical Centre, Amersham, Bucks., U.K.) in 0.5ml of water and the 24h urine collected for use as a control. The next day the rabbit was given another injection of the same amount of $Na_2{}^{35}SO_4$ followed 1h later by an oral dose (2g) of 1-phenylpropan-2-one. The urine (65ml) was collected for the next 24h. Samples (0.01ml) of both urines were chromatographed on paper strips (Watman no. 1) in solvents *B* and *C*. After development and drying, the strips were scanned for ^{35}S. The urine, after the administration of 1-phenylpropan-2-one, showed two ^{35}S peaks not present in the control urine, of R_F 0.57 and 0.7 in solvent *B* and R_F 0.31 and 0.48 in solvent *C*. The R_F values of 0.57 in solvent *B* and 0.31 in solvent *C* correspond to those of metabolite X formed from [^{14}C]amphetamine and 1-phenyl[1-^{14}C]propan-2-one.

A portion (2ml) of the urine after treatment with $Na_2{}^{35}SO_4$ and the ketone was streaked across Whatman 3MM paper (39.7cm×42.3cm) and chromatographed in solvent *C*. The paper was dried and a suitable strip of it scanned to locate metabolite X, which was then eluted with 95% ethanol. Some of the eluate was then chromatographed on an alumina plate, when it showed a single

spot, R_F 0.18 in solvent *C* and R_F 0.7 in pyridine–pentan-1-ol–water (7:7:6, by vol.). The ethanol eluate was evaporated to dryness, the residue dissolved in water (1 ml) and the solution treated with 10% $Ba(NO_3)_2$ (1 ml) and 10% Na_2SO_4 (1 ml). The precipitated $BaSO_4$ was centrifuged and washed twice with water, but was found to contain no ^{35}S. The supernatant was mixed with an equal volume of 10M-HCl and heated under reflux for 2 h. The cooled solution was extracted with ether and then the ether carefully evaporated. The residue was treated with 2,4-dinitrophenylhydrazine (10 mg) in ethanol (1 ml) and 10M-HCl (0.05 ml). The mixture was extracted with $CHCl_3$ (25 ml) andthe extract washed with 2M-HCl (2×10 ml). The $CHCl_3$ was removed and the residue taken up in ethanol. The absorption spectrum of this solution showed $\lambda_{max.}$ 360 nm and ~260 nm, which corresponds to that of the 2,4-dinitrophenylhydrazone of 1-phenyl-propan-2-one. The supernatant left after ether extraction was now treated with carrier 10% Na_2SO_4 (1 ml) and 10% $Ba(NO_3)_2$ (1 ml) as before. The precipitate of $BaSO_4$ was found to contain ^{35}S with a specific radioactivity of 2 μCi/g.

Separation of the basic metabolites. The 24 h urine of rats given orally (±)-[^{14}C]amphetamine sulphate (10 mg/kg; 5 μCi/rat) was collected. The urine (5 ml) was brought to pH 5 with 2M-acetic acid and incubated at 37°C for 24 h with 5 ml of β-glucuronidase solution (Ketodase; Warner–Chilcott Laboratories). With phenolphthalein glucuronide as a control. The solution was then adjusted to pH 6 with M-ammonia, and 0.5 mg each of (±)-amphetamine, (±)-norephedrine, (±)-4-hydroxyamphetamine and (±)-4-hydroxynorephedrine was added. It was then passed through a column (1 cm × 11 cm) of the acid form of IRC-50 resin (standard grade) (recycled as described by Hirs, Moore & Stein, 1953) at the rate of 4 ml/h. The column was then washed with water (20 ml) and eluted with 2M-HCl (40 ml). The eluate was freeze-dried and the residue extracted with ethanol (2 ml) and portions (0.05–0.2 ml) of the extract were chromatographed on Whatman no. 1 paper with solvent *D* (Table 1). The basic metabolites on the chromatogram were detected by radiochromatogram scanning (see Table 1 for R_F values and colour reaction).

RESULTS

Man. The results for three male subjects are shown in Tables 2 and 3. In man, the ^{14}C is excreted largely (about 90%) in the urine, about 60%

Table 2. *Excretion of* ^{14}C *in man receiving* [^{14}C]*amphetamine orally*

Three subjects (R.S., L.D. and S.F.) took orally 5 mg of [^{14}C]amphetamine sulphate in water. The dose of ^{14}C was 6 μCi for the (±)-isomer and 2 μCi for the (+)- and (−)-isomers. ^{14}C was determined daily in the urine. Average values are given with ranges in parentheses.

	% of dose of ^{14}C found in the urine		
Day after dosing	(±)-Amphetamine	(+)-Amphetamine	(−)-Amphetamine
1	66 (61–73)	65 (63–72)	58 (54–63)
2	20 (17–24)	18 (14–20)	23 (17–29)
3	6.5, 7.6*	4.7, 4.1*	7.5 (7.1–8.0)
4	1.9†	2.4†	—
Total ^{14}C excreted	91 (89–94)	86 (83–89)	89 (87–90)

* Two subjects (L.D. and S.F.).
† One subject (L.D.).

Table 3. *Metabolites of* [^{14}C]*amphetamine found in human urine*

Doses and subjects were as in Table 2. The urine analysed was that collected for the first 24 h after dosing. The metabolites were determined by isotope dilution (see the text). Average values are given to the nearest two numbers with the individual values in the order R.S., G.D. and S.F., given in parentheses.

	% of dose excreted in 24 h as metabolites		
Metabolite sought	(±)-Amphetamine	(+)-Amphetamine	(−)-Amphetamine
Amphetamine (total)	30 (35, 23, 32)	34 (39, 25, 37)	34 (41, 22, 38)
4-Hydroxyamphetamine (total)	2.8 (0.5, 6.0, 1.9)	1.1 (0.7, 2.5, 0.1)	3.9 (0.3, 9.2, 2.3)
Benzoic acid (total)	21 (24, 22, 17)	21 (22, 22, 20)	13 (16, 13, 9)
Hippuric acid	16 (19, 17, 13)	– – – –	– – – –
1-Phenylpropan-2-one*	3.4 (2.0, 1.3, 6.8)	2.2 (1.8, 1.5, 3.4)	1.3 (1.0, 1.1, 1.7)
1-Phenylpropan-2-ol*	0.0 (0, 0, 0)	0.0 (0, 0, 0)	0.0 (0, 0, 0)
Sum of above metabolites	57 (62, 52, 58)	58 (63, 51, 61)	52 (58, 45, 52)
^{14}C in 24 h urine	66 (73, 61, 64)	65 (72, 63, 59)	58 (63, 53, 58)

* Determined after acid hydrolysis (see the text).

of the dose being excreted in the first 24h. There was little difference in ^{14}C excretion after administration of the three optical forms of amphetamine (Table 2). On examination of the first 24h urine by isotope dilution, the main radioactive excretory product is amphetamine, amounting to about 30–35% of the dose or about one-half of the 24h ^{14}C excretion, irrespective of the isomer given

Table 4. *Excretion of ^{14}C radioactivity by various species receiving [^{14}C]amphetamine*

The amphetamine sulphate was administered orally in water, except for the greyhounds and guinea pigs, which received the compound intraperitoneally. All animals were females. The results given are mean values with the ranges in parentheses.

Species	Dose of drug	Route of excretion	Days after dosing	% of ^{14}C in dose excreted (±)-Amphetamine	(+)-Amphetamine	(−)-Amphetamine
Rats (3)	10mg/kg	Urine	0–1	81 (79–82)	79 (72–90)	68 (60–76)
	[(±), 10μCi/kg;		0–3	86 (82–89)	88 (78–102)	81 (67–94)
	(+), 10μCi/kg;	Faeces	0–3	4.6 (4.1–5.6)	3.1 (2.2–3.8)	4.9 (4.3–5.3)
	(−), 10μCi/kg]		Total	90	91	86
Mice (3)	10mg/kg	Urine	0–1		78 (74–83)	77 (74–79)
	[(+), 25μCi/kg;		0–3	—	87 (79–96)	82 (77–87)
	(−), 65μCi/kg]	Faeces	0–3		0.7 (0.2–1.4)	3.8 (0.5–7.3)
			Total		87	86
Guinea pigs* (3)	5mg/kg	Urine	0–1		86 (84–87)	83 (80–84)
	[(+), 6μCi/kg;		0–2	—	88 (85–89)	85 (81–88)
	(−), 8μCi/kg]		Total		88	85
Rhesus					no. 1 no. 2	no. 1 no. 2
monkeys* (2)	0.66mg/kg	Urine	0–1		42 73	48 69
	[(+), 1.5μCi/kg;		2–3		16 7	11 13
	(−), 3μCi/kg]		Total		58 80	59 82
Rabbits (3)	10mg/kg	Urine	0–1	72 (54–82)		
	[(±), 10μCi/kg]		0–3	86 (57–108)	—	—
		Faeces	0–3	7.2 (1.7–16)		
			Total	94		
Greyhounds (3)	5mg/kg	Urine	0–1	75 (54–89)		
	[(±), 1μCi/kg]		0–3	78 (56–92)	—	—
		Faeces	0–3	0		
			Total	78		

* Faecal excretion was not examined in these species.

Table 5. *Metabolites of [^{14}C]amphetamine in rhesus monkeys*

See Table 4 for dosage. Two female monkeys, nos. 1 and 2, were used. The metabolites were determined in the urine of the first 24h after dosing by radiochromatogram scanning.

Metabolites sought	% of dose excreted (+)-Amphetamine No. 1	(+)-Amphetamine No. 2	(−)-Amphetamine No. 1	(−)-Amphetamine No. 2
Amphetamine (free)	3.8	31	23	34
(total)	3.8	31	23	34
4-Hydroxyamphetamine (free)	0	0	0	5
(total)	0	11	1	6
Benzoic acid (free)	5	18	14	19
Benzoylglucuronide	14	13	0	0
Hippuric acid	20	0	6	7
Benzoic acid (total)	38	31	20	27
1-Phenylpropan-2-one	0	0	0	0
1-Phenylpropan-2-ol	0	0	0	0
Sum of above metabolites*	42	73	44	67
Total ^{14}C in the urine	42	73	48	69

* Amphetamine + hydroxyamphetamine + total benzoic acid.

(Table 3). Table 3 gives the individual results for the three subjects, and it will be noted that subject G.D. is excreting less amphetamine and more *p*-hydroxyamphetamine than the other two. The significance, if any, of these differences is not clear. The other major metabolite is benzoic acid and its conjugates, which amount to about 20% of the dose for the (±)- and (+)-isomers and rather less (about 13%) for the (−)-isomer. Human urine also contains a substance that yields 1-phenylpropan-2-one on acid hydrolysis. The amount of this substance is small (1–3%), but the third subject (S.F.) is producing more than the other two from all three forms of the drug.

Rhesus monkey. Two female monkeys were used (Tables 4 and 5) and were given the labelled (+)- and (−)-isomer. Both isomers appeared to be treated similarly, but one monkey excreted the ^{14}C more rapidly than the other (Table 4). The composition of the 24h urine of these monkeys is shown in Table 5. The main metabolites are benzoic acid and its conjugates, and possibly less benzoic acid is produced from the (−)- than from the (+)-isomer. 4-Hydroxyamphetamine is a minor metabolite, although monkey no. 1 produced no hydroxyamphetamine from the (+)-isomer. Unchanged amphetamine is a major excretory product except in monkey no. 1 receiving (+)-amphetamine. Monkey urine did not contain a precursor of 1-phenylpropan-2-one.

Rat. In this species some 90% of the ^{14}C of each of the three isomers can be accounted for in 3 days, largely in the urine (Table 4). The main metabolite of amphetamine in the rat is 4-hydroxyamphetamine, which amounts to about 60% of the dose in 2 days for the (±)- and (−)-isomers and about 50% for the (+)-isomer (Table 6). The hydroxy compound is excreted largely as a conjugate that appears, from chromatographic studies before and after treatment of the urine with β-glucuronidase, to be a glucuronide. Benzoic acid and its conjugates are minor metabolites (about 2–3%), and amphetamine itself amounts to about 10–20%, its excretion being essentially the same for the three isomers. A precursor of 1-phenylpropan-2-one did not occur in rat urine and a number of other possible metabolites of amphetamine were absent.

The basic metabolites in rat urine were examined in detail in separate experiments. The chromatograms of the basic metabolites on being scanned for radioactivity showed five peaks of R_F 0.10, 0.30, 0.44, 0.57 and 0.98. The large peaks at R_F 0.30 and 0.57 were identified as 4-hydroxyamphetamine and amphetamine respectively. The minor peaks at R_F 0.10 and 0.44 corresponded to norephedrine and 4-hydroxynorephedrine (see Table 1). Their identity and amount were determined by isotope dilution

Table 6. *Metabolites of [^{14}C]amphetamine in the urine of various species*

Doses and animals were as in Table 4. The values for each metabolite are in percentages of dose excreted in the first 24h after dosing, except for the rat where a 48h urine was analysed. The metabolites for rat, rabbit and greyhound were determined by isotope dilution and for mouse and guinea pig by radiochromatogram scanning. The results in each column are the averages for three animals with ranges in parentheses. —, Not determined.

Species	Rat*			Mouse		Guinea pig		Rabbit	Greyhound
Optical form of drug	(±)	(+)	(−)	(+)	(−)	(+)	(−)	(±)	(±)
Metabolites sought									
Amphetamine (free)	11 (10–11)	12 (9–15)	15 (10–19)	—	—	—	—	—	—
(total)	13 (12–13)	14 (10–17)	17 (13–20)	33 (31–35)	28 (21–35)	22 (18–23)	15 (14–16)	3.5 (2.2–4.8)	30 (20–38)
4-Hydroxyamphetamine (free)	7.3 (3.4–11)	3.4 (2.1–5.8)	4.7 (3.7–5.9)	3.1 (1.2–6.6)	6.2 (0–12)	0	0	—	—
(total)	60 (58–62)	48 (44–50)	63 (58–67)	14 (10–19)	17 (10–21)	0	1.2 (0.5–2.0)	6.2 (5.0–7.6)	5.9 (5.1–7.1)
Benzoic acid (free)	0	0	0	0	0	12 (9–16)	40 (18–68)	—	—
Benzoyl glucuronide	0	0	0	8.5 (6.1–11)	12 (8.6–16)	29 (24–39)	10 (0–30)	—	—
Hippuric acid	3.3 (2.5–3.6)	2.2 (1.1–3.0)	2.4 (1.7–2.8)	23 (18–26)	21 (17–24)	21 (14–30)	18 (0–37)	10 (2–18)	20 (17–24)
Benzoic acid (total)	3.3 (2.5–3.6)	2.2 (1.1–3.0)	2.4 (1.7–2.8)	31 (23–37)	32 (28–37)	62 (55–67)	68 (67–68)	25 (17–35)	28 (20–32)
1-Phenylpropan-2-one	0	0	0	0	0	0	0	22 (18–25)	1.4 (0–2.5)
1-Phenylpropan-2-ol	0	0	0	0	0	0	0	7.5 (6.5–7.9)	1 (0–2)
Sum of above metabolites	76	64	82	78	77	84	84	64	66
Total ^{14}C in the urine	85	85	80	78	77	83	83	72	75

* See the text for norephedrine and 4-hydroxynorephedrine in rat urine.

(see the Materials and Methods section). The amount of norephedrine found was 0.3% of the dose in each of two rats, and of 4-hydroxynorephedrine 0.3% in one rat, no carrier being available for an estimation in the second rat. The minor peak at R_F 0.98 also had this value in solvents *B* and *C* (Table 1), and it corresponded to *N*-acetylamphetamine in amounts of about 1–2% of the dose (Table 6 shows that 2% of the amphetamine excreted by the rat is in a combined form). Identification of *N*-acetylamphetamine has not been confirmed, since the compound showed no colour reactions and its melting point, which we found to be 66–67°C (see Hey, 1930) after long desiccation, was unreliable for isotope dilution.

Rats receiving [^{14}C]amphetamine were also kept in a chamber suitable for collecting expired air. No radioactive CO_2 was detected in the expired air in two separate experiments.

Mouse. In the mouse, over 80% of the ^{14}C is excreted in the urine (Table 4). But the mouse differs from the rat in that 4-hydroxyamphetamine is not the major metabolite in the urine, although it is present in appreciable amounts (10–19%) compared with other species. In the mouse, the main excretory products are amphetamine (about 30%) and benzoic acid and its conjugates (about 30%). Mouse urine, like that of the rat, contains no precursor of 1-phenylpropan-2-one (Table 6).

Guinea pig. In the guinea pig, the major metabolites are benzoic acid and its conjugates, which amount to 60–70% of the dose (Table 6). No 4-hydroxyamphetamine is formed from the (+)-isomer, but about 1% (conjugated) is formed from the (−)-isomer. Amphetamine itself is also excreted, more of the (+)-isomer (22%) being excreted unchanged than the (−)-isomer (15%); possibly the (−)-isomer is metabolized more readily in this animal than the (+)-isomer. The guinea pig did not excrete a precursor of 1-phenylpropan-2-one.

Rabbit. The metabolism of (±)-amphetamine in the rabbit differs from that in most of the other species (Table 6). There is little unchanged amphetamine (3%) or 4-hydroxyamphetamine (6%) excreted. The main metabolites are benzoic acid and its conjugates (25%) and a precursor of 1-phenylpropan-2-one (22%) (see below). Some conjugated 1-phenylpropan-2-ol (7%) is also excreted.

Greyhound. The excretory products of (±)-amphetamine are very similar to those found in man (Table 6).

DISCUSSION

The quantitative aspects of the main metabolites of amphetamine in the urine of various species are summarized in Table 7, in which the metabolites of (±)- or (+)-amphetamine are compared. This seems permissible, since the quantitative differences in the metabolism of the optical forms are not very marked within a species. Table 7 shows that there are considerable species differences in the metabolism of amphetamine, but it must be kept in mind that man and the rhesus monkey received much smaller doses than the other four species.

Man, monkey and dog appear to metabolize the drug in an approximately similar manner. In these species, about half of the excreted drug is unchanged. Although *N*-methyl- and *N*-acetylamphetamine are possible metabolites, chromatographic studies suggested that they were probably absent from the urine of these species. An isotope dilution for *N*-methylamphetamine in human urine

Table 7. *Summary of the quantitative aspects of the main metabolites of* [^{14}C]*amphetamine in the urine of different species*

The values are quoted to the nearest whole number for comparison and taken from Tables 3, 5 and 6.

		% of dose excreted in 24 h						
	Species ...	Man	Rhesus monkey*	Dog†	Rabbit	Rat‡	Mouse	Guinea pig†
	Isomer	(±)	(+)	(±)	(±)	(±)	(+)	(+)
Metabolite	Oral dose (mg/kg) ...	0.07	0.66	5	10	10	10	5
Amphetamine		30	31	30	4	13	33	22
4-Hydroxyamphetamine		3	11	6	6	60	14	0
Benzoic acid		21	31	28	25	3	31	62
1-Phenylpropan-2-one§		3	0	1	22	0	0	0
1-Phenylpropan-2-ol		0	0	1	7	0	0	0
Total		57	73	66	64	76	78	84

* Values for monkey no. 2 (see Table 5).
† Injected intraperitoneally.
‡ Values for 48 h urine.
§ Derived from a precursor (see the text).

gave a negative result. Amphetamine was 4-hydroxylated to a minor extent (1–6%). The major transformation product in these species was benzoic acid and its conjugates. A precursor of 1-phenylpropan-2-one was found in small amounts in human and dog urine, but not in monkey urine.

The four other species (Table 7), which received comparable doses, differ from one another and from man, monkey and dog. In the rat, the major metabolite in the urine is 4-hydroxyamphetamine conjugated with glucuronic acid. Aromatic hydroxylation is thus the major metabolic reaction of the drug in the rat, while deamination and oxidation to benzoic acid are relatively minor. Rats also excrete some unchanged amphetamine (11%) together with what appears chromatographically to be *N*-acetylamphetamine (about 2%). The rat produced no 1-phenylpropan-2-one precursor. It has been reported that (+)-amphetamine [but not the (−)-isomer] and 4-hydroxyamphetamine are substrates for dopamine (3,4-dihydroxyphenethylamine) β-hydroxylase of ox adrenal glands (Goldstein & Contrera, 1962; Goldstein, McKereghan & Lauber, 1964), and Sjoerdsma & von Studnitz (1963) have shown that 4-hydroxyamphetamine is converted to a small extent in man into 4-hydroxynorephedrine, a reaction that also occurs in mice (Iversen, Glowinski & Axelrod, 1966). The rat urine was therefore examined by isotope dilution for the products of the hydroxylation of the methylene group of amphetamine and its 4-hydroxy derivative. Small amounts of conjugated 2-amino-1-phenylpropan-1-ol (norephedrine) (0.3% of the dose in each of two rats) and of 2-amino-1-(4′-hydroxyphenyl)propan-1-ol (4-hydroxynorephedrine) (0.3% in one rat) were found.

In the guinea pig, the metabolism of amphetamine appears to be relatively simple. The major route of metabolism leads to benzoic acid and its conjugates. About 22% of the drug is excreted unchanged. Hydroxylation does not occur to any appreciable extent with the (+)-isomer, but about 1% of the (−)-isomer was hydroxylated. There appears to be no 1-phenylpropan-2-one precursor.

The metabolism of amphetamine in the mouse might be expected to be similar to the rat, but Table 7 shows that this is not so. There is a high excretion of unchanged amphetamine and of benzoic acid, both of which are excreted in low amounts in the rat. Although the mouse is a better hydroxylator of amphetamine than the other species except the rat, the extent of hydroxylation is only about one-quarter of that in the rat.

The rabbit appears to be unique among the species studied, since it excretes little unchanged amphetamine or 4-hydroxyamphetamine. The major route of metabolism is by deamination, leading to 1-phenylpropan-2-one and the corresponding carbinol, and benzoic acid. The ketone was not found as such in the urine, but as an acid-labile precursor, compound X. The latter was not isolated in a pure form, but it was shown to contain 1-phenylpropan-2-one and sulphate. The report in the literature (Gero, 1954) that the ketone occurs to a small extent in the enol form suggests that X might be a salt of 1-phenylprop-1-en-2-yl hydrogen sulphate, $C_6H_5 \cdot CH : C(O \cdot SO_3H) \cdot CH_3$.

The n.m.r. spectrum of compound X in D_2O was consistent with the structure $C_6H_5 \cdot CH : \overset{|}{C} \cdot CH_3$, the τ values being 2.66, 2.76 (phenyl), 2.55 (CH) and 7.6 (CH_3) and the corresponding relative integrated intensities being 5:1:3. The n.m.r. spectrum of 1-phenylpropan-2-one, $C_6H_5 \cdot CH_2 \cdot CO \cdot CH_3$, has been recorded (Sadtler Research Laboratories, 1966), the τ values being 2.8 (phenyl), 6.42 (CH_2) and 7.98 (CH_3) and the corresponding intensities being 5:2:3. The n.m.r. spectrum of 1-phenylprop-1-ene, $C_6H_5 \cdot CH : CH \cdot CH_3$, was recorded for us, but the interpretation was rendered difficult since the compound was a mixture of *cis* and *trans* isomers. However, the doublet with τ values 8.25 and 8.35 due to the CH_3 in the system $=CH \cdot CH_3$ was obvious. A little of compound X was methylated in methanol with diazomethane and the product subjected to mass spectrometry. There was a very small peak at m/e 227, which could correspond to the molecular ion, $C_6H_5 \cdot CH : C(O \cdot SO_3\overset{+}{C}H_2) \cdot CH_3$. According to Reisch, Pagnucco, Alfes, Jantos & Möllmann (1968), who studied the mass spectra of a number of phenylalkylamines including amphetamine, the molecular ion of these compounds either does not appear or is very small in amount. Attempts to synthesize the enol sulphate of 1-phenylpropan-2-one have so far been unsuccessful, so that further support for the tentative structure suggested for compound X is not yet forthcoming. However, Roy (1960) has postulated the formation of 17-enol sulphates of steroids to account for the increased rate of synthesis in the presence of certain 17-oxo steroids of 2-naphthyl sulphamate from 2-naphthylamine in rat liver preparations. Such enol sulphates could behave as 'active sulphate'.

(+)- and (−)-Amphetamine show different pharmacological properties. The acute toxicity of (+)-amphetamine in mice is about ten times that of the (−)-isomer (Fairchild & Alles, 1967) and it is possible that a difference in the metabolic fate of the isomers might be involved in the difference in pharmacological activity. It has been reported that in man and the rat more of the (−)-isomer is excreted unchanged than the (+)-isomer when the (±)-isomer is administered (Gunne, 1967; Gunne & Galland, 1967). Our results in man (Table 3) show that during the first 24h after dosing, the amount of amphetamine excreted unchanged is about the

same for both optically active isomers when given separately. The differences may not be significant in rat, mouse and guinea pig, for the rat excretes in 48h slightly more (−)-isomer than (+)-isomer, whereas the mouse and guinea pig excrete in 24h slightly more (+)- than (−)-amphetamine. More 4-hydroxyamphetamine is excreted after (−)-amphetamine administration in man (Table 3), rat and mouse (Table 6), whereas in the guinea pig 4-hydroxyamphetamine was detected in the urine only after dosing with (−)-amphetamine (Table 6). This could mean that (−)-amphetamine is more readily 4-hydroxylated than (+)-amphetamine, or that both isomers are 4-hydroxylated equally well, but that the 4-hydroxyamphetamine formed from (+)-amphetamine is more readily further metabolized than that from (−)-amphetamine. It is known that (+)-4-hydroxyamphetamine but not its (−)-isomer is a substrate for the dopamine β-hydroxylase of the rat (Goldstein & Anagnoste, 1965). Since 4-hydroxyamphetamine is a major metabolite in the rat, but not in other species, further metabolites of 4-hydroxyamphetamine might be more likely to be found in the rat than the other species. This point, however, has not been investigated, although 4-hydroxynorephedrine has been found as a minor metabolite in the urine of the rat (Table 6), but other species were not examined.

This paper shows that there are wide species variations in the metabolism of amphetamine, but these variations cannot be correlated with any possible species variations in the activity of the drug, since no reliable information on this point is available, except that the drug appears to be more toxic to the rat and mouse than to the rabbit (see Spector, 1956).

This work was supported by a grant from Shell Research Ltd.

REFERENCES

Alleva, J. J. (1963). *J. mednel Chem.* **6**, 621.

Asatoor, A. M., Galman, B. R., Johnson, J. R. & Milne, M. D. (1965). *Br. J. Pharmac. Chemother.* **24**, 293.

Axelrod, J. (1954*a*). *J. Pharmac. exp. Ther.* **110**, 2.

Axelrod, J. (1954*b*). *J. Pharmac. exp. Ther.* **110**, 315.

Axelrod, J. (1955). *J. biol. Chem.* **214**, 753.

Beckett, A. H. & Rowland, M. (1965). *J. Pharm. Pharmac.* **17**, 628.

Blackburn, D. & Burghard, G. (1965). *J. pharm. Sci.* **54**, 1586.

Bridges, J. W., Davies, D. S. & Williams, R. T. (1967). *Biochem. J.* **105**, 1261.

Bridges, J. W., Kibby, M. R. & Williams, R. T. (1965). *Biochem. J.* **96**, 829.

Curry, A. S. & Powell, H. (1954). *Nature, Lond.*, **173**, 1143.

Dring, L. G., Smith, R. L. & Williams, R. T. (1966). *J. Pharm. Pharmac.* **18**, 402.

Dring, L. G., Smith, R. L. & Williams, R. T. (1968). *Biochem. J.* **109**, 10P.

Ellison, T., Gutzait, L. & Van Loon, E. J. (1966). *J. Pharmac. exp. Ther.* **152**, 383.

Ellman, G. L., Burkhalter, A. & La Dou, J. (1961). *J. Lab. clin. Med.* **57**, 813.

El Masry, A. M., Smith, J. N. & Williams, R. T. (1956). *Biochem. J.* **64**, 50.

Fairchild, M. D. & Alles, G. A. (1967). *J. Pharmac. exp. Ther.* **158**, 135.

Fewster, M. E. & Hall, D. A. (1951). *Nature, Lond.*, **168**, 78.

Fodor, G. (1943). *Ber. dt. chem. Ges.* **76**, 1216.

Gaffney, G. W., Schreier, K., DiFerrante, N. & Altman, K. I. (1954). *J. biol. Chem.* **206**, 695.

Gero, A. (1954). *J. org. Chem.* **19**, 1960.

Goldstein, M. & Anagnoste, B. (1965). *Biochim. biophys. Acta*, **107**, 166.

Goldstein, M. & Contrera, J. F. (1962). *J. biol. Chem.* **237**, 1898.

Goldstein, M., McKereghan, M. R. & Lauber, E. (1964). *Biochim. biophys. Acta*, **89**, 191.

Gunne, L. M. (1967). *Biochem. Pharmac.* **16**, 863.

Gunne, L. M. & Galland, L. (1967). *Biochem. Pharmac.* **16**, 1374.

Hey, D. H. (1930). *J. chem. Soc.* p. 18.

Hirs, C. H. W., Moore, S. & Stein, W. H. (1953). *J. biol. Chem.* **200**, 493.

Hoover, F. W. & Hass, H. B. (1947). *J. org. Chem.* **12**, 501

Iversen, L. L., Glowinski, J. & Axelrod, J. (1966). *J. Pharmac. exp. Ther.* **151**, 273.

Knight, R. H. & Young, L. (1958). *Biochem. J.* **70**, 111.

Kratzl, K. & Schweers, W. (1956). *Ber. dt. chem. Ges.* **89**, 186.

Kumler, W. D., Strait, L. A. & Alpen, E. L. (1950). *J. Am. chem. Soc.* **72**, 1463.

Le Brazidec, E. (1922). *Bull. Soc. chim. Fr.* **31**, 258.

Mannich, C. & Jacobsohn, W. (1910). *Ber. dt. chem. Ges.* **43**, 189.

Opieńska-Blauth, J., Sakláwska-Szymonowa, O. & Kanski, M. (1951). *Nature, Lond.*, **168**, 511.

Reisch, J., Pagnucco, R., Alfes, H., Jantos, N. & Möllmann, H. (1968). *J. Pharm. Pharmac.* **20**, 81.

Robinson, R., Lowe, A. & Imperial Chemical Industries Ltd. (1940). *Br. Patent* 519894.

Roy, A. B. (1960). *Biochem. J.* **74**, 49.

Sadtler Research Laboratories (1966). *Nuclear Magnetic Resonance Spectra: Phenylacetone*, no. 1855 M. Philadelphia: Sadtler Research Laboratories.

Smith, J. N., Smithies, R. H. & Williams, R. T. (1954). *Biochem. J.* **57**, 74.

Sjoerdsma, A. & von Studnitz, W. (1963). *Br. J. Pharmac. Chemother.* **20**, 278.

Späth, E., Orechoff, A. & Kuffner, F. (1934). *Ber. dt. chem. Ges.* **67**, 1214.

Spector, W. S. (Ed.) (1956). *Handbook of Toxicology*, vol. 1, p. 40. Philadelphia and London: W. B. Saunders Co.

Van Dijk, J. & Moed, H. D. (1959). *Recl Trav. chim. Pays-Bas Belg.* **78**, 22.

Wichstrom, A. & Salversen, B. (1952). *J. Pharm. Pharmac.* **4**, 631.