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METABOLISM OF 3-*O*-METHYLDOPA BY THE ISOLATED PERFUSED RAT LIVER

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Abstract—The disposition and metabolism of L-3-methoxy-4-hydroxyphenylalanine (3-*O*-methyldopa) in the isolated perfused rat liver system is described. When [^{14}C]3-*O*-methyldopa (5 μCi, 1.9 mg, uniformly labeled except in the *O*-methyl carbon) was added to the perfusate, in the first 5 min the liver took up 10 per cent of the dose, and erythrocytes took up 30 per cent. After 5 min of perfusion, 3-*O*-methyldopa disappeared from plasma in a diphasic fashion; its half-life was 25 min between 5 min and 2 hr, and 360 min between 2 and 5 hr of perfusion. Cumulative excretion of radioactivity in bile accounted for 20 per cent of the dose. After 5 hr of perfusion, 35 per cent of the dose remained as unmetabolized 3-*O*-methyldopa (22 per cent in plasma, 8.5 per cent in erythrocytes, 3.4 per cent in liver and 1 per cent in bile). Known demethylated metabolites accounted for 6 per cent of the dose. The early appearance of 3,4-dihydroxyphenylalanine in erythrocytes, and its formation when the system lacked a liver, indicated that these cells were the site of demethylation. Free and conjugated 3-methoxy-4-hydroxyphenylacetic acid were major metabolites in the system. These compounds could have been metabolites of dopa. If this were so, demethylation of 3-*O*-methyldopa accounted for an additional 12.3 per cent of the dose. Transaminated metabolites (free and conjugated 3-methoxy-4-hydroxyphenyllactic acid) accounted for 19.0 per cent of the dose.

When patients with Parkinson's disease are treated with L-3,4-dihydroxyphenylalanine (L-dopa), 3-methoxy-4-hydroxyphenylalanine (3-*O*-methyldopa) accumulates in plasma [1] and in cerebrospinal fluid [2]. On abrupt cessation of L-dopa therapy, considerable amounts of 3-*O*-methyldopa are present in plasma for several days [3], whereas dopa concentrations rapidly decrease to barely detectable levels. During this time, the clinical condition of the patient correlates with the 3-*O*-methyldopa concentration in plasma rather than with the dopa concentration. It seemed possible, therefore, that demethylation of 3-*O*-methyldopa might provide an additional source of dopa for patients during dopa treatment. Subsequently, treatment of patients with Parkinson's disease with L-3-*O*-methyldopa was tried but proved ineffective [4, 5]. However, as pointed out by Hornykiewicz [6], this does not preclude the possibility that *in situ* formation of dopa from 3-*O*-methyldopa occurs during L-dopa treatment, and that this may be important in the treatment of the disease.

In rats, demethylation of 3-*O*-methyldopa was demonstrated with the ultimate formation of dopamine in brain [7]. Although it was shown that trace amounts of [^{14}C]dopa contaminating [^{14}C]3-*O*-methyldopa could be a serious source of error in such experiments [8], subsequent work using highly purified [^{14}C]3-*O*-methyldopa has shown clearly that demethylation of 3-*O*-methyldopa does occur in rats [9–11].

The enzyme responsible for demethylation of 3-*O*-methyldopa is not yet known. Relatively nonspecific demethylases in liver microsomes demethylate guaiacols [12], but Chalmers *et al.* [13] were not able to detect any demethylation of 3-*O*-methyldopa by rat liver microsomal enzyme preparations. In fact, these investigators found that no demethylation of 3-*O*-methyldopa occurred in rats with external bile fistulas. They therefore concluded that intestinal flora were mainly responsible for demethylation of 3-*O*-methyldopa and that it was unlikely that this reaction would benefit patients.

In the present experiments, the metabolism of 3-*O*-methyldopa in the isolated perfused rat liver system was studied to define the extent and location of demethylation, to determine the half-life of 3-*O*-methyldopa in plasma of the perfusate, and to separate and identify metabolites in perfusate, bile and liver. Preliminary reports of this work have been made [14, 15].

METHODS

Materials. L-[^{14}C]3-*O*-methyldopa (uniformly labeled except in the *O*-methyl carbon; sp. act. 298 (Ci/mole) was obtained from New England Nuclear. This material was examined critically for the presence of contaminating [^{14}C]dopa. Carrier dopa and 3-*O*-methyldopa (0.1 μmole of each) were added to aliquots of the radioactive 3-*O*-methyldopa and analyses were done on an amino acid analyzer and by paper chromatography in *n*-butanol–acetic acid–water (12:3:5). It was not possible to detect any [^{14}C]dopa by these methods, which would have detected 0.2 per cent contamination. L-[3-^{14}C]dopa (5.2 Ci/mole) and [2-^{14}C]3,4-dihydroxyphenylethylamine ([2-^{14}C]dopamine) (55 Ci/mole) were obtained from Amersham-Searle Corp. Stable L-3-*O*-methyldopa was obtained

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from Regis Chemical Co., and 3-methoxy-4-hydroxyphenyllactic acid (vanillactic acid, VLA) from Sigma Chemical Co. Bacterial β-glucuronidase was purchased from Sigma, and Glusulase (160,000 units of β-glucuronidase and 50,000 units of arylsulfatase/ml) from Endo Laboratories, Inc.

Perfusion of the isolated rat liver. Livers were removed from rats and continuously perfused with warmed oxygenated rat blood by methods previously described in detail [16, 17]. In the present experiments, the substrate [^{14}C]3-*O*-methyldopa (5 μCi, 1.9 mg) was dissolved in 2 ml saline with warming. An equal volume of rat plasma was then added and the mixture was adjusted to pH 7.0 to 7.2. This was injected into the perfusate over a period of 2 min, after the removal of the first liver and before the introduction of the second experimental [16] liver. The initial concentration of 3-*O*-methyldopa in the 120 ml of the perfusate was thus 7×10^{-5} M (16 μg/ml). The mean weight of livers used was 10.99 g (S. D. = 1.29, n = 9).

Sampling and measurements of radioactivity were done as described previously [17, 18] except that, in addition, two samples of perfusate were withdrawn at 5 and 10 min after injection of substrate and before the introduction of the experimental liver. In addition, Insta-Gel (Packard) was used as the scintillation mixture.

Separation and identification of metabolites. Separations of catechol and *O*-methylated amino acids, amines, and their acidic metabolites in protein-free extracts of plasma, whole blood, erythrocytes and liver were done by ion exchange (Dowex-50) and adsorption (alumina) chromatography, and by solvent extraction as previously described [18]. At the end of each experiment, methanol–acetone (1:1) [17] extracts of bile, plasma and liver were prepared, and these were examined by paper chromatographic methods, together with fractions which contained more than one ^{14}C-labeled metabolite (amino acid, acid and conjugate fractions). The paper chromatographic systems used were: A, *n*-butanol–acetic acid–water (12:3:5); B, *n*-butanol–pyridine–water (1:1:1); C, isopropanol–ammonia–water (20:1:2); D, toluene–methanol–ethyl acetate–water (1:1:1:1); E, *n*-butanol saturated with 0.1 M potassium acetate buffer, pH 4.5; and F, ethyl acetate–acetic acid–water (5:1.5:3).

Distribution of radioactivity on paper chromatograms was determined with a 4π strip scanner. Radioactive areas on paper strips were eluted with 0.1 N acetic acid. Aliquots of these eluates were incubated with β-glucuronidase or Glusulase and appropriate buffers [17] and the products of enzyme action were co-chromatographed with authentic marker compounds in several solvent systems.

The mean recoveries of 5 μg of [^{14}C]3-*O*-methyldopa and of [^{14}C]L-dopa through the Dowex-50 and alumina separations were 91.2 per cent (S. D. = 3.0, n = 10) and 70.7 per cent (S. D. = 3.2, n = 10) respectively. The recovery of 5 μg of [^{14}C]dopamine through the Dowex-50 separation was 70.0 to 85.0 per cent (n = 4). Fluorometric methods were used to determine the recoveries of 3-methoxy-4-hydroxyphenylacetic acid (HVA), 3,4-dihydroxyphenylacetic acid (dopac) and VLA [19–21] through the Dowex-50 and subsequent ether and Tris buffer extractions; these ranged from 75 to 85 per cent, 60 to 60.3 per cent and 74.6 to 81.1 per cent, respectively (n = 4). The recovery of dopac through alumina adsorption was 60.0 to 81.7 per cent. No corrections were made for these recoveries.

Calculations. In each experiment, the injected "dose" of [^{14}C]3-*O*-methyldopa was obtained by multiplying the average amount of ^{14}C present in the aliquots of blood removed at 5 and 10 min after injection (but before the introduction of the experimental liver) by the total volume of perfusate. ^{14}C in the total volume of each compartment (bile, plasma and whole blood) was calculated at each time interval and expressed as a percentage of the injected dose. Total ^{14}C in liver at intermediate time intervals was calculated by subtracting from the injected dose the amount of ^{14}C in blood and in bile and the amount removed in sampling. Total ^{14}C in erythrocytes at most intermediate times was obtained by subtracting total ^{14}C in plasma from total ^{14}C in whole blood. However, at 5 min and at 5 hr after injection, blood samples were removed and centrifuged for 30 min, plasma and buffy coat were removed by aspiration, and aliquots of erythrocytes were removed for the direct measurement of total ^{14}C (and for the separation of metabolites). Total ^{14}C in erythrocytes obtained in these direct measurements did not differ by more than ± 10 per cent from the calculated values.

RESULTS

Effects of 3-O-*methyldopa on the isolated perfused rat liver.* The addition of 3-*O*-methyldopa did not cause any changes in the flow of blood through the liver which is normally 1–3 $ml \cdot g^{-1} \cdot min^{-1}$ [16]. However, the mean volume (± S. D.) of bile produced in 5 hr was 3.39 ± 0.45 ml (n = 9). This was significantly greater than that formed when L-dopa or thyroxine were substrates (1.98 ± 0.30 ml, n = 9 [17], and 2.83 ± 0.45 ml, n = 20 [16] respectively).

Disposition of ^{14}*C after addition of* [^{14}*C*]3-O-*methyldopa to liver perfusions.* After injection of [^{14}C]3-*O*-methyldopa to the isolated perfused rat liver system, total ^{14}C in whole blood decreased steadily to 62 per cent of the dose in 5 hr (Fig. 1). After 5 min of perfusion, ^{14}C in plasma accounted for 60 per cent of the dose, decreasing slowly during the subsequent 5 hr to 46 per cent of the dose. Erythrocyte ^{14}C accounted for 30 per cent of the dose after 5 min of perfusion and 16 per cent after 5 hr. Total ^{14}C in liver accounted for 10 per cent of the dose early in the experiment, decreasing to a final 6 per cent. Cumulative excretion of ^{14}C into bile during 5 hr accounted for 20 per cent of the dose.

In four experiments in which no liver was present in the system, ^{14}C in whole blood accounted for 96 per cent (S. D. = 7.1) of the dose after 5 min. After 5 hr this fraction had decreased to 88 per cent (S. D. = 0.8) of the dose. This decrease could be entirely accounted for by the ^{14}C which had been removed in sampling. ^{14}C in red blood cells fluctuated little throughout the experiment (after 5 min it was 27.8 per cent, S. D. = 5.9, and after 5 hr 27.8 per cent, S. D. = 6.4).

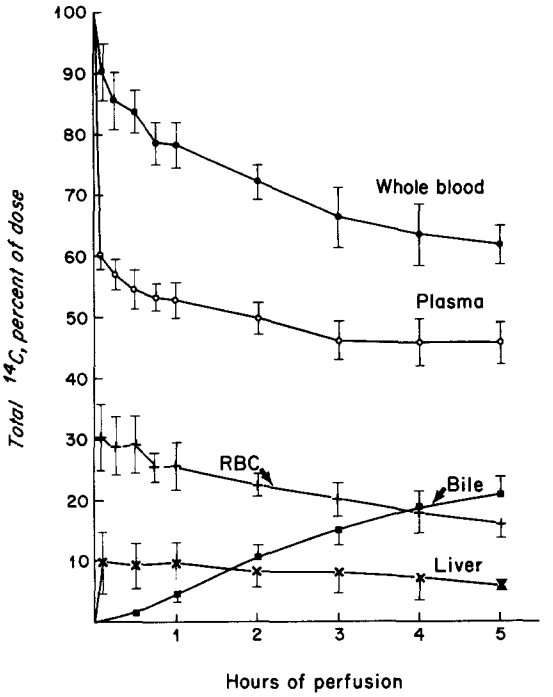

Fig. 1. Disposition of radioactivity in whole blood, plasma, red blood cells (RBC) and liver, and cumulative excretion of ^{14}C in bile, at different times after perfusion of the isolated rat liver was begun with blood containing 5 μCi (1.9 mg) of [^{14}C]3-*O*-methyldopa. In Figs. 1, 2 and 3, data represent means ± S. D. of nine experiments.

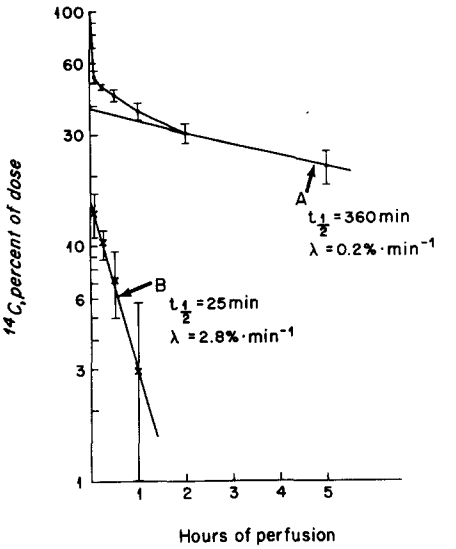

Fig. 2. [^{14}C]3-*O*-methyldopa in plasma at different times during the perfusion of the isolated rat liver. Lines of best fit were drawn by the method of least squares.

Disappearance of [^{14}C]3-O-*methyldopa from plasma in the isolated perfused rat liver system.* 3-*O*-methyldopa disappeared from plasma in a triphasic fashion (Fig. 2). After 5 min of perfusion, only 52.5 per cent of the dose remained as unmetabolized 3-*O*-methyldopa. Between 5 min and 2 hr, 3-*O*-methyldopa disappeared less rapidly (phase B, Fig. 2) and after 2 hr yet more slowly (phase A, Fig. 2). During the slowest phase, the half-life was 360 min and the rate of disappearance was 0.2 per cent·min^{-1}. By subtracting the slowest component A from the observed values, the more rapid phase B was obtained, with a half-life of 25 min and a disappearance rate of 2.8 per cent·min^{-1}.

Metabolites of 3-O-*methyldopa in plasma, erythrocytes, bile and liver.* During the perfusion, the most abundant compound in plasma was unmetabolized [^{14}C]3-*O*-methyldopa (Fig. 3). Traces of dopa were present in plasma, slightly larger amounts being present in the first 30 min of perfusion. Acidic metabolites accounted for 5 per cent of the dose after 2 hr, decreasing to 3 per cent after 5 hr. Conjugated metabolites accumulated during the experiment, accounting finally for 16 per cent of the dose. There was no significant radioactivity in the amine fraction (eluted with strong acid from the Dowex-50) at any time during the perfusion.

At the end of the perfusion, the major metabolites in plasma were VLA and VLA-sulfate, and HVA and HVA-sulfate (Table 1). Smaller amounts of dopa, dopac and dopac-sulfate were found in plasma. Also present was a compound which was hydrolyzed by β-glucuronidase to a product isographic with 3-*O*-methyldopa in solvents A, C and E. This compound was partially retained by Dowex-50 when applied at pH 2, and was eluted with the neutral buffer used to elute amino acids. The glucuronides of dopamine and of 3-*O*-methyldopamine have also been shown to be isolated in this fraction [22].

In erythrocytes, 3-*O*-methyldopa was the most abundant radioactive compound. [^{14}C]dopa accounted for 1.4 per cent of the dose, and small amounts of free VLA were present. The total conjugated compounds in the effluent from the Dowex-50

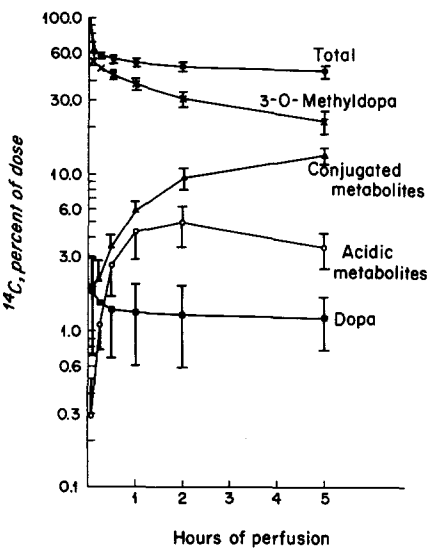

Fig. 3. ^{14}C metabolites of [^{14}C]3-*O*-methyldopa (5 μCi, 1.9 mg) in plasma at different times after perfusion of the isolated rat liver was begun.

Table 1. Metabolites of 3-*O*-methyldopa in the isolated rat liver system after perfusion for 5 hr*

Fraction	^{14}C as per cent of dose			
	Plasma	Erythrocytes	Combined bile	Liver
Total ^{14}C†	45.8 ± 3.32	16.1 ± 2.19	19.7 ± 2.77	5.6 ± 0.40
Perchloric acid extract	41.7 ± 2.94	15.4 ± 2.47	19.1 ± 2.48	5.2 ± 0.22
3-*O*-methyldopa	22.0 ± 3.57	8.5 ± 2.00	1.0 ± 0.60	3.4 ± 0.31
3-*O*-methyldopa-G	2.0 ± 0.67	ND	3.6 ± 0.87	Trace
Dopa	1.2 ± 0.45	1.4 ± 0.39	0	0.1 ± 0.07
Dopac	0.3 ± 0.08	Trace	0.2 ± 0.09	Trace
Dopac-S	0.8 ± 0.40	‡	ND	‡
Dopamine-G	ND	ND	2.0 ± 0.43	ND
Total known demethylated products	2.3	1.4	2.2	0.1
VLA	3.1 ± 0.93	0.8 ± 0.41	2.7 ± 0.46	0.3 ± 0.04
VLA-S	8.0 ± 1.71	‡	4.4 ± 1.36	‡
Total transaminase products	11.1	0.8	7.1	0.3
HVA	0.3 ± 0.11	ND	0.7 ± 0.31	ND
HVA-S	7.5 ± 2.63	‡	3.8 ± 1.00	‡
HVA + HVA-S (metabolic origin in question)	7.8		4.5	
Unidentified-S	ND	ND	1.9 ± 0.55	ND
Total conjugated compounds	18.3 ± 1.60	3.5 ± 1.16‡	15.7 ± 2.33	1.3 ± 0.50‡

* Data are expressed as means ± S. D. Abbreviations used are: S, sulfate; G, glucuronide; ND, not detected.
† No corrections have been made for 10–11 per cent of the dose removed in sampling the perfusate.
‡ The conjugated fraction was not fractionated because it contained low amounts of radioactivity.

accounted for 3.5 per cent of the dose, but further isolation of compounds in this fraction was not attempted because the amounts of radioactivity present were too low to allow for good separation and quantitation.

Only small amounts of free 3-*O*-methyldopa were excreted in bile (Table 1, Fig. 4). Considerable amounts of free and conjugated HVA and VLA were present, and also the glucuronides of dopamine and 3-*O*-methyldopa. Also present, accounting for 1.9 per cent of the dose, was a compound hydrolyzed by Glusulase but not by β-glucuronidase to a product which was not identified. Paper chromatography in systems A, B, C, D, E and F with authentic standards indicated that this product was not 3-methoxy-4-hydroxymandelic acid (VMA), 3,4-dihydroxymandelic acid, 3-methoxy-4-hydroxyphenylglycol, 3-methoxy-4-hydroxyphenylethanol, 3,4-dihydroxyphenylethanol, dopamine, 3-*O*-methyldopamine or 3-*O*-methyldopa. Boiling *in vacuo* for 6 hr at pH 0.5 did not alter the chromatographic properties of the product, and this suggested that it was not an *N*-acetyl compound.

In liver, at the end of 5 hr of perfusion the major radioactive compound present was unmetabolized 3-*O*-methyldopa. Small amounts of dopa, VLA and conjugated metabolites were also present.

Analyses were done of plasma and erythrocytes in four experiments in which no liver was attached in the system (Table 2). The major ^{14}C compound present in both compartments was unmetabolized 3-*O*-methyldopa, but significant amounts of dopa were also present. Only small amounts of radioactivity were present in the conjugated and acid fractions, and so further analyses of these were not attempted. When no liver was attached to the perfusate, 27 per cent of the radioactivity in the erythrocytes was associated with the precipitated protein. This did not occur when a liver was present in the system (Table 1).

Demethylation of 3-O-*methyldopamine: fact or artifact* [8]. Although the [^{14}C]3-*O*-methyldopa used in these experiments was shown by analysis on the amino acid analyzer to contain no dopa, 2 per cent of the total radioactivity emerged as an artifact in the catechol fraction during the separation from guaiacols by adsorption on alumina (Table 3). A significantly larger proportion of the radioactivity in the amino acid fraction was present in the catechol fraction in plasma extracts after 300 min and in erythrocytes after both 5 and 300 min, both in the presence and absence of a liver in the system. At both time intervals, a greater percentage of ^{14}C was present in the catechol fraction in erythrocyte extracts than in plasma extracts.

DISCUSSION

Approximately 6 per cent of a dose of 1.9 mg of 3-*O*-methyldopa was demethylated in the isolated perfused rat liver system. The early appearance of dopa in the erythrocytes suggested that these cells were the site of demethylation. Also, when no liver was in the system, at least 3.9 per cent of the dose of 3-*O*-methyldopa was demethylated in the circulating perfusate. It is probable that larger amounts of dopa were, in fact, formed in the system lacking a liver, because 7.5 per cent of the dose was precipitated with the proteins in erythrocytes. This fraction probably represents [^{14}C]dopa because it has been shown previously that dopa quickly binds to proteins in erythrocytes [17, 18]. Demethylation of normetanephrine by erythrocytes has previously been noted [23].

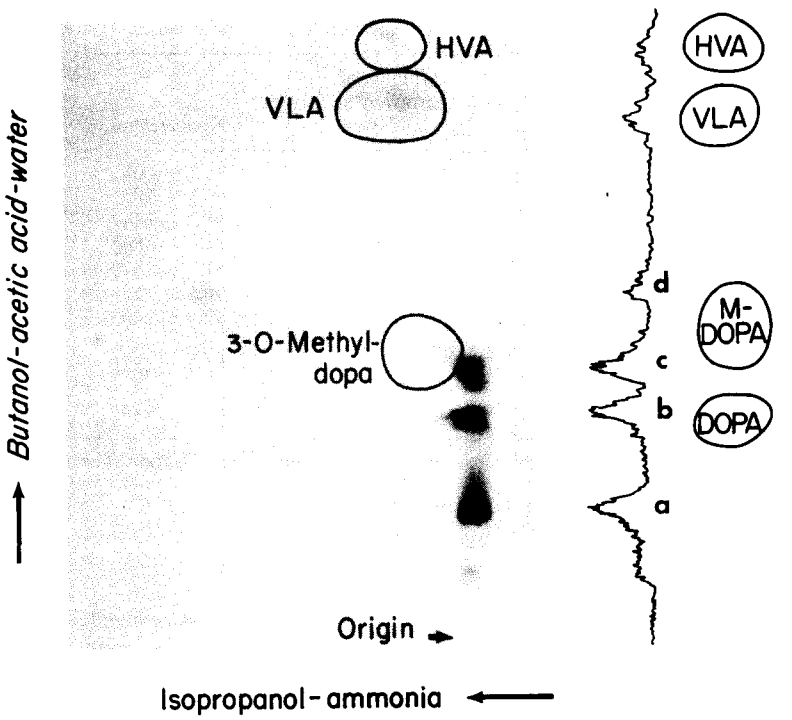

Fig. 4. Metabolites of [^{14}C]3-*O*-methyldopa in bile. Autoradiograph (left) of a two-dimensional paper chromatogram of a methanol–acetone extract of bile run in *n*-butanol-acetic acid–water (12:3:5) and in isopropanol–ammonia–water (20:1:2), and a scan of radioactivity on a one-dimensional chromatogram run in *n*-butanol–acetic acid–water (right). Standards were: HVA, homovanillic acid; VLA, vanillactic acid; M-DOPA, 3-methoxy-4-hydroxyphenylalanine; DOPA, 3,4-dihydroxyphenylalanine. The radioactive compounds were identified after elution, incubation with enzyme preparations, and co-chromatography with authentic standards in several solvent systems; thus: Band a, a mixture of the glucuronides of 3-*O*-methyldopa and of dopamine; Band b, the sulfate of VLA; Band c, the sulfate of HVA; and Band d, the sulfate of an unidentified metabolite.

Dopa metabolites were not detected in the system unless a liver was present, and it is suggested that, in the complete system, dopa was formed in the erythrocytes, it diffused into the plasma, and it was rapidly metabolized by liver [17]. However, the present experiments in no way exclude the possibility that some 3-*O*-methyldopa was demethylated in liver.

Chalmers *et al.* [13] have suggested that, in the intact rat, bacteria in the gastrointestinal tract were mainly responsible for the demethylation of 3-*O*-methyldopa. In the present experiments demethylation by these bacteria was excluded, but the perfusions were not carried out under sterile conditions so that some metabolism by other invading bacteria might have occurred. However, bacteria growth would be most likely to occur during the later hours of the perfusion. The early formation of dopa in red blood cells, 5 min after the injection of 3-*O*-methyl-

Table 2. Metabolites of 3-*O*-methyldopa in the perfusate which had circulated for 5 hr without a liver in the system*

Fraction	^{14}C as per cent of dose	
	Plasma	Erythrocytes
Total ^{14}C†	60.5 ± 5.60	27.8 ± 6.36
Perchloric acid extract	56.1 ± 2.11	20.3 ± 3.18
3-*O*-methyldopa	51.4 ± 2.93	15.3 ± 2.95
Dopa	2.0 ± 0.42	1.9 ± 0.22
Conjugated fraction	1.9 ± 0.88	0.7 ± 0.28
Acid fraction	0.4 ± 0.26	0.1 ± 0.1

* Data are shown as means ± S. D. of four experiments.
† The unaccounted for 11.7 per cent was removed by sampling.

Table 3. Is dopa formation from [^{14}C]3-*O*-methyldopa in the isolated rat liver system an artifact? Distribution of radioactivity from [^{14}C]3-*O*-methyldopa before and after addition to the perfusate of the isolated rat liver*

Compartment	Minutes of perfusion or circulation	Noncatechol	Catechol
Injected [^{14}C]3-*O*-methyldopa (10)	0	96.3 ± 4.04	2.0 ± 1.08
Plasma (9)	5	96.3 ± 3.85	3.2 ± 1.80
Plasma (9)	300	91.8 ± 4.44†	5.1 ± 1.74‡
Erythrocytes (9)	5	84.6 ± 2.84‡	11.2 ± 1.94‡
Erythrocytes (9)	300	81.2 ± 5.87‡	13.8 ± 2.69‡
Plasma (no liver in system) (4)	5	96.6 ± 1.52	3.4 ± 1.51
Plasma (no liver in system) (4)	300	99.1 ± 4.40	3.7 ± 0.70†
Erythrocytes (no liver in system) (4)	5	85.2 ± 1.51‡	13.5 ± 1.83‡
Erythrocytes (no liver in system) (4)	300	89.2 ± 5.88‡	10.9 ± 1.58‡

* Data are shown as mean (± S. D.) per cent of the total ^{14}C in the amino acid fraction. The number of determinations is given in parentheses.

† $P < 0.05$ for difference from injected [^{14}C]3-*O*-methyldopa.

‡ $P < 0.001$ for difference from injected [^{14}C]3-*O*-methyldopa.

dopa, strongly suggested that erythrocyte enzymes were responsible for the demethylation rather than contaminating bacteria.

The origin of the HVA and HVA-sulfate in the system is in question. These compounds are major metabolites of dopa, but it has also been thought that HVA could be formed by transamination of 3-*O*-methyldopa to vanilpyruvic acid, followed by the decarboxylation of this compound [24]. However, Fellman *et al.* [25] administered [carboxyl-^{14}C]vanilpyruvic acid to mice and found that no [^{14}C]CO_2 was produced during the following 2 hr. If this finding applies also to rats, it can be concluded that HVA is a metabolite of dopa in our system, and demethylation would account, therefore, for a further 12.3 per cent of the dose.

Transamination was the major route of metabolism for 3-*O*-methyldopa in our system, with the ultimate production of VLA. It accounted for 19.3 per cent of the dose and undoubtedly occurred in liver.

3-*O*-methyldopa is a poor substrate for the enzyme L-aromatic amino acid decarboxylase [26], and there was no evidence that it was decarboxylated in the isolated perfused rat liver system. However, several of the ^{14}C metabolites (HVA, dopac, dopamine and their conjugates) were ultimate products of decarboxylations. These compounds together accounted for a total of 15.6 per cent of the dose. Presumably the 1 C fragment was lost as [^{14}C]CO_2, which was not measured because our perfused liver is an open system. However, we did not find any difference between initial total radioactivity present in the perfusate before introduction of the liver, and the sum, after 5 hr, of total ^{14}C in perfusate, liver, bile and in the aliquots removed for sampling. However, if 15.6 per cent of the injected [^{14}C]3-*O*-methyldopa, labeled with nine ^{14}C atoms, had lost one ^{14}C atom during metabolism, then only 1.7 per cent of the dose would have been lost as [^{14}C]CO_2. It is not surprising that we did not detect such a small loss.

When 3-*O*-methyldopa was administered to man, significant increases were observed in the amounts of VMA excreted in urine [21]. It was not known whether VMA was a metabolite of 3-*O*-methyldopa or was formed from norepinephrine released by 3-*O*-methyldopa or by one of its metabolites. No [^{14}C]VMA was detected as a metabolite of [^{14}C]3-*O*-methyldopa in the present experiments.

The amount of 3-*O*-methyldopa in liver was not more than 10 per cent of the dose at any time. More 3-*O*-methyldopa than this was taken up by liver, however, because 20 per cent of the dose was excreted into bile. The hepatic uptake of L-dopa has been shown to be much greater than the uptake of 3-*O*-methyldopa in the present experiments [17]. This could merely be because lower concentrations of substrate were used in the dopa experiments (190 μg of dopa and 1900 μg of 3-*O*-methyldopa), and it has been shown by Pardridge and Jefferson [27] that uptake by liver of many amino acids is by a saturable transport system. However, these workers also showed that there was great differences in the uptakes of amino acids, and it is possible that 3-*O*-methyldopa is poorly taken up by liver.

The half-life of 3-*O*-methyldopa in the isolated perfused rat liver system was considerably shorter than that reported in the intact rat (12–13 hr) [28]. It is possible that in the intact animal hepatic uptake of 3-*O*-methyldopa is not great, and that the amino acid is taken up and held in extrahepatic tissues and protected from hepatic metabolism. Previous work [28] has shown that metabolism of 3-*O*-methyldopa in heart is much less rapid than the hepatic metabolism in the present experiments.

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