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The Metabolites of Cyclohexylamine in Man and Certain Animals

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1. [1-^{14}C]Cyclohexylamine hydrochloride was synthesized and given orally or intraperitoneally to rats, rabbits and guinea pigs (dose 50–500mg/kg) and orally to humans (dose 25 or 200mg/person). The ^{14}C is excreted mainly in the urine, most of the excretion occurring in the first day after dosing. Only small amounts (1–7%) are found in the faeces. 2. In the rat, guinea pig and man, the amine is largely excreted unchanged, only 4–5% of the dose being metabolized in 24h in the rat and guinea pig and 1–2% in man. In the rabbit about two-thirds of the dose is excreted unchanged and about 30% is metabolized. 3. In the rat, five minor metabolites were found, namely cyclohexanol (0.05%), *trans*-3- (2.2%), *cis*-4- (1.7%), *trans*-4- (0.5%) and *cis*-3-aminocyclohexanol (0.1% of the dose in 24h). 4. In the rabbit, eight metabolites were identified, namely cyclohexanol (9.3%), *trans*-cyclohexane-1,2-diol (4.7%), cyclohexanone (0.2%), cyclohexylhydroxylamine (0.2%) and *trans*-3- (11.3%), *cis*-3- (0.6%), *trans*-4- (0.4%) and *cis*-4-aminocyclohexanol (0.2%). 5. In the guinea pig, six minor metabolites were found, namely cyclohexanol (0.5%), *trans*-cyclohexane-1,2-diol (2.5%) and *trans*-3- (1.2%), *cis*-3- (0.2%), *trans*-4- (0.2%) and *cis*-4-aminocyclohexanol (0.2%). 6. In man only two metabolites were definitely identified, namely cyclohexanol (0.2%) and *trans*-cyclohexane-1,2-diol (1.4% of the dose), but man had been given a smaller dose (3mg/kg) than the other species (50mg/kg). 7. The hydroxylated metabolites of cyclohexylamine were excreted in the urine in both free and conjugated forms. 8. Although cyclohexylamine is metabolized to only a minor extent, in rats the metabolism was mainly through hydroxylation of the cyclohexane ring, in man by deamination and in guinea pigs and rabbits by ring hydroxylation and deamination.

Cyclohexylamine has several industrial uses (Browning, 1953) but in recent years it has become of considerable toxicological interest because it has been found in the urine of man and animals as a metabolite of the sweetening agent cyclamate (Kojima & Ichibagase, 1966; Leahy *et al.*, 1967; Oser *et al.*, 1968; Renwick & Williams, 1969; Prosky & O'Dell, 1970), and because it was suspected of being a weak carcinogen (Price *et al.*, 1970). Its metabolism has not been fully investigated. Bernhard (1937) gave it orally and injected it into dogs and concluded that it was probably completely destroyed in these animals. Elliott *et al.* (1968) briefly reported that in rabbits much of the base was excreted unchanged and that *N*-hydroxycyclohexylamine was a minor metabolite. It was further briefly reported by Sonders *et al.* (1968) that, in the only human subject tested, the base was almost entirely excreted unchanged in the urine.

Cyclohexylamine is a fairly strong base (pK_a 10.6) and therefore it is unlikely to be readily metabolized (see Williams, 1970). In the present paper it is shown that in man, rat and guinea pig, cyclohexylamine, as expected, is excreted unchanged in the urine to the extent of 90% or more, but in the rabbit some 20–30% of the dose may be metabolized. Minor metabolites of cyclohexylamine have been found in various numbers and amounts in the urine of the four species and they include cyclohexanol, cyclohexanone, *trans*-cyclohexane-1,2-diol, *cis*-3-, *trans*-3-, *cis*-4- and *trans*-4-hydroxycyclohexylamine and probably *N*-hydroxycyclohexylamine.

Materials and Methods

Chemicals

Cyclohexylamine (Fisons Scientific Apparatus Ltd., Loughborough, Leics., U.K.), cyclohexanol, *trans*-2-chlorocyclohexanol (Koch–Light Laboratories, Colnbrook, Bucks., U.K.), cyclohexanone (British Drug Houses Ltd., Poole, Dorset, U.K.), *trans*-cyclohexane-1,2-diol (Aldrich Chemical Co., Milwaukee, Wis., U.S.A.) and an aq. 50% (w/v) 4-aminocyclohexanol soln. (K. & K. Laboratories Inc., Plainview, N.Y., U.S.A.) were purchased. Cyclohexylamine hydrochloride, m.p. 203°C, was prepared from cyclohexylamine in acetone and 11M-HCl and recrystallized from acetone.

[1-^{14}C]Cyclohexylamine. [1-^{14}C]Cyclohexanone (10mg; 1mCi; International Chemical and Nuclear Corp., City of Industry, Calif., U.S.A.) was mixed with unlabelled cyclohexanone (1g). The ketone was

added drop by drop with vigorous shaking to a solution of hydroxylamine hydrochloride (1g) and sodium acetate (1.6g) in water (4ml) and then warmed to 40°C. The mixture was cooled in ice and the crystalline cyclohexanone oxime that separated (0.55g) was filtered off and dried overnight *in vacuo* over anhydrous $CaCl_2$. The oxime was dissolved in dry ethanol (15ml), and sodium metal (1.4g) was added in small pieces at such a rate that the reaction could be controlled. After dissolution of the sodium, water (20ml) was added and the cyclohexylamine steam-distilled into 6M-HCl (3ml). The distillate was adjusted to pH12 with 10M-NaOH, saturated with NaCl and extracted with ether (5×25ml). The extract was dried (anhydrous Na_2SO_4), filtered and neutralized with a solution of hydrogen chloride in ether. The ether solution was evaporated at reduced pressure to 10ml and acetone (10ml) added. The [1-^{14}C]cyclohexylamine hydrochloride crystals that separated were filtered, washed with acetone and dried *in vacuo* for 24h over anhydrous $CaCl_2$. The yield was 0.51g (specific radioactivity 0.484μCi/mg) or 37% of theory and the crystals had m.p. 201°C [Wallach (1905) gives m.p. 203–204°C]. Further material of lower specific radioactivity was obtained by working up the mother liquors. The compound was characterized by i.r. spectroscopy and on chromatography in solvents A, B and C (see Table 1) it gave a single spot. Its purity was 98.9% as determined by isotope dilution.

A small quantity of *N*-acetyl[1-^{14}C]cyclohexylamine, m.p. 105°C, was prepared by standard methods from [^{14}C]cyclohexylamine for use in paper chromatography, since the unlabelled acetyl derivative had no sensitive colour reaction for its detection. *N*-Benzoylcyclohexylamine, m.p. 148°C, was prepared by standard methods.

Cyclohexylglucuronide was prepared as a gum for chromatography by feeding rabbits with cyclohexanol as described by Elliott *et al.* (1959). The gum, which was mainly cyclohexylglucuronide, contained some *trans*-cyclohexane-1,2-diol monoglucuronide (see Table 1).

Cyclohexylhydroxylamine, m.p. 140–141°C from water, was prepared as described by List (1957).

trans-2-Aminocyclohexanol. The hydrochloride of this compound, m.p. 171°C, was prepared from *trans*-2-chlorocyclohexanol as described by Zinner & Schritt (1962). Lapporte & Ferstandig (1961) gave m.p. 171–172°C.

cis- and trans-4-Aminocyclohexanol. The commercial aq. 50% 4-aminocyclohexanol soln. (12g) was mixed with water (20ml) and acetylated by shaking the solution with acetic anhydride (6g). The solution was adjusted to pH12 with 10M-NaOH, saturated with NaCl and extracted with ether (4× 50ml). The ether was evaporated and the dry solid fractionally recrystallized from acetone to separate the isomers.

trans-4-Acetamidocyclohexanol (1g) had m.p. 163–165°C and the *cis*- isomer had m.p. 105°C. Billman & Buehler (1953) gave m.p. 165–167°C for the *trans* isomer and Hartmann *et al.* (1939), m.p. 135°C, for the *cis* isomer. Each isomer (20mg) was then refluxed for 4h with 1M-NaOH (10ml). The solution was saturated with NaCl and continuously extracted with ether. The ether solutions of the free 4-aminocyclohexanols were examined by g.l.c. (see below). The *trans*-4-acetamidocyclohexanol was found to contain 4% *cis* isomer, and the *cis* product contained 29% of *trans* isomer.

cis- and trans-3-Aminocyclohexanol. 3-Acetamidophenol (20g) in methanol (75ml) was hydrogenated for 100h at a pressure of 35lb/in^2 (241kN·m^{-2}) at room temperature by using a 5% rhodium–alumina catalyst (Freifelder *et al.*, 1965). Analysis by g.l.c. showed that the reduction was essentially complete, and the solution was filtered and evaporated to dryness *in vacuo*. The 3-acetamidocyclohexanols were hydrolysed with 2.5M-NaOH and the free amines separated as described by Burckhardt *et al.* (1967). *trans*-3-Aminocyclohexanol was obtained as the white crystalline hydrogen oxalate (6g), m.p. 193–195°C, and *cis*-3-aminocyclohexanol as a gum of its hydroperchlorate salt [Burckhardt *et al.* (1967) gave m.p. 193–194°C for the *trans* salt and 144–145°C for the *cis* salt]. By g.l.c. analysis, the *trans* isomer contained 5% of the *cis* form, and the gummy *cis* isomer salt contained 33% of the *trans* form.

Animals

The animals used were Wistar albino rats, albino guinea pigs (Duncan–Hartley strain) and New Zealand White rabbits, which were kept on an appropriate diet. Cyclohexylamine hydrochloride in water was administered by stomach tube or by intraperitoneal injection. The animals were kept in appropriate metabolism cages, which allowed the separate collection of urine and faeces.

Collection of expired air. The collection of CO_2 from the expired air of the animals was carried out essentially as described by Elliott *et al.* (1959).

Isotope-dilution methods

Radioactivity in urine and faeces and in isotope-dilution procedures was determined with a Packard Tri-Carb liquid-scintillation spectrometer (model 3214 or 3320) by using the dioxan scintillator fluid used in this laboratory (see Bridges *et al.*, 1967).

Cyclohexylamine. To the urine (0.5–80ml) was added 0.5–1.0g of cyclohexylamine hydrochloride. It was adjusted to pH12 with 10M-NaOH, saturated with NaCl and extracted with dichloromethane (3× 25ml). The extract was shaken with 2M-HCl (20ml), the acid layer separated and adjusted to pH12 with

10M-NaOH. The solution was then treated with 10M-NaOH (1ml) and benzoyl chloride (0.5–1.0ml) and shaken for 5min. The *N*-benzoylcyclohexylamine (m.p. 149°C) that separated was recrystallized to constant specific radioactivity from aq. ethanol.

Cyclohexanol. The urine (1.5–250ml) was treated with redistilled cyclohexanol (1g) and 2–12M-HCl (25ml) depending on the volume of urine. The solution was heated under reflux for 4h and, after cooling, was extracted with ether (4×20ml). The ether layer was dried (anhydrous Na_2SO_4), filtered and evaporated under reduced pressure. The residual cyclohexanol was dissolved in light petroleum (b.p. 80–100°C; 50ml) and the solution dried (anhydrous Na_2SO_4) and filtered. α-Naphthyl isocyanate (1.45ml) was added and the whole refluxed for 3h. The cyclohexyl α-naphthylurethane, m.p. 130°C, was recrystallized to constant specific radioactivity from aq. ethanol.

Cyclohexylhydroxylamine. The carrier (0.5 or 1g) and 2–12M-HCl (25ml) were added to the urine (1.5–250ml) and the solution refluxed for 4h. After cooling and adjustment to pH8 with 10M-NaOH, the mixture was extracted with chloroform (4× 25ml). After drying (anhydrous Na_2SO_4), the solution was evaporated at 35°C under reduced pressure and the residue recrystallized from light petroleum (b.p. 80–100°C) to constant specific radioactivity. It had m.p. and mixed m.p. 139°C and was characterized by its i.r. spectrum.

Cyclohexanone. The carrier (1g) was added to the urine sample (2–250ml) and the mixture adjusted to 2M-HCl with 12M-HCl and refluxed for 4h. After cooling, the solution was extracted with ether (4× 25ml), the extract was dried (anhydrous Na_2SO_4) and evaporated at 30°C. The residual ketone was converted into its semicarbazone, m.p. 166°C, and recrystallized from light petroleum (b.p. 100–120°C) to constant specific radioactivity.

Chromatography and mass spectrometry

Paper chromatography. The R_F values and colour reactions of cyclohexylamine and some of its possible metabolites are shown in Table 1. This table also contains the values for cyclamate, which is considered in the next paper (Renwick & Williams, 1972). Urine containing about 2×10^4 d.p.m. from animals dosed with [^{14}C]cyclohexylamine was chromatographed as a band (3cm wide) on a strip of Whatman no. 1 paper

Table 1. *R_F values and colour reactions of cyclamate, cyclohexylamine and related compounds*

The descending technique on Whatman no. 1 paper was used. Solvents: A, butan-1-ol–acetic acid–water (4:1:2, by vol.); B, propan-1-ol–aq. NH_3 soln. (sp.gr. 0.88) (7:3, v/v); C, butan-1-ol–propan-2-ol–water–acetic acid (8:4:2:1, by vol.); D, butan-1-ol–aq. NH_3 soln. (sp.gr. 0.88)–water (10:1:1, by vol.). The following sprays were used: (1) 0.2% ninhydrin in propan-2-ol containing 1% acetic acid, then drying and heating the paper in an oven at 120°C for 5min; (2) a solution of 0.1% $AgNO_3$ and 0.1% pyrogallol in 1M-HNO_3, then heating the paper in an oven at 120°C for 5–10min (Ko *et al.*, 1959); (3) 2% naphtharesorcinol in aq. 33% (w/v) trichloroacetic acid, then heating at 120°C for up to 10min. Abbreviations: v, volatilizes in alkaline solvents; h, hydrolyses, owing to instability in acid solvents; —, not determined.

Compound Solvent ...	R_F A	B	C	D	Colour reactions Spray (1)	Spray (2)	Spray (3)
Cyclamate	0.66	0.76	0.44	0.39	None	Orange on yellow background	—
Cyclohexylamine	0.79	v	0.62	v	Blue	—	—
N-Acetylcyclohexylamine*	0.91	0.85	—	0.86	None	—	—
N-Cyclohexylhydroxylamine	0.88	—	—	0.86	Pale blue	—	—
Cyclohexylglucuronide	0.72	0.62	0.58	0.17	None	—	Blue
trans-Cyclohexane-1,2-diol monoglucuronide	0.53	0.41	0.41	0.09	None	—	Blue
Cyclohexylamine-*N*-glucuronide†	h	0.10	h	0.00	—	—	Blue
trans-2-Aminocyclohexanol	0.57	0.81	0.53	0.82	Blue	—	—
cis-3-Aminocyclohexanol	0.59	0.78	0.55	0.70	Blue	—	—
trans-3-Aminocyclohexanol	0.43	0.78	0.43	0.72	Blue	—	—
cis-4-Aminocyclohexanol	0.40	0.78	0.42	0.68	Blue	—	—
trans-4-Aminocyclohexanol	0.49	0.78	0.50	0.68	Blue	—	—

* Detected by using the ^{14}C-labelled compound.
† Prepared as described by Takitani (1959).

Table 2. *Gas–liquid chromatography of trimethylsilyl derivatives of cyclohexylamine and related compounds*

A 5ft (1.54m) glass column (3mm internal diam.) packed with silicone gum rubber E 301 (3%) on Chromosorb G (100–120) was used. Column temp. 100°C; detector temp. 160°C; flash heater temp. 150°C; hydrogen pressure, 20lb/in² (138kN·m⁻²); air pressure, 25lb/in² (172kN·m⁻²); nitrogen pressure, 40lb/in² (276kN·m⁻²), flow rate, 38ml/min.

Compound	Retention time (min)
Cyclohexanol	2
Cyclohexylamine	3
trans-Cyclohexane-1,2-diol	11
trans-2-Aminocyclohexanol	16
cis-3-Aminocyclohexanol	19
trans-3-Aminocyclohexanol	15
cis-4-Aminocyclohexanol	21
trans-4-Aminocyclohexanol	25

(5cm wide) with the solvents given in Table 1 and the front was allowed to move 35±5cm from the origin. The ^{14}C peaks were located with a Packard radiochromatogram scanner (model 7200) and the ^{14}C activity was measured by cutting the chromatogram into suitable pieces and counting them in a Packard Tri-Carb liquid-scintillation spectrometer (model 3214 and 3320).

Gas–liquid chromatography. A urine sample from individuals given the larger doses of cyclohexylamine (see Table 5), containing the equivalent of about 50mg of [^{14}C]cyclohexylamine, was made 2M with respect to HCl by adding 12M-HCl. It was heated under reflux for 4h to hydrolyse conjugates. The hydrolysed urine was adjusted to pH12 with 10M-NaOH, saturated with NaCl and continuously extracted with ether for 16h. A portion of the ether solution containing the equivalent of about 20mg of cyclohexylamine was evaporated at 30°C under reduced pressure until just dry and the residue was immediately dissolved in 1ml of ether. The recovery of ^{14}C at each stage was determined. A portion of the last ether solution (0.1ml) was treated with bis(trimethylsilyl)acetamide (0.1ml) and the mixture kept overnight at room temperature. For g.l.c. 5μl of the trimethylsilylated solution was used. The areas of the peaks of the trimethylsilyl derivatives were determined and the percentage present as each peak was calculated from the percentage of the ^{14}C in the final solution. Unconjugated metabolites were determined by omission of the acid-hydrolysis stage. Peaks arising from normal urine constituents were identified by subjecting an equal volume of control urine to the same procedure. Reference compounds were similarly treated with bis(trimethylsilyl)acetamide to obtain their retention times under the standard conditions used. Retention times were measured in a Hewlett Packard Chromatograph (F. and M. Scientific 402 High Efficiency Gas Chromatograph) fitted with a flame ionization detector. The conditions and retention times are given in Table 2.

Gas–liquid chromatography–mass spectrometry. A Varian Aerograph 1700 gas chromatograph with a Varian MAT CH5 Mass Spectrometer was used. A 5ft (1.54m) glass column (2mm internal diam.) packed with SE 30 (3%) on Chromosorb W-AW/DMCS was used for chromatographic separation. The column was set at 115°C, the injection port at 260°C, the detector at 250°C, the interface at 260°C, the Biemann–Watson-type separator at 250°C, the line-of-sight inlet line at 200°C and the source at 200°C. The electron-beam energy was 70eV and the helium carrier-gas flow rate was 33ml/min at 50lb/in² (345kN·m⁻²).

The standards and the 24h urine samples were prepared by the method for g.l.c. determination of metabolites in urine and a sample (5μl) was injected. For compounds representing less than 1% of the dose, the remaining untreated ether extract (0.9ml) was evaporated at 30°C under reduced pressure, dissolved in ether (0.1ml), treated with bis(trimethylsilyl)acetamide (0.1ml) and a sample (5μl) was injected. The retention times and mass spectra of the peaks were recorded (Table 3).

Results

Table 4 shows the gross recovery of ^{14}C in 3 days after oral and intraperitoneal administration of [^{14}C]cyclohexylamine hydrochloride to rats and guinea pigs, intraperitoneal administration to rabbits and oral administration to man. The recovery of ^{14}C was 90–100%, most of it being found in the urine in the four species. The faecal excretion of ^{14}C was 1% or less in man, the rat and rabbit, and some 4–7% in the guinea pig. In the experiment with rats and guinea pigs, the elimination of ^{14}C in the expired air was also examined. In the rat the amount of $^{14}CO_2$ formed was negligible, but in the guinea pig it was significant, amounting to about 0.5% of the dose. The urine of the first 24h, which contained most of the ^{14}C administered, was analysed for cyclohexylamine by reverse isotope dilution. Over 90% of this ^{14}C was accounted for as unchanged cyclohexylamine in the rat, guinea pig and human. The results suggest that some 10% of the ^{14}C excreted in 24h was in the form of metabolites in the rat and guinea pig and some 5% in man. In the rabbit, however, cyclohexylamine was more extensively metabolized, and more than one-half of the ^{14}C excreted in 24h was present in the urine as metabolites.

Table 3. *G.l.c.–mass spectrometry of trimethylsilyl ethers of cyclohexylamine metabolites*

Reference compounds and urine extracts were treated with bis(trimethylsilyl)acetamide (see the text) and the retention times and mass spectra of the trimethylsilyl ethers measured. The results given are the relative abundances of the important ions. The table also contains the retention times and relative abundances of one metabolite from each species to illustrate the method of identification. Abbreviations: *t*-diol, *trans*-cyclohexane-1,2-diol; *t*-2, *trans*-2-aminocyclohexanol; *c*-3 and *t*-3, *cis*-3- and *trans*-3-aminocyclohexanol; *c*-4 and *t*-4, *cis*-4- and *trans*-4-aminocyclohexanol.

								Rat metabolite	Rabbit metabolite	Guinea-pig metabolite	Human metabolite
	Retention time (min) ...	*t*-diol 9	*t*-2 14	*c*-3 16	*t*-3 13	*c*-4 17.5	*t*-4 20.5		no. 1 13	no. 3 16	no. 4 17.5	9.5
m/e												
261		0.1										0.3
260		0.3*	8	2	2	1	1		3	2	1	0.7
259			32*	9*	11*	5*	6*		10	9	5	
245		40	2	2	2				2	2		27
217			5	19	21				19	21		
216			22	100	100				98	100		
170		6	4	21	40		2		33	24		4
169		4		10	14		6		14	10		2
147		80	17	19	6	7	2		10	21	12	58
143		8		3	14				12	3		5
142		56	6	7	11				9	8		33
129		29	14	9	11	13	14		10	9	14	19
128			100	18	24	100	100		22	20	100	
115		3	18	20	22	8	10		21	20	10	2
102			16	7	8				8	6		
100			11	18	21	7	9		20	17	8	
96			2	4	21				18	4	2	
81		52	3	11	2	8	2		5	13	10	27
75		28	10	17	21	7	9		23	21	28	42
73		100	77	93	98	36	48		100	82	58	100
								Identification:	*t*-3	*c*-3	*c*-4	*t*-diol

* Parent ions.

Table 4. *Excretion of ^{14}C in animals and man receiving [^{14}C]cyclohexylamine hydrochloride*

[^{14}C]Cyclohexylamine hydrochloride dissolved in water was administered to animals by stomach tube or by intraperitoneal injection, the dose being 50mg/kg. The human subjects took the compound by mouth, dissolved in water, the dose being 25mg per person. ^{14}C was determined by scintillation counting and cyclohexylamine in urine by isotope dilution (see the text). The results are the averages for three animals with ranges in parentheses; ranges are given only where relevant. n.d., Not determined.

Animal (sex)	Dose of ^{14}C (μCi/animal)	Route of administration	Time after dosing (days)	^{14}C excreted (% of dose)					Unchanged cyclohexylamine found in 24h urine sample	
				Urine	Faeces	Expired air as CO_2	Cage wash	Total recovered	% of dose	% of ^{14}C in 24h urine sample
Rat (F)	5.3	Intraperitoneal	1	91.2 (82.5–98.2)	0.8 (0–2.5)	n.d.			80.9 (72.8–87.2)†	88.7
			2	92.3	0.9					
			3	92.5	1.0		1.5	94.9 (89.3–99.2)		
Rat* (F)	6.0	Oral	1	86.4 (84.4–88.2)	0.5 (0.2–1.0)	<0.01			83.4 (81.4–85.6)	96.5
			2	86.8	0.6					
			3	87.0	0.6		5.0	92.6 (91.1–94.5)		
Guinea pig (F)	2.5	Intraperitoneal	1	94.7 (87.4–99.3)	3.9 (0.1–10.5)	0.5 (0.2–0.8)			86.7 (73.5–95.8)	91.6
			2	95.3	4.0					
			3	95.4	4.0		0.1	100 (99.4–100.5)		
Guinea pig (F)	2.5	Oral	1	89.8 (80.9–98.8)	6.9 (1.2–12.3)	0.4 (0.2–0.8)			78.2 (70.8–83.8)	87.1
			2	90.7	7.0					
			3	90.9	7.0		0.1	98.4 (95.4–101.1)		
Rabbit (F)	5.0	Intraperitoneal	1	88.1 (82.5–95.2)	0.5 (0–1.3)	n.d.			38.4 (31.0–51.1)	43.6
			2	91.9	0.7					
			3	92.6	0.7		0.7	94.2 (91.0–99.3)		
Man (M)	12.2	Oral	1	92.1 (89.7–94.1)		n.d.			87.0 (84.9–89.3)	94.5
			2	94.4						
			3	94.8	<1			94.8 (93.8–95.6)		

* Rats on a cyclamate diet for 4 months (see the text and Renwick & Williams, 1972).
† Result obtained by counting the radioactivity of the relevant portion of paper chromatograms (see the text).

Paper chromatography

Rat urine. Radiochromatogram scans of the 24h urine samples of rats given [^{14}C]cyclohexylamine hydrochloride (50mg/kg, or about 12mg/animal) showed two peaks of ^{14}C radioactivity. The main peak corresponded to cyclohexylamine and represented 81% (range 77–87% in three animals) of the dose after intraperitoneal injection and 79% (77–81%) after oral dosing. The smaller peak (R_F 0.58, 0.71, 0.42 and 0.59 in solvents A, B, C and D respectively; see Table 1) represented 10% (9–11%) after intraperitoneal and 7% (6–8%) after oral administration. After a dose of 100mg/animal, the scans showed three peaks of R_F 0.73 (67% of dose; identified as cyclohexylamine), 0.46 (3%) and 0.56 (2%) in solvent A. The peak at R_F about 0.56 in solvent A may be conjugated aminocyclohexanols, since it gave a positive naphtharesorcinol test for glucuronic acid, and that at R_F 0.46 may be free aminocyclohexanols (see R_F values in Table 1).

Guinea-pig urine. The urine samples of three guinea pigs given cyclohexylamine hydrochloride (50mg/kg) were examined as described above and showed three peaks of ^{14}C radioactivity. The main one was cyclohexylamine, accounting for 87% of the intraperitoneal and 81% of the oral dose. The other peaks had R_F 0.48 (3%) and 0.64 (6%) in solvent A and 0.05 and 0.22 in solvent D. These values corresponded approximately to the glucuronides of cyclohexane-1,2-diol and cyclohexanol (see Table 1 for R_F values). This was confirmed by g.l.c. and mass spectrometry.

Rabbit urine. Radiochromatograms of rabbit urine (dose of cyclohexylamine hydrochloride, 50mg/kg) showed five peaks of ^{14}C radioactivity in solvent D, of R_F 0.03, 0.08, 0.18, 0.45 and 0.63, and three peaks in solvents C (R_F 0.15, 0.46 and 0.68) and B (R_F 0.58, 0.65 and 0.80). The peak at R_F 0.68 in solvent C was cyclohexylamine [40% (31–51% in three rabbits) of the dose] and that of R_F 0.65 in solvent B and R_F 0.18 in solvent D corresponded to cyclohexylglucuronide [12% (8–17%) of the dose]. Cyclohexanol obtained after acid hydrolysis was determined by isotope dilution (11%; range 9–15%). Most of the remaining ^{14}C, which represented 28% (19–36%) of the dose, could be separated from cyclohexylamine and cyclohexylglucuronide in solvents A, B and D, in which it had R_F 0.56, 0.80 and 0.63 respectively. These R_F values are in the region of those for the aminocyclohexanols (see Table 1) and these were identified by g.l.c. and mass spectrometry (see below and Tables 3 and 5).

Human urine. Each of three subjects took 25mg of [^{14}C]cyclohexylamine hydrochloride orally. In solvent A, the main peak had R_F 0.74 and corresponded to cyclohexylamine, which amounted to 91% (89–93%) of the dose. Cyclohexylglucuronide (about 0.2%) was detected by chromatography with solvents B and D and confirmed by isotope dilution for cyclohexanol after hydrolysis.

Gas–liquid chromatography

Rat urine. The urine samples of animals given cyclohexylamine contained four compounds, with retention times 15, 18.5, 20.5 and 25min, in addition to cyclohexylamine. These corresponded to *trans*-3-, *cis*-3-, *cis*-4- and *trans*-4-aminocyclohexanol and represented 0.5, 0.02, 0.3 and 0.1% of the dose respectively before hydrolysis and 2.2, 0.1, 1.7 and 0.5% of the dose after hydrolysis.

Rabbit urine. G.l.c. showed the presence of six compounds with retention times 2, 11, 15, 18.5, 20.5 and 24.5min, in addition to cyclohexylamine. These corresponded to cyclohexanol, *trans*-cyclohexane-1,2-diol, *trans*-3-, *cis*-3-, *cis*-4- and *trans*-4-aminocyclohexanol and represented 0.2, 0.5, 5.1, 0.2, 0.1 and 0.2% of the dose respectively before hydrolysis and 8.0, 4.7, 11.3, 0.6, 0.2 and 0.4% of the dose after acid hydrolysis.

Guinea-pig urine. The g.l.c. traces for guinea-pig urine were similar to those for rabbit urine. Thus the urine contained cyclohexanol, *trans*-cyclohexane-1,2-diol, *trans*-3-, *cis*-3-, *cis*-4- and *trans*-4-aminocyclohexanol to the extents of <0.2, 0.4, 1.2, 0.2, 0.2 and 0.3% of the dose respectively before hydrolysis and 0.5, 2.5, 1.2, 0.2, 0.2 and 0.2% of the dose after hydrolysis.

Human urine. The only apparent difference between extracts of the urine of subjects given cyclohexylamine and normal urine was the presence of unchanged cyclohexylamine. However, the normal urine contained compounds giving g.l.c. peaks coincident with the reference compounds given in Table 2, with an especially large peak, with a retention time of 11min, present after acid hydrolysis. The urine extracts containing the equivalent of about 50mg of [^{14}C]cyclohexylamine were evaporated at 30°C under reduced pressure in the presence of 2M-HCl (0.5ml). These solutions were chromatographed in solvent A on Whatman 3MM paper and three regions, R_F 0.36–0.60, 0.60–0.85 and 0.85–1.00, were removed and eluted with water (40ml). These regions represented 0.3, 90.3 and 0.5% of the dose respectively before hydrolysis and 0.3, 90.7 and 1.4% of the dose after hydrolysis. Each eluate was made alkaline, saturated with NaCl, extracted and treated with bis(trimethylsilyl)acetamide as described above, and then examined by g.l.c. The regions corresponding to aminocyclohexanols (R_F 0.36–0.60) did not differ from those of normal urine either before or after acid hydrolysis. This region contained the endogenous material that interfered with the determination of *trans*-cyclohexane-1,2-diol after acid hydrolysis. The region corresponding to cyclohexylamine (R_F 0.60–0.85) contained cyclohexylamine (retention

Table 5. *Metabolites of cyclohexylamine in the urine of various species*

The rats (five) each received orally 100mg of cyclohexylamine hydrochloride (500mg/kg) containing 2.3 μCi of ^{14}C, the rabbits (three) received 200mg (100mg/kg) containing 9.4 μCi, the guinea pigs received 100mg (450mg/kg) containing 3.9 μCi, and the humans received 200mg containing 6.2 μCi. The urine samples were 24h collections and were analysed separately for ^{14}C, but were pooled for the determination of metabolites. Abbreviations: r.i.d., reverse isotope dilution; <, not detected.

		Content in 24h urine sample (% of dose)							
		Rat		Rabbit		Guinea pig		Man	
Metabolite sought	Method of analysis	Free	Total	Free	Total	Free	Total	Free	Total
Cyclohexylamine	r.i.d.	68.2	68.2	58.0	58.0	67.8	67.8	86.9	86.9
Cyclohexanol	r.i.d.	0.0	0.05	<0.2	9.3*	0.0	0.5	0.0	0.2
		(g.l.c.)		(g.l.c.)		(g.l.c.)		(g.l.c.)	
trans-Cyclohexane-1,2-diol	g.l.c.	0.0	0.0	0.5	4.7	0.4	2.5	0.3	1.4
trans-2-Aminocyclohexanol	g.l.c.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
trans-3-Aminocyclohexanol	g.l.c.	0.5	2.2	5.1	11.3	1.2	1.2	0.0	0.0
cis-3-Aminocyclohexanol	g.l.c.	0.02	0.1	0.2	0.6	0.2	0.2	0.0	0.0
trans-4-Aminocyclohexanol	g.l.c.	0.1	0.5	0.2	0.4	0.3	0.2	0.0	0.0
cis-4-Aminocyclohexanol	g.l.c.	0.3	1.7	0.1	0.2	0.2	0.2	0.0	0.0
Cyclohexylhydroxylamine	r.i.d.		<0.05		0.2		<0.05		<0.02
Cyclohexanone	r.i.d.		<0.05		0.2		<0.05		<0.03
Sum of metabolites			72.7		84.9		72.6		88.5
^{14}C in the urine			72.1 (67.0–75.7)†		95.2 (90.4–97.7)†		71.1 (51.2–86.2)†		92.7 (88.4–95.0)†

* Value found by g.l.c. was 8%.

† Ranges for five rats, three rabbits, three guinea pigs and three humans.

time 3 min) and *trans*-cyclohexane-1,2-diol (retention time 11 min). This region contained 90% of the dose as cyclohexylamine and 0.3% as *trans*-cyclohexane-1,2-diol from unhydrolysed urine, and cyclohexylamine and trace amounts of the diol from hydrolysed urine. The region of R_F 0.85–1.00 did not contain detectable cyclohexylamine, but the extract from hydrolysed urine contained a material with a retention time of 11 min, similar to *trans*-cyclohexane-1,2-diol, that was not present in the corresponding extract of normal urine.

Thus the urine contained, in addition to cyclohexylamine, *trans*-cyclohexane-1,2-diol, corresponding to 0.3 and 1.4% of the dose in unhydrolysed and hydrolysed urine respectively, and possibly traces of unidentified aminocyclohexanols.

G.l.c.–mass spectrometry

Rat urine. The silylated extracts of rat urine showed peaks on the total-ion-current monitor of retention times 13, 15.5, 17 and 20.5 min. Reference to Table 3 shows that these corresponded to the trimethylsilyl ethers of *trans*-3-, *cis*-3-, *cis*-4- and *trans*-4-aminocyclohexanol respectively. The *m/e* values for one of these metabolites are given in Table 3. The other metabolites were identified similarly. The mass spectra of *trans*-3-, *cis*-4- and *trans*-4-aminocyclohexanol from the urine corresponded with those of the reference compounds, but that of *cis*-3-aminocyclohexanol, which occurs in small amounts, was considerably contaminated although consistent with standard *cis*-3-aminocyclohexanol. *trans*-2-Aminocyclohexanol (retention time of trimethylsilyl ether, 14 min) is not completely separated from the *trans*-3-isomer (retention time 13 min), but a mass spectrum of the extract taken after 14 min was essentially similar to that of the *trans*-3- isomer and indicated the absence of the *trans*-2- isomer.

Rabbit urine. For rabbit urine extracts the total-ion-current monitor showed peaks of retention times 9, 13.5, 16, 17.5 and 20.5 min corresponding to *trans*-cyclohexane-1,2-diol, *trans*-3-, *cis*-3-, *cis*-4- and *trans*-4-aminocyclohexanol respectively. The mass spectra were essentially the same as the corresponding reference compounds and the results for one metabolite are given in Table 3. A spectrum taken at the tail of the *trans*-3- isomer peak at a retention time of 14.5 min suggested that this region was a mixture of *trans*-2- and *trans*-3-aminocyclohexanol and that the *trans*-2-isomer was probably a trace metabolite of cyclohexylamine in the rabbit.

Guinea pig urine. The total-ion-current monitor showed the presence of peaks with retention times 9.5, 13, 16, 17.5 and 21 min, corresponding to *trans*-cyclohexane-1,2-diol, *trans*-3-, *cis*-3-, *cis*-4- and *trans*-4-aminocyclohexanol respectively. The mass spectrum of the peak corresponding to *trans*-3-aminocyclohexanol showed that although this compound was present there was considerable contamination. The spectra of the other metabolites were essentially the same as the corresponding reference compound and the results for one of these are given in Table 3.

Human urine. The eluates of the paper chromatograms from the regions of R_F 0.60–0.85 before hydrolysis and R_F 0.85–1.00 after hydrolysis were analysed by g.l.c.–mass spectrometry. These regions each contained a peak with retention time 9.5 min, corresponding to *trans*-cyclohexane-1,2-diol. The mass spectrum of the peak in unhydrolysed urine was very similar to that of the reference material, although the presence of additional small peaks indicated some contamination. The peak in the hydrolysed urine had a spectrum almost identical with that of *trans*-cyclohexane-1,2-diol (Table 3).

The nature and amounts of the metabolites of cyclohexylamine are shown in Table 5. Rat urine contained very small amounts of conjugated cyclohexanol together with larger amounts (about 4% of the dose) of *cis*-3-, *trans*-3-, *cis*-4- and *trans*-4-aminocyclohexanol, mainly in conjugated form, the main metabolite being *trans*-3-aminocyclohexanol. In rabbit urine eight metabolites were found, amounting together to nearly 27% of the dose. These were,

Table 6. *Positions at which cyclohexylamine is metabolized*

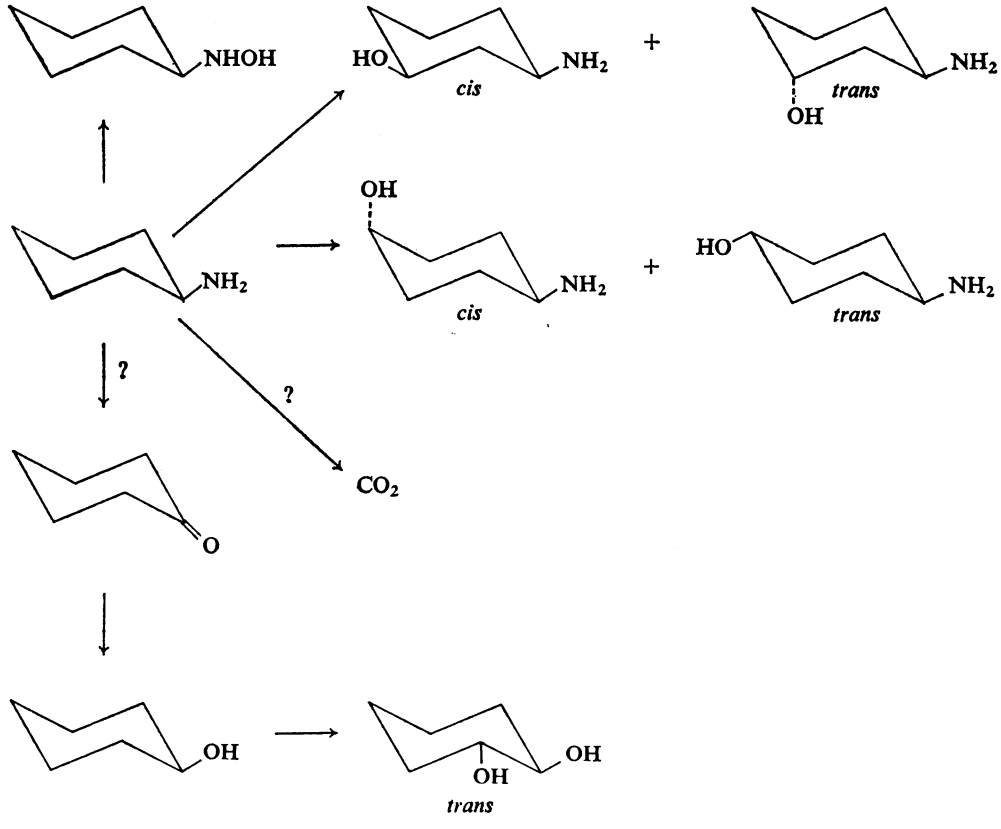
The extent of attack at each position is expressed as % of dose.

	Rabbit		Rat		Guinea pig		Man	
Position	Total	*trans/cis* ratio	Total	*trans/cis* ratio	Total	*trans/cis* ratio	Total	*trans/cis* ratio
N { deamination	14	—	0.05	—	3	—	1.6	—
N { hydroxylation	0.2	—	0	—	0	—	0	—
1	0	—	0	—	0	—	0	—
2	0	—	0	—	0	—	0	—
3	11.9	19	2.3	22	1.4	6	0	—
4	0.6	2	2.2	0.3	0.4	1	0	—

in quantitative order, *trans*-3-aminocyclohexanol (11.3%), cyclohexanol (9.3%), *trans*-cyclohexane-1,2-diol (4.7%), *cis*-3-aminocyclohexanol (0.6%), *cis*-4-aminocyclohexanol (0.4%), *trans*-4-aminocyclohexanol, cyclohexylhydroxylamine and cyclohexanone (0.2% each). Six metabolites totalling 4.8% of the dose were found in guinea-pig urine, the main one being *trans*-cyclohexane-1,2-diol (2.5%), with *trans*-3-aminocyclohexanol (1.2%), cyclohexanol (0.5%) and small amounts (0.2% each) of the *cis*-3-, *trans*-4- and *cis*-4-aminocyclohexanol. Only two metabolites were detected in human urine, these being *trans*-cyclohexane-1,2-diol (1.4%) and cyclohexanol (0.2%).

Discussion

It is clear that in the rat, guinea pig and human, cyclohexylamine is excreted unchanged to the extent of some 90% or more of the dose, but in the rabbit about one-third of the dose is metabolized and the rest is excreted unchanged in the urine. However, the proportion of the cyclohexylamine that is metabolized gives rise to several products. In the rat five metabolites were found, these being mainly aminocyclohexanols, there being very little deamination to cyclohexanol. In the rabbit and guinea pig both deamination and ring hydroxylation occurred. In the rabbit slightly more deamination (about 14%; see Table 6) occurred than ring hydroxylation (about 12%), and this was true also for the guinea pig, but the values were lower, i.e. 3% deamination and nearly 2% ring hydroxylation. The mechanism of this deamination is obscure, since cyclohexylamine is not a substrate for but an inhibitor of monoamine oxidase (Sarker *et al.*, 1960). If cyclohexylamine were deaminated, cyclohexanone would be formed and was, in fact, found as a metabolite of the amine in rabbits. However, this ketone was obtained only after acid hydrolysis of the urine and could therefore be an artifact, since Elliott *et al.* (1959) have shown that it is produced by acid hydrolysis of *trans*-cyclohexane-1,2-diol monoglucuronide, which is probably present in the urine from



Scheme 1. *Probable routes of metabolism of cyclohexylamine*

rabbits given cyclohexylamine (see Table 5). *N*-Hydroxylation was also detected in the rabbit, since a small amount of *N*-hydroxycyclohexylamine was found in the urine, thus confirming the observation of Elliott *et al.* (1968). *N*-Hydroxycyclohexylamine was not detected in the urine of the rat, guinea pig or human.

The main aminocyclohexanol formed in the species examined is the *trans*-3-isomer. The next in quantity is the *cis*-4-isomer. Table 6 indicates that the 3-position (of which there are two) of cyclohexylamine is more readily hydroxylated than the 4-position and that the enzyme attack on these positions is mainly axial, since the *trans*/*cis* ratio for the 3-position is large (6–22) and for the 4-position is small (0.3–2) (see Table 6). The probable routes of metabolism of cyclohexylamine are shown in Scheme 1, and it is clear from the diagrams that the addition of the hydroxyl group to the amine is mainly axial, as in *trans*-3-aminocyclohexanol and *cis*-4-aminocyclohexanol.

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