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PHARMACOKINETICS OF METHYLDOPA IN MAN

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ABSTRACT

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Single doses of methyldopa were administered orally and intravenously as aqueous solutions to 12 healthy volunteers in a crossover study. Serial plasma and urine samples were analyzed specifically for methyldopa and its O-sulfate conjugate. Kinetic analyses of the results indicated that methyldopa disposition could be adequately represented by a two-compartment open model. Renal excretion accounted for about two-thirds of the plasma clearance of methyldopa. Absorption profiles were constructed with the aid of the pharmacokinetic model and contrasted with estimates of absorption which were model-independent. The mean fraction reaching the systemic circulation as methyldopa was estimated to be 0.25 (range 0.08-0.62 for $n = 11$). Although most of the absorption occurred within the first 5 hours oral administration, a minor component, suggestive of limited enterohepatic circulation, persisted from 9 to 36 hours. O-sulfate conjugation was route-dependent and appeared to be derived predominantly, if not exclusively, as a first-pass effect of absorption and/or enterohepatic circulation.

Methyldopa[2] is an effective antihypertensive agent which has been available for about a decade. During that time, it has been associated with a wide range of effective oral doses. Pharmacologic studies in man have suggested considerable variation in absorption on the basis of urinary recovery (Sjoerdsma *et al.*, 1963; Prescott *et al.*, 1966). Metabolism studies with methyldopa-^{14}C in man have indicated that the mono-O-sulfate conjugate is the predominant product of biotransformation (Buhs *et al.*, 1964; Au *et al.*, 1972). Variability in both absorption and metabolism may be contributing to the range in effective dosage.

A series of studies have been undertaken to elucidate the pharmacokinetics of methyldopa in man. The first of these studies, which is reported herein, is an attempt to define the kinetics of absorption and disposition after the oral and intravenous administration of methyldopa as an aqueous solution to healthy volunteers. It will serve as a reference point for subsequent studies which will be more concerned with metabolism and biopharmaceutics of methyldopa.

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Methods and Materials

Clinical

In an open crossover study, 12 healthy, male subjects were selected from a group of healthy prison volunteers; these men were not receiving medications

previously and had normal results for laboratory studies including complete blood count, differential, blood urea nitrogen, creatinine, urinalysis, and liver function. The men were divided into two groups of six for the initiation of the study on two different days; each group of six men was further divided into two groups of three, each of whom received either one of the two treatments according to a random allocation. They reported in the fasting state, and except for medications, remained fasting for the first 4 hours.

Control blood samples were collected prior to drug administration, and a 12-hour urine collection was taken prior to the administration of drug to serve as base line or test blanks. Each of three men on each test day received a 750 mg oral dose of methyldopa as a solution in 120 ml of water; this was followed by another 120 ml of water to wash the dose into the gastric lumen. The other three men were started on a 250 mg i.v. dose of methyldopa in 240 ml of sterile dextrose water and infused over 90 minutes. A second test day followed 4 days later with crossover of dosage forms so that each man received both preparations.

Blood samples in heparin-treated vacutainers were collected periodically up to 9 hours after oral medication and up to 450 minutes after the initiation of the intravenous dose. Plasma was immediately harvested after centrifugation, was frozen and was maintained in the frozen state until time of analysis. Total urine collections were carried out for 36 hours postdrug administration in fractions representing output from 0 to 2, 2 to 4, 4 to 8, 8 to 12, 12 to 24 and 24 to 36 hours; collections were made in bottles containing 1 ml of 0.1 N HCl solution. Aliquots were kept frozen until analyzed. Blood pressure and pulse rates were measured at 0, 3, 6 and 24 hours after doses.

Analytical

Plasma and urine were analyzed for free and conjugated methyldopa. Free methyldopa was adsorbed on alumina. After hydrolysis, conjugated methyldopa was again adsorbed on alumina, eluted with acid and determined by the fluorescence of the hydroxyindole formed by an oxidation reaction (Laverty and Taylor, 1968).

To tubes containing 2 ml of plasma were added 10 ml of 0.4 N perchloric acid. The tubes were mixed (Vortex), let stand for 5 minutes, mixed again, and centrifuged for 15 minutes. Ten milliliters of the centrifugates were transferred to 30-ml centrifuge tubes, each of which contained 200 mg of alumina (prepared according to Anton and Sayre, 1962), 5 mg of sodium metabisulfite and 2.5 ml of a 4% solution of disodium edetate. Urine was diluted with 0.01 N hydrochloric acid and 1 ml of this diluted urine and 10 ml of 0.4 N perchloric acid were added directly to centrifuge tubes containing alumina, sodium metabisulfite and disodium edetate as above.

The pH of each solution was then adjusted to 9.0 ± 0.1 with 1 N NaOH. The tubes were stoppered, shaken for 5 minutes and centrifuged. A 15-ml aliquot of the centrifugates, containing methyldopa conjugates, was transferred to a second set of 30-ml glass stoppered centrifuge tubes each of which contained 5 ml of 0.8 N perchloric acid. These latter tubes were then placed in a boiling water bath. After 30 minutes, the tubes were removed and cooled, 200 mg of alumina was added and the adsorption of the liberated methyldopa was carried out as for the first set of tubes. The alumina from both sets was washed with 20 ml of distilled water which was discarded. The methyldopa from each set was eluted from the alumina with 3 ml of 0.05 N hydrochloric acid. The tubes were shaken for 5 minutes and centrifuged.

One-milliliter aliquots were added to tubes containing 1 ml of 4% disodium edetate and 1 ml of 0.5 M potassium phosphate pH 6.5 buffer. Iodine solution[3] (0.4 ml) was added to each tube, which was then mixed. Alkaline sulfite solution[4] (0.5 ml) was added, the tubes were mixed and again after 2 minutes, 0.6 ml of 5 N acetic acid was added and the tubes were placed in a boiling water bath for 5 minutes. The tubes were cooled and the fluorescence of the solutions was determined with an Aminco-Bowman spectrophotofluorometer at 325-nm activation and 375-nm emission wavelengths.

Methyldopa standards in perchloric acid solution were added to control plasma or urine and were taken through the procedure simultaneously with the samples. The recovery of methyldopa-^{14}C added to control plasma was 64 ± 5% and was concentration-independent up to 20 mg/ml.

The background fluorescence in plasma and urine due to endogenous catecholamines for 24 control samples from the 12 subjects was equivalent to 0.28 ± 0.03 μg/ml of methyldopa for both the free and conjugated plasma fractions and 0.62 ± 0.12 μg/ml for the urine. The background fluorescence for each control sample was subtracted from the reading obtained for the subsequent samples from that same treatment for each subject.

The conditions employed for the hydrolysis of the conjugates of methyldopa were determined by heating in a boiling water bath for various lengths of time the free and conjugated fractions of pooled plasma and urine from human subjects given methyldopa. Maximal hydrolysis of conjugated methyldopa and minimal degradation of free methyldopa were found to occur with perchloric acid solutions heated for 30 minutes. Hydrochloric acid did not hydrolyze the conjugates as efficiently as perchloric acid, and

[3] Dissolve 635 mg of iodine in 10 ml of water containing 12 g of KI, q.s. with water to 250 ml.

[4] Dissolve 1.26 g of sodium sulfite in 10 ml of water, q.s. to 50 ml with 5 N NaOH. This solution is prepared fresh daily.

enzymatic hydrolysis resulted in substantial degradation of methyldopa.

Results

Mean plasma concentrations of free and conjugated methyldopa for the orally-administered dose of 750 mg and for the intravenous dose of 250 mg are shown in table 1. Values for free methyldopa are plotted in figure 1. A peak concentration of 2.62 μg/ml was obtained at 3 hours after the administration of an oral solution of methyldopa. A peak concentration of 7.50 μg/ml was achieved at the end of the 250 mg intravenous infusion.

The plasma samples from the two routes of administration showed a marked difference in the proportion of methyldopa present as conjugated material. For the first 150 minutes after the start of the intravenous dosage, all of the methyldopa in the plasma was unconjugated. With the oral solution, the earliest plasma sample at 15 minutes, contained only 86% of methyldopa in the free state and this declined over the first 2 hours postdrug to 70%. The decline in free methyldopa when expressed as percentage of total circulating drug is shown in figure 2.

The mean incremental urinary recoveries of free and conjugated methyldopa are given in table 2. The urinary recovery in 36 hours of methyldopa when given intravenously averaged 66% of the dose of 250 mg in these volunteers. The range included a low of 52% and a high of 82%. Individual 36-hour urinary recovery data are summarized in table 3. The major portion of this recovery (98%) was identified as free methyldopa.

When the oral dose of 750 mg as a solution was given to the same volunteers, the urinary recovery averaged 27% in 36 hours. The range of recoveries varied from a low of 9% to a high of 57% of the dose. Of that which was recovered, 61% was identified as free methyldopa; in comparison with intravenous dosage, a larger fraction (39% *vs.* 2%) of this recovery was conjugated. Hence, urinary results are in agreement with plasma results in that conjugation occurs to a much greater extent for drug administered by the oral route.

Blood pressure data indicated a minor, insig-

TABLE 1

Mean plasma concentrations of free and conjugated methyldopa after the administration of 250 mg intravenously and 750 mg orally to 12 subjects

Time	Intravenous Dosage		Oral Dosage	
	Free	Conjugated	Free	Conjugated
min	*μg/ml*		*μg/ml*	
15	2.07 (0.30)[a]	0 (0)	0.18 (0.09)	0.03 (0.02)
30	3.59 (1.26)	0 (0)	0.74 (0.36)	0.12 (0.08)
45			1.33 (0.71)	0.30 (0.19)
60	6.01 (1.45)	0 (0)	1.84 (1.24)	0.52 (0.31)
90	7.50 (1.46)	0 (0)	2.40 (1.95)	0.84 (0.37)
105	5.42 (1.16)	0 (0)		
120	4.59 (0.93)	0 (0)	2.47 (1.66)	1.07 (0.42)
135	3.73 (0.79)	0 (0)		
150	3.30 (0.89)	0.01 (0.03)		
180			2.62 (1.52)	1.29 (0.45)
210	2.05 (0.53)	0.03 (0.03)		
270	1.26 (0.37)	0.04 (0.04)		
300			1.81 (1.35)	1.01 (0.42)
330	0.90 (0.28)	0.04 (0.04)		
390	0.57 (0.17)	0.05 (0.04)		
420			1.10 (1.09)	0.74 (0.36)
450	0.39 (0.12	0.06 (0.06)		
480			0.81 (0.77)	0.63 (0.36)
540			0.59 (0.54)	0.51 (0.32)

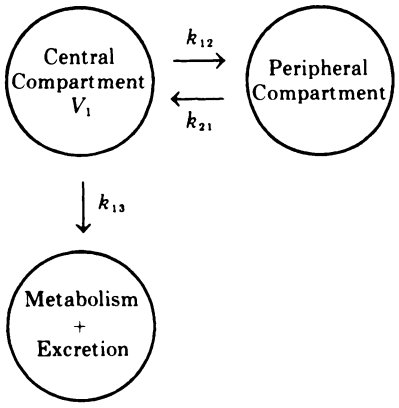
[a] Standard deviation.

nificant decline in mean pressure after the intravenous dose. Oral doses were accompanied by less change in blood pressure, and some subjects showed little or no change. Hence, this study corroborates the commonly recognized and documented (Cannon *et al.*, 1962; Theilen *et al.*, 1963; Wilson *et al.*, 1961; Lindmar *et al.*, 1968) limited action of methyldopa on the blood pressure of normotensives in spite of the presence of the drug in the body in a similar magnitude for the effective management of hypertension (Buhs *et al.*, 1964; Prescott *et al.*, 1966).

Pharmacokinetic Analysis

Distribution, Metabolism and Excretion

The kinetics of methyldopa disposition after intravenous infusion can be represented by a two-compartment open model; *i.e.*,



where k_{12}, k_{21} and k_{13} are first-order rate constants for the designated processes, and V_1 is the apparent volume of distribution for the central compartment which includes blood plasma. Model parameters, estimated from the postinfusion plasma concentration curve for 12 individual subjects, are shown in table 4. Plasma half-lives and other pharmacokinetic parameters are shown in table 5. The adequacy of the model can be verified in several ways. First, individual infusion profiles are constructed by applying parameters in table 4 to the corresponding methyldopa plasma concentration data in accordance with the method of Loo and Riegelman (1968). A comparison of expected and observed infusion profiles is shown in figure 3. It is noted that the infusion curves rise linearly during the infusion period, and plateau at 90 minutes, corresponding to the end of infusion. The observed average of 244 mg for the plateau is in excellent agreement with the dose of 250 mg. Individually, greater deviations from linearity are seen during

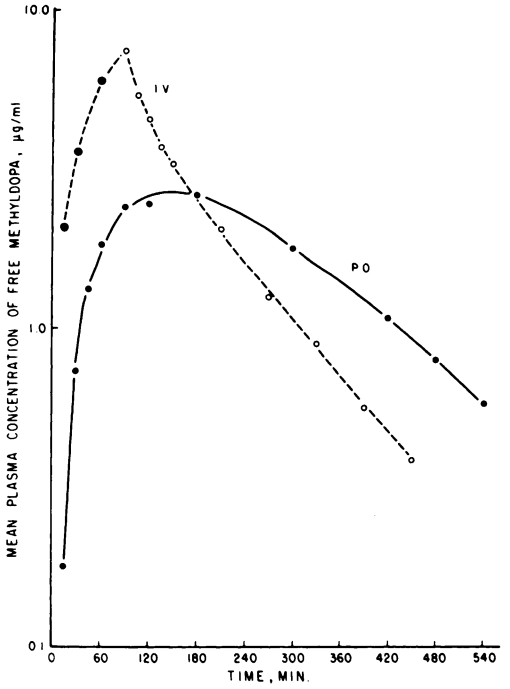


FIG. 1. Mean plasma concentration of methyldopa after a 750 mg oral dose and an intravenous infusion of 250 mg in 12 subjects.

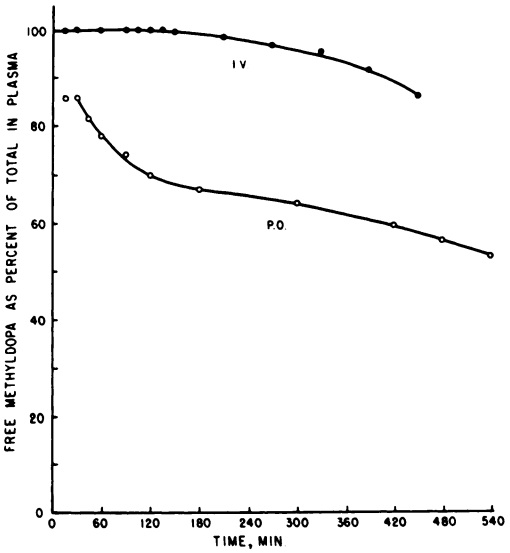


FIG. 2. Relative amounts of free methyldopa in plasma after oral and intravenous dosage. Mean of 12 subjects.

TABLE 2

Mean urinary recovery in milligrams of methyldopa after the administration of 250 mg intravenously and 750 mg orally to 12 subjects

Time	Intravenous Dosage			Oral Dosage		
	Free	Conjugated	Total	Free	Conjugated	Total
hr		*mg*			*mg*	
0-2	83.7 (15.6)[a]	0.2 (0.3)	83.9 (8.1)	26.3 (20.0)	7.4 (4.1)	33.7 (22.6)
2-4	42.3 (8.1)	0.5 (0.6)	42.8 (8.3)	44.1 (33.3)	18.1 (7.9)	62.2 (39.0)
4-8	23.9 (6.0)	1.2 (1.3)	25.0 (6.7)	44.2 (37.0)	27.2 (11.7)	71.4 (47.4)
8-12	4.8 (2.4)	0.8 (0.6)	5.6 (2.9)	11.2 (9.2)	10.1 (5.9)	21.3 (14.5)
12-24	4.4 (2.3)	1.3 (0.8)	5.7 (2.6)	6.4 (5.2)	5.4 (5.6)	11.9 (10.1)
24-36	1.2 (1.1)	0.5 (0.7)	1.8 (1.5)	2.5 (1.3)	1.5 (2.5)	4.0 (3.4)

[a] Standard deviation.

TABLE 3

Summary of urinary recovery after the administration of methyldopa 250 mg intravenously and 750 mg orally

Subject	0-36 Hr Recovery						Recovery Methyldopa	
	Methyldopa Equivalent			Dose				
	Free	Conjugated	Total	Free	Conjugated	Total	Free	Conjugated
		mg			%			%
Intravenous								
301	148.4	3.4	151.8	39.4	1.4	60.8	97.8	2.2
302	141.9	1.5	143.4	56.8	0.6	57.4	99.0	1.0
303	197.9	7.2	205.1	79.2	2.9	82.1	96.5	3.5
304	146.4	6.9	153.3	58.6	2.0	60.6	96.6	3.4
305	168.3	8.1	176.4	67.3	3.2	70.5	95.4	4.6
306	164.3	7.9	170.5	65.7	2.5	68.2	96.4	3.4
307	169.8	2.0	171.8	67.9	0.8	68.7	98.8	1.2
308	174.1	1.1	175.2	69.6	0.4	70.0	99.4	0.6
309	155.3	3.1	158.4	62.1	1.2	63.3	98.0	2.0
310	127.4	2.1	129.5	51.0	0.8	51.8	98.4	1.6
311	178.4	3.4	181.8	71.4	1.4	72.8	98.1	1.9
312	151.9	5.4	157.3	60.8	2.2	63.0	96.6	3.4
Mean	160.3	4.3	164.6	64.2	1.6	65.8	97.6	2.4
Oral								
301	59.0	35.7	95.0	7.9	4.8	12.7	62.1	37.9
302	26.1	40.3	66.4	3.5	5.4	8.9	39.3	60.7
303	200.8	61.0	261.8	26.8	8.1	34.9	76.7	23.3
304	62.4	58.2	120.6	8.3	7.8	16.1	51.7	48.3
305	144.4	79.0	223.4	19.3	10.5	29.8	64.6	35.4
306	286.2	111.3	397.5	38.2	14.8	53	72.0	28.0
307	48.9	47.1	96.0	6.5	6.3	12.8	50.9	49.1
308	226.5	83.5	310.0	30.2	11.1	41.3	73.1	26.9
309	290.4	134.4	424.8	38.7	17.9	56.6	68.4	31.6
310	53.5	58.9	112.4	7.1	7.9	15.0	47.6	52.4
311	120.7	37.8	158.5	16.1	5.0	21.1	76.2	23.8
312	97.8	95.0	192.8	13.0	12.7	25.7	50.7	49.3
Mean	134.7	70.1	204.8	18.0	9.4	27.4	61.2	38.8

TABLE 4

Pharmacokinetic parameters of methyldopa in man

Subject	Rate Constants			Volume of Distribution	Plasma Clearance
	k_{12}	k_{21}	k_{13}	V_1	$k_{13}V_1$
		min^{-1}		l	ml/min
301	0.00714	0.00992	0.0195	14.03	273.4
302	0.0175	0.0122	0.0189	14.87	281.1
303	0.0123	0.0137	0.0142	11.65	165.4
304	0.0183	0.0236	0.0145	13.35	194.2
305	0.00895	0.0205	0.0127	17.23	219.3
306	0.00879	0.0182	0.0107	15.19	162.5
307	0.0133	0.0236	0.0141	16.79	237.1
308	0.00770	0.0189	0.00954	21.36	203.8
309	0.0139	0.0194	0.0141	21.34	300.9
310	0.00889	0.0157	0.0138	15.79	217.9
311	0.0137	0.0126	0.0182	9.23	167.8
312	0.0124	0.0191	0.0140	17.34	217.9
Mean	0.0119	0.0173	0.0145	15.68	220.1
Standard deviation	0.00367	0.00445	0.00302	3.54	46.1
C.V. (%)	30.1	25.7	20.8	22.6	20.9

TABLE 5

Individual weight-adjusted volumes of distribution, surface-adjusted renal clearance and plasma half-life of methyldopa

Subject	Body Weight	Apparent Volume of Distribution[a]	Renal Clearance[b]	Plasma Half-life
	kg	l/kg	$ml/min/m^2$	min
301	67.7	1.40	67.6	108.0
302	67.3	1.22	60.4	130.0
303	91.8	0.45	54.1	123.1
304	63.2	0.54	70.7	100.0
305	65.0	0.55	69.1	92.1
306	114.1	0.28	60.7	112.0
307	74.1	0.51	45.6	90.0
308	89.5	0.45	59.0	116.1
309	69.1	0.83	106.2	103.0
310	65.5	0.67	59.5	101.0
311	68.6	0.66	85.1	117.1
312	61.4	0.73	119.4	100.0
Mean	71.8	0.69	71.4	106.4[c]
S.D.	15.7	0.33	21.8	12.5
C.V. (%)	21.9	47.8	30.5	11.7

[a] V_d, extrapolation = $V_1(\alpha-\beta)/(k_{21}-\beta)$.

[b] Body surface area (cm^2) = $11.0 \times$ [body weight, g]$^{0.66}$.

[c] Harmonic mean.

the infusion period. This, too, is expected since occasional readjustments in infusion rates were made during gravity feed. The validity of the model depends greatly on the proper execution of the experimental plan because the shape of the post-infusion curve, from which pharmacokinetic parameters are derived, is affected by the timing and the constancy of the infusion.

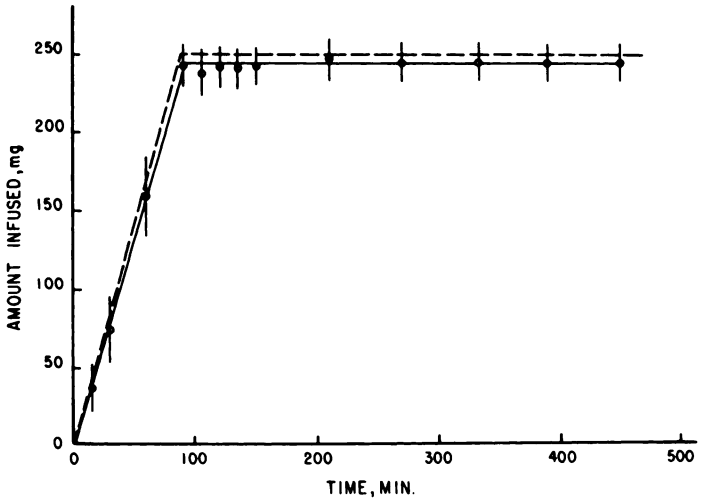


FIG. 3. Mean infusion profile. Planned (----); calculated (O——O); length of vertical lines represents ± 1 standard deviation of the mean 12 subjects.

Thus, the propinquity between the simulated and the expected infusion profiles provide insight concerning not only the pharmacokinetic model but also the implementation of the study plan. Both appear adequate.

A further verification of the model is provided by comparisons between the observed plasma and urinary excretion profiles and those calculated in accordance with the two-compartment open model (Benet, 1972). Figure 4 is typical of the type of agreement found. The goodness of fit for the plasma concentration profiles is not unexpected, because it is already implicit in the agreement between observed and expected infusion profiles which are generated from pharmacokinetic parameters derived from plasma concentration data. However, an adequate model should also predict the time course of urinary excretion. Table 6 shows individual urinary excretion profiles calculated with parameters from table 5, and their agreement with those observed. It has been shown that this type of agreement is possible only when plasma and renal clearances are sufficiently constant within a given treatment period (Kwan *et al.*, 1971).

A final demonstration of the appropriateness of the model is the fact that plasma clearances, $\dot{V}_{cl,p}$, estimated from model parameters ($k_{13}V_1$) are in agreement with those calculated without the aid of a model. The proximity of these estimates is shown by comparing the individual entries in the last column of table 4 with those in column 3 ($\dot{V}_{cl,p} = \dot{V}_{cl,r}/f$) of table 7, where

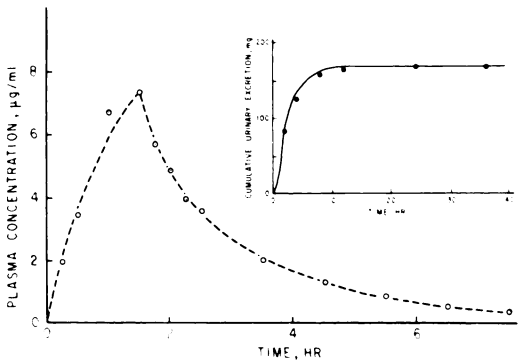


FIG. 4. Typical individual plasma concentration and urinary excretion profiles after an intravenous infusion of methyldopa, 250 mg. Observed (O, ●); predicted (----,——) with the aid of pharmacokinetic parameters in table 5. (Subject 305).

f is the fraction of the dose recovered in the urine as free methyldopa.

Absorption

Two different types of analyses were performed on the plasma concentration and urinary excretion data after oral administration in the same subjects. The first is a kinetic analysis based on the two-compartment open model for methyldopa disposition and the second a model-independent method based on estimates of plasma clearance and total urinary recovery of unchanged drug. It will be shown that the conclusions from the two methods of analysis are compatible and mutually reinforcing.

For a drug whose disposition can be represented by a two-compartment open model, the time course of oral absorption can be estimated by the technique of Loo and Riegelman (1968). This method attempts a material balance between the central compartment, the peripheral compartment and that which is already metabolized and excreted at each sample point; it assumes that each of the model parameters derived from intravenous administration (table 4) is equally applicable for a given subject after oral doses. Lack of constancy in some of these parameters between treatments is generally manifested as changes in plasma half-life ($t_{\frac{1}{2}}$) or renal clearance or both. If a disparity occurs between treatments, an adjustment in the parameters can be introduced.

An independent estimate of the extent of absorption can be obtained by the method of Kwan and Till (1973), which is model-independent. This method is based on the fact that total urinary recovery, U_∞, from an oral dose, D, is the product of that fraction which is absorbed (F) and that which is excreted (f) unchanged in the urine, *i.e.*,

$$U_\infty = fFD \quad (1)$$

Since f is just the ratio of renal clearance to plasma clearance,

$$f = \frac{\dot{V}_{cl,r}}{\dot{V}_{cl,p}} \quad (2)$$

an estimate of the amount absorbed, FD, can be obtained from observed values of U_∞ and $\dot{V}_{cl,r}$ and assumptions concerning $\dot{V}_{cl,p}$. The presumption is that $\dot{V}_{cl,p}^{\text{i.v.}}$ is an estimate of $\dot{V}_{cl,p}^{\text{p.o.}}$ and that changes in $\dot{V}_{cl,p}$ following oral and intravenous administration would be manifested as observed differences between $\dot{V}_{cl,r}^{\text{i.v.}}$ and $\dot{V}_{cl,r}^{\text{p.o.}}$. If necessary, appropriate adjustments can be made (Kwan and Till, 1973).

It should be noted that assumptions concerning renal and plasma clearances are common to the kinetic and the model-independent methods

TABLE 6

Urinary excretion of methyldopa after intravenous infusion in man (dose: 250 mg)

Subject	Source	Amount mg Excreted after					
		2 hr	4 hr	8 hr	12 hr	24 hr	36 hr
				mg			
301	Observed	93.1	129.0	143.0	145.8	148.2	148.4
	Predicted[a]	95.3	128.8	144.2	147.5	148.4	148.4
302	Observed	53.9	117.2	139.5	141.9	141.9	141.9
	Predicted[a]	76.7	109.3	132.8	139.4	141.8	141.9
303	Observed	98.4	145.7	175.3	185.8	195.0	197.9
	Predicted[a]	99.2	150.8	185.8	194.8	197.9	197.9
304	Observed	66.5	112.0	139.8	144.8	146.4	146.4
	Predicted[a]	72.4	114.8	140.3	145.2	146.4	146.4
305	Observed	82.0	124.8	156.2	162.2	168.3	168.3
	Predicted[a]	86.0	136.3	163.1	167.4	168.3	168.3
306	Observed	76.1	121.1	151.0	158.5	163.1	164.3
	Predicted[a]	74.6	123.3	155.0	162.2	164.3	164.3
307	Observed	105.2	140.8	159.0	163.1	167.2	169.8
	Predicted[a]	88.0	138.1	164.8	169.0	169.8	169.8
308	Observed	92.7	131.7	163.4	165.7	171.2	174.1
	Predicted[a]	74.6	127.2	162.9	171.4	174.1	174.1
309	Observed	86.5	123.4	145.0	148.8	153.8	155.3
	Predicted[a]	78.1	122.0	148.7	154.0	155.3	155.3
310	Observed	70.4	102.1	119.1	122.1	126.7	127.4
	Predicted[a]	67.1	102.8	122.7	126.5	127.4	127.4
311	Observed	102.6	146.1	167.4	173.3	177.9	178.4
	Predicted[a]	100.2	142.9	169.8	176.3	178.4	178.4
312	Observed	77.1	117.7	139.2	143.8	149.0	151.9
	Predicted[a]	77.6	120.8	146.0	150.8	151.9	151.9

[a] Calculated with the aid of parameters from table 4.

of analysis. In terms of the two-compartment open model,

$$\dot{V}_{cl,p} = k_{13}V_1 \quad (3)$$

and

$$\dot{V}_{cl,r} = fk_{13}V_1. \quad (4)$$

Thus, the two methods of analysis can be performed in parallel provided adjustments for changes in disposition between intravenous and oral treatments reflected by $t_{1/2}$ or $\dot{V}_{cl,r}$ are made consistently between methods.

In the present study, even though incremental renal clearances were acceptably constant within a given treatment, the observed means for each subject do vary between study days. The magnitude of these variations is seen by comparing columns 2 and 4 in table 7. To compensate for these observed differences, adjustments in $\dot{V}_{cl,p}$ and k_{13} were made on the assumption that the nonrenal components of elimination did not change; hence,

$$\dot{V}'_{cl,p} = \dot{V}^{\text{i.v.}}_{cl,p} - \dot{V}^{\text{i.v.}}_{cl,r} + \dot{V}^{\text{p.o.}}_{cl,r} \quad (5)$$

$$k'_{13} = \dot{V}'_{cl,p}/V_1 \quad (6)$$

Model-independent estimates of absorption are obtained by the appropriate substitution of the adjusted plasma clearance, $\dot{V}'_{cl,p}$, and the corresponding values of $\dot{V}^{\text{p.o.}}_{cl,r}$ and $U^{\text{p.o.}}_{\infty}$ into equations 1 and 2. The results are shown in the last column of table 8. The adjusted elimination rate constants, k'_{13}, together with the corresponding k_{12}, k_{21} and V_1 from table 4, are then applied to the oral plasma concentration data to construct absorption profiles according to the kinetic method of Loo and Riegelman (1968). Individual time courses of oral absorption are shown in table 8. It is evident that there is good agreement between the two methods in estimating absorption.

It should be noted that the kinetic method can only yield information on absorption up to the 9th hour, the last plasma sample point. By contrast, the model-independent method estimates total absorption up to 36 hours, the last urine collection period. If the absorption process were complete by the 9th hour, the two estimates for absorption would be expected to be identical. However, in spite of the relatively short plasma half-life (106 minutes), there is evidence that small amounts of unchanged drug continued to be excreted in the urine beyond 24 hours. This suggests that absorption, albeit minor, may continue beyond the 9th hour. Under these circumstances, the amount absorbed up to 9 hours calculated by kinetic analysis is expected to be slightly below or just

TABLE 7

Model-independent estimates of amounts absorbed after oral administration of methyldopa (dose: 750 mg)

Subject	Clearances				Urinary Recovery $U_\infty^{\text{p.o.}}$	Amount Absorbed $FD^{\text{p.o.}}$
	$\dot{V}_{cl,r}{}^{\text{i.v.}}$	$\dot{V}_{cl,p}{}^{\text{i.v.}}$	$\dot{V}_{cl,r}{}^{\text{p.o.}}$	$\dot{V}_{cl,p}{}^{\text{p.o.}}$		
	ml/min				*mg*	*mg*
301	152.9	255.9	115.0	218.0	59.0	111.8
302	176.3	310.6	106.9	241.2	26.1	58.9
303	129.7	163.8	117.3	151.4	200.8	259.2
304	111.6	190.6	120.2	199.2	62.4	103.4
305	138.3	205.4	119.6	186.7	144.4	225.4
306	99.0	150.6	152.4	204.0	286.2	383.1[a]
307	151.0	222.3	86.2	157.5	48.9	89.3
308	140.8	202.2	125.6	187.0	226.5	337.2
309	184.8	297.5	191.2	303.9	290.4	461.6
310	118.5	232.5	103.6	217.6	53.5	112.4
311	125.0	175.2	152.4	202.6	120.7	160.5
312	151.9	250.0	199.4	297.5	97.8	145.9
Mean	140.4	221.4	132.5	213.9	134.7	187.7
S.D.	24.8	50.4	34.7	47.5	94.6	123.0
C.V. (%)	17.7	22.8	26.7	22.2	70.2	65.5

[a] Not included in the mean.

TABLE 8

Estimated oral absorption profiles of methyldopa after single 750-mg doses as an aqueous solution in man

Subject	Adjusted Elimination Rate k'_{13}	Cumulative Absorption[a] at Checked Time Stages (Minutes after Administration)											Total Amount Absorbed[b]
		15	30	45	60	90	120	180	300	420	480	540	
	min						*mg*						*mg*
301	0.0161	2.2	6.6	17.7	22.0	39.2	53.7	79.8	99.2	101.8	102.7	102.8	111.8
302	0.0154	0.7	2.9	4.8	8.9	18.4	29.4	43.1	48.3	48.4	49.2	49.8	58.9
303	0.0130	2.7	15.9	28.1	34.5	56.6	83.5	164.5	235.1	248.5	251.1	255.5	259.2
304	0.0150	4.8	17.1	27.1	33.3	41.6	49.5	74.0	93.1	93.4	93.6	93.6	103.4
305	0.0111	3.9	15.7	31.9	49.4	68.2	88.5	129.6	189.5	223.6	230.2	231.0	225.4
306	0.0137	4.5	17.9	39.6	60.2	102.1	144.8	213.9	331.2	397.5	414.6	423.9	383.1
307	0.0120	4.2	17.4	38.8	41.2	55.4	67.0	79.3	83.6	82.6	83.1	83.6	89.3
308	0.00878	4.1	28.8	74.9	142.9	260.0	286.5	302.7	312.3	314.7	317.8	319.2	337.2
309	0.0143	3.1	16.8	35.2	60.8	112.2	151.2	221.8	330.2	442.9	460.9	466.2	461.6
310	0.0133	3.1	17.1	28.2	37.9	56.5	69.4	95.0	108.1	107.3	106.9	107.9	112.4
311	0.0217	1.4	8.2	20.0	33.8	50.3	64.1	101.4	131.2	140.5	142.0	144.5	160.5
312	0.0170	0.2	2.3	8.1	17.3	33.1	46.3	70.2	112.2	125.1	132.7	137.2	145.9

[a] Estimated by the kinetic method of Loo and Riegelman (1968).
[b] Estimated by the model-independent method of Kwan and Till (1973).

TABLE 9

The absorption, urinary excretion and renal clearance of methyldopa and its conjugate after oral administration

Subject	Absorption of Unchanged Drug		Urinary Recovery, Free + Conjugates	Renal Clearance	
	Method A[a]	Method B[b]		Free[c]	Conjugate
	%		%	*ml/min*	
301	13.7	14.9	12.6	115.0	183.9
302	6.6	7.9	8.9	106.9	164.2
303	34.1	34.6	34.9	117.3	116.2
304	12.5	13.8	16.1	120.2	138.2
305	30.8	30.0	29.8	119.6	118.7
306	56.5[d]	51.1[d]	53.0[d]	152.4	150.6
307	11.1	11.9	12.8	86.2	119.9
308	42.5	44.9	41.3	125.6	108.2
309	62.1	61.5	56.6	191.2	140.7
310	14.4	15.0	15.0	103.6	92.2
311	19.3	21.4	21.1	152.4	97.3
312	18.3	19.4	25.7	199.4	156.8
Mean	24.1	25.0	25.0	132.5	132.2
S.D.	16.7	16.4	14.6	34.7	28.2
C.V. (%)	69.3	65.4	58.6	26.2	21.3

[a] Kinetic method for absorption up to 9 hours.
[b] Model-independent method for absorption up to 36 hours.
[c] Same as Column 4 of table 7.
[d] Not included in the mean.

approaching the model-independent estimate. Within limits of experimental error, such is indeed the case for 11 of the 12 subjects (columns 2 and 3 in table 9). Attempts to seek better agreement for subject 306 by considering alternate corrective measures (Till *et al.*, 1974) for changes in elimination and/or renal clearance were not successful. It is suspected, therefore, that urinary recovery after oral dosage may be incomplete or that k_{12}, k_{21} or both might not have been sufficiently constant between intravenous and oral administrations in this subject

to yield valid estimates by the kinetic treatment.

Conjugation

An interesting observation from this study is that the sum of the urinary recoveries of methyldopa and its conjugate closely approximates the amount of unchanged methyldopa absorbed from an oral dose (table 9). A reasonable explanation for this fortuitous result appears to be as follows. Approximately two-thirds of an intravenous dose is excreted in the urine unchanged, 1.6% as the conjugate (table 3). By analogy, approximately two-thirds of that which is absorbed unchanged from an oral dose is expected to appear in the urine unchanged and very little as the conjugate. Thus, in order for total urinary recovery (free + conjugate) to approximate the amount absorbed, a quantity equivalent to one-third of the amount absorbed unchanged must have been absorbed and excreted as the conjugate. Qualitatively, figure 2 can be interpreted to mean that conjugation occurs in the intestinal lumen, the intestinal wall, and/or the liver during the first pass after an oral dose of methyldopa. Quantitatively, it can be seen in table 3 that on the average approximately two-thirds of that which is recovered in the urine from an oral dose is unchanged drug; the remainder is the conjugate.

Finally, the renal clearance of the conjugate was estimated for each subject after oral administration (table 9). On the average, the conjugate appears to be cleared by the kidney at about the same rate as methyldopa, but the two clearance rates do not necessarily coincide for a given individual. Conjugate concentrations were too low to attempt similar comparisons for the intravenous dose.

Discussion

Metabolism and Excretion

Plasma and urine samples in this study were assayed for methyldopa and its mono-O-sulfate conjugate. It is known from the work of others that these constitute about 90% of the radioactivity in the urine and probably everything in plasma (Sjoerdsma *et al.*, 1963; Buhs *et al.*, 1964; Au *et al.*, 1972). The remaining metabolites in urine are 3-O-methyl-α-methyldopa or its conjugates (4%), α-methyldopamine or its conjugates (2%), 3-O-methyl-α-methyldopamine or its conjugates (0.3%) and 3,4-dihydroxyphenylacetone or its conjugates (2.5%). Of these, only α-methyldopamine and its conjugates would be detected by the analytical method used in the present study. Thus, the urinary methyldopa values reported herein may be slightly biased to the extent of about 2% on the high side. By comparison, it is noted that the same kind of bias is inherent in the values reported by Myhre *et al.* (1972a,b) whose assay method excluded separation by alumina and therefore, presumably would include the 3-O-methyl metabolites of α-methyldopa and of α-methyldopamine and their conjugates as additional potential contaminants, which is minor.

Total (free + conjugate) urinary recoveries of methyldopa were 52 to 82% after intravenous dosage and 9 to 57% after oral administration. These results are consistent with those observed in hypertensive patients (Sjoerdsma *et al.*, 1963; Buhs *et al.*, 1964; Prescott *et al.*, 1966; Au *et al.*, 1972; Myhre *et al.*, 1972a). Relatively low and highly variable urinary recoveries after oral dosage probably reflects mostly manifestations of variable absorption rather than disposition since there is no apparent relationship between the amount excreted in the urine and the patients' responsiveness to the antihypertensive activity of methyldopa (Au *et al.*, 1972) or their blood urea nitrogen levels (Buhs *et al.*, 1964).

Of that which is excreted in the urine, 39% is conjugated after oral dosage whereas only 2% is conjugated after intravenous administration. Thus, it would appear that a significant portion of the oral dose is conjugated as it crosses the gastrointestinal wall or in its first cycle through the hepatic circulation. The possibility of conjugation by lumenal contents (microbial) is not totally excluded but appears unlikely since only free α-methyldopa is found in the feces (Buhs *et al.*, 1964). It has been shown that the proportion of conjugate to free drug increases on repeated dosing and in patients with impaired renal function (Buhs *et al.*, 1964; Myhre *et al.*, 1972b). Subsequent discussion will attempt to show that these observations are consistent with the hypothesis of enterohepatic circulation and of conjugation being predominantly a first-pass effect.

On the average, plasma and renal clearances of methyldopa did not differ for the two treat-

ments in the crossover study; their respective values of approximately 218 and 136 ml/min are consistent with the fact that the renal component represents about two-thirds of the overall elimination. The renal clearance rate for the mono-O-sulfate was 132 ml/min, about the same as that for the free drug. In contrast, Myhre *et al.* (1972b) had reported renal clearance values for the conjugate to be only one-half those for free methyldopa. However, it is evident that renal clearances for the free and the conjugated drug should approach equality since they also reported that the same ratio of conjugate to free drug was found in both plasma and urine (Myhre *et al.*, 1972c).

Absorption

The absorptive behavior of methyldopa was studied by estimating the time course of change in the amount of drug having reached the systemic circulation unchanged. On the average, 25% of a 750 mg oral dose was absorbed in 36 hours; the range among subjects was 7.9 to 61.5%. Although most of the absorption took place during the first 5 hours, there were indications of continued absorption beyond the 9th hour.

The chance agreement between amount absorbed and total urinary recovery after a single oral dose has several important implications. First, the fractional recovery of the mono-O-sulfate conjugate in the urine after single oral dosages may be taken as an estimate of the extrarenal component of methyldopa elimination. Secondly, the results from the present study may be compared with previous studies at comparable dosages based on the urinary excretion of total radioactivity. This is so because free and conjugated methyldopa constitute more than 90% of all metabolites in the urine. Finally, both of the above cited properties can be utilized in the planning of future studies in which the inclusion of an intravenous dose may not be practical or desirable.

The variability in absorption among subjects is consistent with the reported range of urinary recovery of total radioactivity after a single oral dose of methyldopa-^{14}C (Sjoerdsma *et al.*, 1963; Buhs *et al.*, 1964; Prescott *et al.*, 1966; Au *et al.*, 1972). Published data would also suggest that intrasubject variations in absorption may be much less, about a 2-fold range on repeated administration (Sjoerdsma *et al.*, 1963; Prescott *et al.*, 1966; Myhre *et al.*, 1972c).

Enterohepatic Circulation

Conjugation is route-dependent; much greater quantities of the mono-O-sulfate are found in plasma and urine after oral administration, indicating that most of the conjugates are formed before reaching the general circulation. A minor fraction of methyldopa absorption seems to persist for a long time. These observations suggest that the limited extent of conjugation after intravenous administration may be construed as that being derived from the enterohepatic circulation of methyldopa. The temporal relationships of free and conjugated drug after intravenous dosage are at least qualitatively compatible with the sporadic nature of biliary secretion and the variable nature of methyldopa absorption. In terms of material balance, it should be recalled that extrarenal elimination of methyldopa is about one-third of the total and that gastrointestinal absorption is about 25% of the dose on the average. As a first approximation, if 33% of an intravenous dose were excreted by the liver and secreted *via* the bile into the gastrointestinal tract, 25% of the 33%, or about 8.2% of the dose, would be reabsorbed as unchanged drug. Furthermore, because of the first-pass effect, a quantity equivalent to one-third of the 8.2%, or about 2.7% of the dose, would be expected to be absorbed and excreted in the urine as the conjugate. The observed value is 1.6%.

Enterohepatic circulation and first-pass conjugation can be further supported by observations of disproportionately greater conjugate accumulation on repeated dosing and in renal failure. The average fractional recovery of conjugates in the urine increases from 0.34 to 0.69 after single and repeated oral dose in subjects with normal kidney function and further increases to >0.90 in patients whose creatinine clearances are 5 to 15 ml/min (Buhs *et al.*, 1964; Myhre *et al.*, 1972c). In other words, the average ratio of conjugate to free drug in the urine increases on repeated oral administration from 0.5 to 2.2 in normal kidney function and from 0.5 to about 9 in renal insufficiency. Since renal clearances of the free and conjugated drug are equal on the average, the observed ratio in the urine is directly related to the corresponding ratio of their mean plasma concentrations.

In the absence of enterohepatic circulation, the relationship between the mean plasma concentration after the first dose, Cp (1), and that at steady state after chronic administration, Cp (∞), can be approximated[5] by

$$\bar{C}p(\infty) = \frac{\bar{C}p(1)}{(1-e^{-\delta\tau})} = \frac{\int_0^\tau Cp\,(t)dt}{\tau(1-e^{-\delta\tau})} \qquad (7)$$

where τ is the dosing interval; δ is the slowest disappearance rate constant; and the integral is the area under the plasma concentration curve over the dosage interval τ after the first dose. Thus, the largest difference in the degree of accumulation between free and conjugated drug would be obtained when the difference in δ is large and τ is small. Based on the largest observed δ for methyldopa and the smallest observed δ for the conjugate (0.139 hr^{-1}) and a reasonable dosage regimen of four times daily, only a ratio of 0.75 for

$$\frac{[\bar{C}p(\infty)]\ \text{conj}}{[\bar{C}p(\infty)]\ \text{free}}$$

can be anticipated for subjects with normal kidney function. Some other factor(s) must be introduced to account for the observed ratio of 2.2.

Similarly in cases of renal dysfunction, maximal difference in accumulation would result if renal excretion were the only route for conjugate elimination, *i.e.*. renal clearance equals plasma clearance. Taking creatinine clearances of 5 to 15 ml/min to mean 10% of normal, plasma clearance would be 13 ml/min for the conjugate and 102 ml/min (13 for renal and 89 for extrarenal) for methyldopa. This kind of change in plasma clearance attributed to renal dysfunction would represent a ratio of 2.3 for

$$\frac{[\bar{C}p(\infty)]\ \text{conj}}{[\bar{C}p(\infty)]\ \text{free}}$$

due to accumulation on a four times daily dosage regimen. Again, additional factor(s) are needed to account for the observed ratio of about 9.

Thus, a single source of methyldopa, namely that derived from the ingested dose, is insufficient to account for the extent of conjugation after repeated oral administration; the deficit is greater for patients with impaired renal function. By considering enterohepatic circulation, a second source of methyldopa is available for conjugation on reabsorption. Unlike the first which is constant for a given dosage regimen, this second source of methyldopa accumulates on chronic administration and is greater in patients with impaired renal function. Hence, enterohepatic circulation is totally compatible with existing data on methyldopa disposition in man. Whether O-sulfate conjugation is solely a manifestation of the first-pass effect of methyldopa absorption or reabsorption cannot be demonstrated unequivocally. However, since enterohepatic circulation is a likely occurrence, it is evident from the limited conjugate recovery in the urine after intravenous dosage that O-sulfation takes place predominantly, if not exclusively, in the gastrointestinal walls.

[5] Equation 7 is strictly valid only in the case of repeated intravenous injections of a one-compartment open model drug. It is believed adequate as an approximation in view of the relative nature of the present argument.

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