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The Metabolism of ^{14}C-Labelled α-Methyldopa in Normal and Hypertensive Human Subjects

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1. The fate of orally administered ^{14}C-labelled L-α-methyldopa has been examined in three normal men and in eight hypertensive patients who responded to the drug and three who did not. 2. The output of ^{14}C in the urine in 2 days and in the faeces in 4 days was not very different in any of the subjects. The normals excreted about 40% of the dose in the urine and 60% in the faeces, the responders 52% (range 35–60%) and 45% and the non-responders 42% and 41%. Most of the urinary ^{14}C radioactivity was eliminated in 24h after dosing. 3. The main metabolite in the urine was free and conjugated α-methyldopa (normal men, 23%; responders, 37%; non-responders, 25% of the dose). Free and conjugated 3-*O*-methyl-α-methyldopa was about 4% in all subjects, total amines (α-methyldopamine and 3-*O*-methyl-α-methyldopamine) about 6% and ketones (mainly 3,4-dihydroxyphenylacetone) about 3%. 4. The output of α-methyldopamine (2–4% of dose), 3-*O*-methyl-α-methyldopamine (0.3%) and 3,4-dihydroxyphenylacetone (3–5%) was similar in the one normal and two responders examined. 5. The faecal ^{14}C in all subjects was unchanged L-α-methyldopa. 6. In general, the amounts of the metabolites in the urine in normal men and in responding and non-responding patients were quantitatively similar, except in one non-responding patient who converted nearly two-thirds of the absorbed drug into amines and ketones. There appeared to be no correlation between metabolites in the urine and response or lack of response to the drug. 7. In two normal subjects 70–80% of D-α-methyldopa was excreted unchanged in the faeces. Of the absorbed compound most (9–14% of the dose) was excreted as the free and conjugated drug together with a small amount (1–2%) of 3-*O*-methyl-α-methyldopa. No amines and only traces of ketone were excreted.

α-Methyldopa [L-3-(3′,4′-dihydroxyphenyl)-2-methylalanine; Aldomet] was found by Oates *et al.* (1960) to be an antihypertensive agent and several studies have been published on its metabolism in man (Sourkes *et al.*, 1962; Gillespie *et al.*, 1962; Sjoerdsma *et al.*, 1963; Buhs *et al.*, 1964; Prescott *et al.*, 1966). In a small proportion of the hypertensive patients treated, methyldopa is ineffective (Dollery & Harington, 1962; Bayliss & Harvey-Smith, 1962) and the present study was undertaken to find out whether there was a metabolic basis for this observation. It is shown that there is no significant difference in the metabolism of α-methyldopa in responding and non-responding hypertensive patients treated with the drug. However, 3,4-dihydroxyphenylacetone has been shown for the first time to be a metabolite of the drug in man, and a study has been made in two human subjects of the metabolism of the D-isomer of the drug, which is inactive as an antihypertensive (Gillespie *et al.*, 1962). The metabolism of the D-isomer is different from that of the active L-isomer.

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Materials and Methods

Compounds

(±)-2-Amino-1-(3′,4′-dihydroxyphenyl)propane (α-methyldopamine) hydrochloride, m.p. 190°C (Mannich & Jacobsohn, 1910), (±)-2-amino-1-(4′-hydroxy-3′-methoxyphenyl)propane (3-*O*-methyl-α-methyldopamine) hydrochloride (Dring *et al.*, 1970), (±)-3-(4′-hydroxy-3′-methoxyphenyl)-2-methylalanine (3-*O*-methyl-α-methyldopa), m.p. 294–295°C (Merck and Co. Inc., 1963), 1-(4′-hydroxy-3′-methoxyphenyl)propan-2-one, b.p. 150°C/0.8mmHg (Pearl & Beyer, 1951) and 1-(3′,4′-dihydroxyphenyl)propan-2-one (Slates *et al.*, 1964) were prepared as described in the literature.

The following compounds were obtained as gifts: 2-amino-1-(4′-hydroxy-3′-methoxyphenyl)propan-1-ol (α-methylnormetanephrine) hydrochloride (Dr. A. Carlsson, University of Gothenburg, Sweden),

2-amino-1-(3′,4′-dihydroxyphenyl)propan-1-ol (α-methylnoradrenaline) hydrochloride, m.p. 176–178°C (Cobefrine; Bayer Products Ltd., Surbiton, Surrey, U.K.), D-α-methyldopa, m.p. 308°C (decomp.), and L-α-methyldopa, m.p. 310°C (decomp.) (Merck, Sharpe and Dohme Ltd., Hoddesdon, Herts., U.K.).

Synthesis of ^{14}C-labelled D- *and* L-α-*methyldopa*

DL-3-(3′,4′-Dihydroxyphenyl)-2-methyl[3-^{14}C]-alanine was prepared by the method of Duhm *et al.* (1965) starting with $Ba^{14}CO_3$ (from The Radiochemical Centre, Amersham, Bucks., U.K.). The amount of DL-[^{14}C]-α-methyldopa obtained was 1.03g, of specific radioactivity 1.15μCi/mg; m.p. 290°C (decomp.).

DL-α-Methyldopa (1g; 0.5g of radioactive compound+0.5g of non-radioactive compound) was treated with pyridine (4ml) and acetic anhydride (4ml). The slurry became quite warm and the solid present dissolved in 15min to a pale-brown solution. The mixture was kept at room temperature for 2 days and then the solvents were removed at reduced pressure. The brown oil was treated with toluene (10ml) and evaporated at reduced pressure to remove residual pyridine and acetic anhydride, a treatment that was repeated three times. The viscous residue was cooled in ice and triturated with 2M-HCl (3ml) until it crystallized. The white crystalline solid was extracted with ethyl acetate (3×15ml) and the extract washed with water (3ml) and dried over anhydrous Na_2SO_4. Removal of the ethyl acetate left white crystals of DL-*N*-acetyl-3-(3′,4′-diacetoxyphenyl)-2-methyl[3-^{14}C]alanine (1.15g; m.p. 194–195°C). Tristram *et al.* (1964) reported m.p. 197–199°C for the non-radioactive compound.

The above-mentioned triacetyl compound (1.15g) and quinine base (1.3g) were stirred till they dissolved (5min) in acetone (11.5ml). On further stirring for 10min a white precipitate separated and the whole was kept at 0°C for 4h. The mixture was filtered and the solid washed with acetone (1ml) and dried *in vacuo* at room temperature. This quinine salt of the L-form of triacetyl-α-methyldopa (0.972g) had m.p. 164–165°C [Tristram *et al.* (1964) gave m.p. 164–166°C].

The above-mentioned quinine salt (0.972g) was suspended in water (2ml), and 2M-HCl (3ml) was added to dissolve it. The solution was extracted with ethyl acetate (4×25ml), and the extract washed with 2M-HCl (3ml) and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to give a pale-yellow viscous oil, which soon crystallized. The crystalline triacetyl-L-α-methyldopa was dissolved in 6M-HCl (20ml) and heated under reflux for 2h. The solution was evaporated to dryness under reduced pressure and then the residue was dissolved in water (10ml) and evaporated to dryness, the treatment with water being repeated three times to remove HCl. The partially crystalline residue was taken up in water (1ml) saturated with SO_2 and the solution adjusted to pH 6.5 by the careful addition of aq. NH_3 soln. (sp. gr. 0.880). The resulting solution was then allowed to crystallize at 0–5°C for 3h. The crystals were filtered and dried *in vacuo* over P_2O_5. The L-3-(3′,4′-dihydroxyphenyl)-2-methyl[3-^{14}C]alanine (0.27g) had m.p. 292°C (decomp.) and specific radioactivity 0.6μCi/mg. It was characterized by its i.r. spectrum. Its radiochemical purity was determined by paper chromatography and optical purity by reverse isotope dilution analysis with both D- and L-isomers by the method of Evans *et al.* (1963). In both cases the purity was >98%.

The acetone mother liquors from the separation of the quinine salt of the triacetyl-L-α-methyldopa were evaporated to dryness and the residue was treated exactly as described for the L-isomer. In this way there was obtained D-3-(3′,4′-dihydroxyphenyl)-2-methyl[3-^{14}C]alanine (0.28g), m.p. 290°C (decomp.) and specific radioactivity 0.6μCi/mg. Determination of purity as above gave the value of 97%.

Procedures with subjects

Administration of drug. Normal volunteers took orally 20mg (12μCi) of ^{14}C-labelled D- or L-α-methyldopa dissolved in water adjusted to pH3 with 0.1M-HCl, together with 230mg of non-radioactive D- or L-α-methyldopa. Hypertensive patients were dosed similarly with the L-isomer, the radioactive compound being part of the daily course of medication.

Collection of excreta. Urine was collected daily for 5 days and treated with sufficient 2M-HCl to give pH2–3. Stools were collected for 5 days. The excreta were stored at −20°C until analysed.

Determinations

^{14}C radioactivity. The radioactivity of urine and stools was determined with a Packard Tri-Carb scintillation spectrometer (model 3214) as described by Bridges *et al.* (1967). The 24h stool collection was homogenized with water (with drops of 10M-HCl added to give pH3) in a Waring Blendor and made up to 1 litre with water. A portion (25ml) was adjusted to pH7 with 2M-NaOH, diluted with water to 50ml, mixed with H_2O_2 (100vol. soln.; 50ml) and then kept at room temperature for 1 week. The radioactivity of a portion of the decolorized faecal suspension was then counted as described for urine.

Chromatography. The R_F values and colour reactions of relevant compounds are given in Table 1. Chromatograms of urine were examined in a Packard radiochromatogram scanner (model 7200).

The chromatograms were prepared by placing 0.3ml of the urine as a band (5cm) on strips of

Table 1. *R_F values and colour reactions of α-methyldopa and its possible metabolites*

The descending technique was used with Whatman no. 1 paper unless otherwise stated. The solvent systems used were: A, butan-1-ol–acetic acid–water (4:1:1, by vol.); B, butan-1-ol saturated with 1M-HCl with Whatman 3MM paper; C, butan-2-ol–pyridine–water–acetic acid (2:6:4:1, by vol.). The chromatograms were run until the solvent front had travelled 35cm from the origin. For t.l.c. the systems used were: D, benzene–dioxan–acetic acid (90:25:4, by vol.) on silica gel G (0.25mm) (E. Merck A.-G., Darmstadt, Germany); E, butan-2-ol–acetic acid–cyclohexane (80:7:10, by vol.) (Segura-Cardona & Soehring, 1964) on aluminium sheets coated with Polyamide $11F_{254}$ (0.15mm) (Merck). These chromatograms were run to 12cm from the origin. The compounds were detected with diazotized *p*-nitroaniline (Wichström & Salvesen, 1952).

	R_F					
	Paper chromatography			T.l.c.		
Compound Solvent ...	A	B	C	D	E	Colour with diazotized *p*-nitroaniline
α-Methyldopa	0.34	—	0.29	—	—	Green–blue
α-Methylnoradrenaline	0.39	0.24	—	—	—	Green
α-Methylnormetanephrine	0.48	—	—	—	—	Purple
3-*O*-Methyl-α-methyldopa	0.49	—	0.34	—	—	Purple
α-Methyldopamine	0.52	0.35	0.59	—	—	Pale pink slowly
3-*O*-Methyl-α-methyldopamine	0.64	0.47	—	—	—	Purple
3,4-Dihydroxybenzoic acid	0.76	—	—	0.47	0.41	Green–blue
3,4-Dihydrophenylacetone	0.81	—	0.91	0.51	0.67	Blue
4-Hydroxy-3-methoxyphenylacetone	0.83	—	0.91	0.81	0.86	Purple
4-Hydroxy-3-methoxybenzoic acid	0.85	—	—	0.75	0.63	Purple

Whatman no. 1 paper and drying in a stream of N_2 at room temperature. For hydrolysed urine, the sample was adjusted to pH1 or less with 2M-HCl and heated under reflux for 30min. After cooling the pH was raised to 3 with 0.1M-NaOH and a sample of the hydrolysed urine containing about the same radioactivity as 0.3ml of unhydrolysed urine was banded on Whatman no. 1 paper as before. The chromatograms were developed with solvent A (Table 1), dried, and cut into 1cm segments. The radioactivity of each segment was determined in the scintillation counter.

Detection and determination of ketones and acids. The urine (10ml), mixed with 10M-HCl (2ml), was heated under reflux for 1h. The cooled hydrolysate was saturated with NaCl and extracted with ether (3×15ml). After drying overnight with anhydrous Na_2SO_4, the ether extract was evaporated under reduced pressure to 1ml of which 0.2ml was subjected to t.l.c. in solvents D and E (Table 1). After drying, 0.5cm bands were scraped from the plates and counted for radioactivity.

G.l.c. and mass spectrometry of 3,4-dihydroxyphenylacetone. A Varian Aerograph 1700 gas chromatograph with a Varian MAT CH5 mass spectrometer was used. The column for g.l.c. consisted of 3% SE-30 on Chromosorb W-AW/DMCS packed in a 1.5m (5ft) glass column (2mm internal diam.). The column temperature was programmed at 4°C/min with an initial temperature of 90°C, the injector port at 205°C, the detector at 250°C, the interface at 270°C, the Biemann–Watson-type molecular separator at 240°C, the line-of-sight inlet line at 200°C and the source temperature at 180°C. The electron-beam energy was 70eV and the helium carrier gas flow rate 20ml/min at 275kN/m² (40lbf/in²).

3,4-Dihydroxyphenylacetone (3mg), benzene (0.4ml) and bis(trimethylsilyl)acetamide (0.1ml) were placed in a stoppered tube and heated at 50°C for 30min. The product (1μl) was injected on to the column.

With urine, extracts were prepared as described above except that before drying with Na_2SO_4 the ether extract was washed with phosphate buffer, pH6, to remove organic acids. The extract was evaporated in a stream of N_2 and the residue silylated as above. Urine from a normal male was treated in the same way, as a control.

Detection and estimation of amines. Amberlite CG-50 ion-exchange resin (100–200 mesh) was prepared as described by Hirs *et al.* (1953). EDTA and ascorbic acid were used to protect catecholamines (cf. Häggendal, 1962) and all water used was glass-distilled. The column (1cm×10cm) was treated in the order of listing with 2M-NaOH containing 1% EDTA (40ml), water containing 0.1% EDTA to remove excess of NaOH, 2M-acetic acid (40ml),

0.1% EDTA in water to remove excess of acid, 0.2M-sodium acetate adjusted to pH6.0–6.2 with acetic acid until the column effluent was about pH6, and finally with 0.1% EDTA in water (10ml).

The urine (10ml) and 10M-HCl (2ml) were heated under reflux for 1h. Excess of acid was removed by evaporation to dryness twice under reduced pressure after addition of 10ml portions of water. The residue was made up to 5ml with water and ascorbic acid (2mg) and EDTA (20mg) were added. The solution was adjusted to pH6 with 2M-NaOH and then added to the above-mentioned Amberlite column. The latter was washed with 0.1% EDTA in water (20ml) and eluted with 1M-HCl (40ml). The eluate was evaporated to dryness under reduced pressure and the residue extracted with 95% ethanol (4×5ml). The extract was evaporated at low pressure to 5ml. Samples (1ml) were chromatographed on Whatman 3MM paper with solvent B (Table 1; Carlsson & Lindquist, 1962). After drying the paper was cut up and the radioactivity located by scintillation counting.

Results

Table 2 shows the clinical features of the hypertensive patients selected for this study and who were being treated with α-methyldopa. It is to be noted that eight patients exhibited a fall of blood pressure while taking methyldopa, which was accepted as being a real and therapeutically useful response. In three patients, however, there was no satisfactory hypotensive response to the drug. All patients showed normal urine analysis, blood urea concentration and intravenous pyelograms.

Excretion of ^{14}C radioactivity

The excretion of ^{14}C radioactivity in three normal subjects and the 11 hypertensive patients receiving L-α-methyl[^{14}C]dopa is shown in Table 3. In the normal subjects nearly 40% of the dose of ^{14}C is excreted in the urine in 2 days, most of it appearing on the first day, and nearly 60% is found in the faeces collected over 4 days. The total recovery of ^{14}C radioactivity is satisfactory. In another experiment two of these subjects were also given D-α-methyl[^{14}C]dopa, and then only 9 and 15% of the ^{14}C appeared in the urine; the rest, 84 and 72%, was in the 4-day stools.

In the eight responding hypertensive patients, the urinary excretion of ^{14}C radioactivity is a little higher, 52% (range 35–60%), than in the normals and the faecal excretion a little lower, i.e. 37–57% in three patients. The recovery of ^{14}C radioactivity was again satisfactory. In the three non-responding patients, the urinary ^{14}C radioactivity was about 40%, the rest being in the faeces (at least in patient B. F.).

Nature of metabolites in urine

Paper chromatography of the urine before and after acid hydrolysis showed that the metabolites were present in free and conjugated forms in patients and normal subjects. The unhydrolysed urines showed four radioactive peaks, and in some subjects five. In solvent A (Table 1) there was a large peak at R_F 0.23 and smaller ones at 0.33, 0.45 and 0.52, and occasionally one at 0.81. On hydrolysis and rechromatography the peaks at 0.33, 0.52 and 0.81 remained unchanged and in solvent C these showed R_F 0.29, 0.59 and 0.91

Table 2. *Clinical features of hypertensive patients treated with methyldopa*

	Sex	Age (years)	Dose of methyldopa (g/day)	Arterial blood pressure (mmHg) Before methyldopa: Systolic/diastolic	Before methyldopa: Mean blood pressure	With methyldopa: Systolic/diastolic	With methyldopa: Mean blood pressure	Change in blood pressure (%)
Responding patients								
M. S.	M	55	0.75	210/120	150	160/90	113	−25
R. P.	F	52	1.25	210/110	143	150/100	117	−18
W. R.	M	56	1.00	220/130	160	150/100	117	−27
M. T.	M	27	1.50	160/115	130	130/100	110	−15
N. P.	M	38	0.50	150/110	123	130/90	103	−16
A. C.	F	34	1.50	190/120	143	160/100	120	−16
S. A.	M	36	0.75	170/110	130	140/90	107	−18
C. H.	F	58	1.50	215/140	165	180/120	140	−15
Non-responding patients								
G. G.	M	54	2.00	210/140	163	200/130	153	−6
T. W.	F	50	2.00	200/125	150	180/130	147	−2
B. F.	M	37	1.50	220/110	147	220/110	147	0

Table 3. *Excretion of ^{14}C radioactivity and metabolites by humans receiving α-methyl[^{14}C]dopa orally*

Each subject received 250mg of α-methyldopa containing 12μCi of ^{14}C. The metabolites were estimated by scintillation counting of segments of radiochromatograms as described in the text. Values are given to the nearest whole number in most instances; —, not determined.

		% of ^{14}C radioactivity dose				% of dose excreted in first day's urine			
		In urine							
Compound	Subject	First day	Second day	In stools 4 days	Total	α-Methyldopa	3-*O*-Methyl-α-methyldopa	Amines*	Ketones†
L-α-Methyldopa	Normal								
	G. D.	37	2	59	98	20	2	9	7
	W. A.	39	2	58	99	27	5	4	1
	D. G.	35	1	53	89	23	5	6	1
D-α-Methyldopa	G. D.	13.5	1.8	71.5	86.8	11	2	0	0.2
	W. A.	8.5	0.9	83.7	93.1	7	1	0	0.2
	Hypertensive patients								
L-α-Methyldopa	(a) Responding								
	M. S.	34	1	—	—	24	4	3	2
	R. P.	45	1	40	86	34	3	3	4
	W. R.	42	2	57	101	31	4	5	3
	M. T.	56	2	37	95	44	6	3	3
	N. P.	52	2	—	—	38	3	9	3
	A. C.	58	1	—	—	45	3	8	1
	S. A.	58	2	—	—	42	3	8	5
	C. H.	53	2	—	—	39	1	9	4
	(b) Non-responding								
	B. F.	43	2	53	98	13	2	15	13
	G. G.	41.5	—	—	—	29	—	4	7
	T. W.	33.6	3.2	30	66.8	20	—	8	5

* α-Methyldopamine plus 3-*O*-methyl-α-methyldopamine (see Table 4).
† 3,4-Dihydroxyphenylacetone (see Table 4).

respectively, which suggests (Table 1) that they are due to unchanged α-methyldopa, α-methyldopamine and 3,4-dihydroxyphenylacetone. The peaks at R_F 0.23 and 0.45 in solvent A were changed on acid hydrolysis. That of R_F 0.23 gave rise to a major peak at R_F 0.34 and two minor peaks at 0.52 and 0.49 in solvent A, whereas that of R_F 0.45 gave one peak at 0.81. This suggests that the peak of R_F 0.23 in solvent A contained conjugates of α-methyldopa, α-methyldopamine and 3-*O*-methyl-α-methyldopa. The peak at R_F 0.45 in solvent A appeared to be a conjugate of 3,4-dihydroxyphenylacetone. This was supported by their R_F values in solvent C. The work of Buhs *et al.* (1964) would suggest that these conjugates are probably *O*-sulphates.

Chromatography of the urine of the normal subjects who had been given D-α-methyldopa showed only three peaks, of R_F 0.23, 0.33 and 0.50 in solvent A. The first peak on hydrolysis gave rise to peaks in solvents A and C consistent with α-methyldopa, thus suggesting that it corresponded to conjugated α-methyldopa. The second peak is consistent with it being α-methyldopa and the third with 3-*O*-methyl-α-methyldopa.

Table 3 also gives the quantitative aspects of the metabolites in the urine based on the distribution of radioactivity on paper chromatograms.

Identification of 3,4-*dihydroxyphenylacetone*

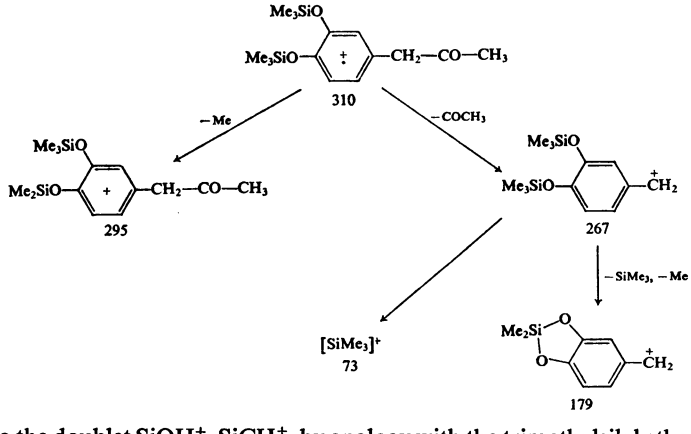
When ether extracts of hydrolysed urine were examined by t.l.c., two radioactive peaks were found, the larger one with R_F 0.51 and a small one with R_F 0.81 in solvent D, and R_F 0.67 and R_F 0.86 respectively in E (Table 1). These R_F values would suggest the presence of 3,4-dihydroxyphenylacetone

Table 4. *Mass spectrum of dihydroxyphenylacetone*

The mass spectra of the trimethylsilyl derivative of 3,4-dihydroxyphenylacetone of retention time 19 min on 3% SE-30 and of the silylated extract of urine of retention time 19 min on 3% SE-30 were determined as described in the text. Values for the main peaks are given.

Bis(trimethylsilyl)-3,4-dihydroxyphenylacetone		Silylated extract of the urine of patient B. F.	
m/e	Relative intensity	*m/e*	Relative intensity
310	32	310	26
295	8	295	7
267	97	267	85
179	29	179	27
73	100	73	100
45	23	45	24

Metastable ions at 230 and 120 were present in both spectra. The cracking pattern is probably as follows; the metastable ion at *m/e* 230 supports the transition *m/e* 310→267 and that at *m/e* 120 the transition *m/e* 267→179.



The peak at 45 is due to the doublet $SiOH^+$, $SiCH_5^+$, by analogy with the trimethylsilyl ether of phenol (see Beynon *et al.*, 1968).

Table 5. *Amines and ketones in the urine of humans receiving α-methyl[^{14}C]dopa orally*

The first 24h urine of selected subjects receiving α-methyl[^{14}C]dopa (250mg) was examined. The metabolites were obtained after acid hydrolysis of the urine and estimated as described in the text.

		% of dose		
Compound	Subject	α-Methyldopamine	3-*O*-Methyl-α-methyldopamine	3,4-Dihydroxyphenylacetone
L-α-Methyldopa	G. D. (normal)	3.9	0.3	4.8
	W. R. } (responding hypertensives)	1.8	0.3	2.7
	M. T. }	2.7	0.3	3.3
	B. F. (non-responding hypertensive)	13.1	0.5	8.2
D-α-Methyldopa	G. D. (normal)	0.0	0.0	0.2

(3′,4′-dihydroxyphenylpropan-2-one) and its 3-*O*-methyl ether. However, on elution of the larger peak of R_F 0.51 in solvent D and rechromatographing it in the same solvent, two peaks (0.51 and 0.81) were obtained. The authentic 3,4-dihydroxyphenylacetone behaved similarly. Furthermore, g.l.c. of the ether extract indicated the presence of only one compound and this corresponded to the 3,4-dihydroxy ketone. It would appear that the small peak found at R_F 0.81 in solvent D is an artifact, which is probably an oxidation product of 3,4-dihydroxyphenylacetone, a catechol, and not 4-hydroxy-3-methoxyphenylacetone.

Authentic 3,4-dihydroxyphenylacetone was examined by g.l.c.–mass spectrometry. The silylated compound gave a single peak, as recorded on the total ion current monitor on the mass spectrometer, with a retention time of 19.0min. The mass spectrum of this peak (see Table 4) was consistent with a bis(trimethylsilyl) derivative. The fragmentation pattern given in Table 4 accounts for all the major peaks in the spectrum. The silylated urine extract also gave a peak with retention time 19.0min and there were no interfering peaks from tests on control urine. The mass spectrum of the silylated urine extract was identical with that of the authentic bis(trimethylsilyl) ether of 3,4-dihydroxyphenylacetone.

There was no evidence to indicate the presence in the urine extract of labelled 3,4-dihydroxybenzoic acid (protocatechuic acid) or its 3-*O*-methyl ether, vanillic acid.

Identification of amine metabolites

The characterization of the amine metabolites in the urine of one normal subject and three hypertensive patients receiving L-α-methyl[^{14}C]dopa and one normal subject receiving the D-isomer was carried out (Table 5). The amine fraction from the L-isomer on paper chromatography in solvent B (Table 1) gave two radioactive peaks, of R_F 0.35 and 0.47, but no amines were found after the D-isomer. These values correspond to α-methyldopamine and 3-*O*-methyl-α-methyldopamine. No evidence was found to indicate the presence of α-methylnoradrenaline, a possible metabolite.

Tables 3 and 5 show that in normal subjects the main metabolite of L-α-methyldopa appearing in urine is a conjugate of the drug, the total methyldopa excreted amounting to just over 20% of the dose. The minor metabolites are 3-*O*-methyl-α-methyldopa (about 4%) and, in the one case examined (Table 5), α-methyldopamine (3.9%), 3-*O*-methyl-α-methyldopamine (0.3%) and 3,4-dihydroxyphenylacetone (4.8%). In two normal subjects, only about 10% of the dose of D-α-methyldopa was excreted in the urine and this was mainly in the form of an acid-hydrolysable conjugate of the unchanged compound. The D-isomer did not appear to give any amines, but two minor metabolites, which were 3-*O*-methyl-α-methyldopa (1–2%) and 3,4-dihydroxyphenylacetone (0.2%).

The fate of L-α-methyldopa in hypertensive patients was in general similar to that in normal subjects, apart from one non-responder, patient B. F., who appeared to decarboxylate L-α-methyldopa more extensively than the others. This patient excreted 43% of the dose of ^{14}C in 24h, 15% as free and conjugated α-methyldopa and its 3-*O*-methyl ether and 28% as decarboxylated metabolites made up of the amines, α-methyldopamine (13% see Table 5) and its 3-*O*-methyl ether (0.5%) and the product of decarboxylation and deamination 3,4-dihydroxyphenylacetone (8%).

Faecal radioactivity

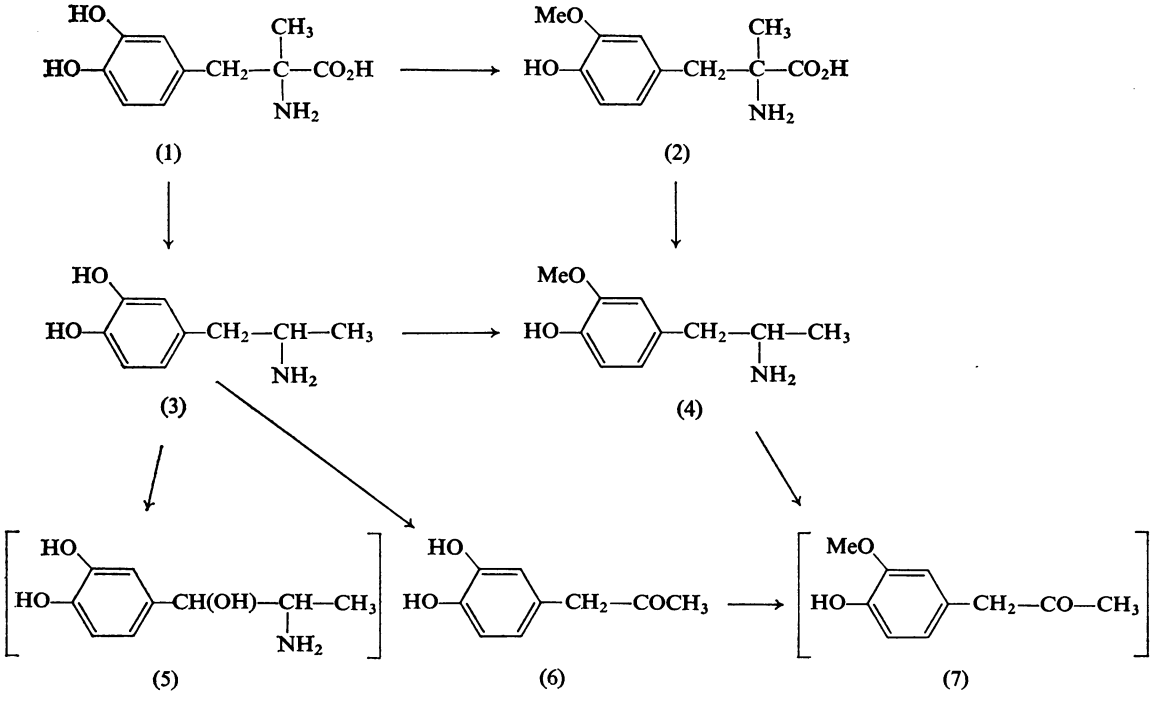
A sample of the faecal homogenate that had been made up to 1 litre was centrifuged and the supernatant was chromatographed in solvent systems A

and C (Table 1). In each case only one radioactive peak was found and this corresponded to unchanged α-methyldopa.

Discussion

About half of the dose of orally administered L-α-methyldopa was absorbed by the hypertensive patients (see Table 3) and this agrees with the observations of other workers (Dollery & Harington, 1962; Sjoerdsma *et al.*, 1963; Buhs *et al.*, 1964; Prescott *et al.*, 1966), although the variations in our results are not as great as those recorded by others. In the three normal volunteers, the absorption (35% of the dose) of the L-isomer appeared to be less than in the patients, but the volunteers took a single dose of the drug whereas the patients were on continuous therapy when they took the radioactive drug. It is noteworthy that the inactive D-isomer is much less readily absorbed than the active L-isomer in the normal volunteers, this absorption being only 10% of the dose as judged from the urinary and faecal excretion of ^{14}C radioactivity. A similar finding has been reported for the rat by Duhm *et al.* (1965) with the labelled D- and L-isomers. Young & Edwards (1964), who also examined the excretion of the D- and L-isomers in the rat, suggest an active transport mechanism that is optically specific, as seen with the D- and L-isomers of natural amino acids (Lin *et al.*, 1962).

The values found here for free and total L-α-methyldopa and its 3-*O*-methyl ether (compound 2, Scheme 1) in normal subjects and in responding hypertensive patients are similar to those found by earlier workers (Buhs *et al.*, 1964; Prescott *et al.*, 1966). The total amines and phenyl ketones (the 'neutral fraction' of earlier workers) are also similar in amounts to those found by these workers who, however, did not separate the components. This has now been done and the amines were found to consist of α-methyldopamine (compound 3) (about 2%) and 3-*O*-methyl-α-methyldopamine (0.3%; compound 4), the latter having previously been identified only in the rat. The phenyl ketone fraction has been identified as 3,4-dihydroxyphenylacetone (about 2.5% of the dose; compound 6) and this ketone has been previously identified as a metabolite of L-α-methyldopa only in the rat (Porter & Titus, 1963), probably as a



Scheme 1. *Metabolic pathways of* L-α-*methyldopa*

The compounds shown in brackets, (5) and (7), were not found in human urine in this study. With D-α-methyldopa, compounds (3), (4), (5) and (7) were not found and only very small amounts of compound (6) were detected.

sulphate conjugate. Buhs *et al.* (1964) did not find this ketone in human urine, probably because it is readily oxidized during isolation and it would appear that their 'neutral metabolite' conjugated with sulphate was probably this ketone.

The metabolites of D-α-methyldopa in human urine seem to be limited to the free and conjugated compound and its 3-*O*-methyl ether, with very small amounts of the phenyl ketone. These findings are similar to those of Sjoerdsma *et al.* (1963), who were unable to detect α-methyldopamine after the administration of the unlabelled D-isomer to man.

Prescott *et al.* (1966) have reported that hypertensive patients who became tolerant to L-α-methyldopa during treatment do not differ markedly in their ability to metabolize the drug from those who respond. The three non-responding patients examined in this study had a slightly lower average urinary excretion of ^{14}C radioactivity (41%) than the responders (51%). Two of the non-responding patients (G. G. and T. W.) have values for the minor metabolites similar to the responders, but one of the non-responders (B. F.) appeared to metabolize the absorbed drug more extensively than any other subject shown in Table 3. In fact in this patient about 65% of the ^{14}C radioactivity excreted in the urine in 24h is in the form of amines and ketones whereas in the other patients only about 10% is degraded to these metabolites (Table 3; see also Table 5).

On the other hand we have found no gross or qualitative difference in non-responding patients and one would expect such differences as have been found to be overcome therapeutically by increasing the dose of methyldopa. It is common clinical experience, however, that in many non-responding patients increasing the dose does not then lead to a therapeutic response. Though it is true that our numbers are small, we think it unlikely that the differences in metabolism of the drug between responders and non-responders could be responsible for the lack of hypotensive effect in certain patients.

It has been suggested that the clinical action of methyldopa could be due to α-methylnoradrenaline (compound 5), which Carlsson & Lindquist (1962) and Maître & Staehelin (1963) have claimed to have detected chromatographically in the central nervous system and peripheral tissues of mice treated with the drug and which Lindmar *et al.* (1968) claim to have detected by a biological method in the urine of humans treated with the drug. Pettinger *et al.* (1965) proposed that α-methylnoradrenaline replaces noradrenaline in storage sites and acts as a false neurotransmitter when released at nerve endings after sympathetic stimulation; Day & Rand (1964) have assumed that the clinical action of methyldopa is due to α-methylnoradrenaline being less effective than noradrenaline in stimulating the α-receptors of blood vessels. α-Methylnoradrenaline, however, could not be detected in the urine in the present radioactive tracer study, but this does not rule out the false-transmitter theory since the minimum amount detectable in this study was 0.1% of the dose whereas Lindmar *et al.* (1968) claim to have detected an amount one-tenth of this.

Sjoerdsma *et al.* (1963) have made the suggestion that the hypotensive action of α-methyldopa is mediated through the metabolite α-methyldopamine, but Buhs *et al.* (1964) were unable to produce any effect in hypertensive patients by either oral administration or intramuscular injection of this amine. The hypotensive effect of α-methyldopa may be due to a central action, and decarboxylation of the drug within the central nervous system is necessary (Henning, 1969; Salmon & Ireson, 1970). It would appear that two compounds have been postulated as the active metabolites of α-methyldopa, namely α-methyldopamine and α-methylnoradrenaline. The former is known to be β-hydroxylated peripherally to the latter in mice (Carlsson *et al.*, 1968), but neither can readily penetrate the blood–brain barrier.

Our non-responding patients were excreting as much α-methyldopamine (in one case much more) as the responding patients. If it is assumed, as suggested by others (see above), that decarboxylation of α-methyldopa must occur in the central nervous system for the drug to be effective, it is possible that the non-responding patients have either a defect in the penetration of α-methyldopa into the central nervous system or, if there is no such fault, a central defect in the decarboxylation and subsequent β-hydroxylation of α-methyldopa.

This investigation was approved by the Ethical Committee of St. Mary's Hospital and Medical School. Permission to administer ^{14}C-labelled α-methyldopa was obtained from the Radioisotopes Committee of the Medical Research Council. Informed consent to the investigation was obtained from all the patients studied. W. Y. W. A. participated in this work during a sabbatical leave supported by the U.S. Public Health Service. We are grateful to the Medical Research Council for a grant for a mass-spectrometer.

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