Biochemical Pharmacology, Vol. 28, pp. 3279–3284.

0006-2952/79/1115-3279 $02.00/0

ADMINISTRATION OF L-3,4-DIHYDROXYPHENYLALANINE TO RATS AFTER COMPLETE HEPATECTOMY—II

EXCRETION OF METABOLITES

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(*Received* 27 *November* 1978; *accepted* 11 *May* 1979)

Abstract—The excretion of metabolites of L-[3-^{14}C]dihydroxyphenylalanine (L-[3-^{14}C]DOPA) was studied after its injection into rats with complete hepatectomies and into control rats. Approximately 60 per cent of the dose (11 mg/kg; 20 μCi) of injected [^{14}C]DOPA was excreted in urine in 24 hr by the control rats, and 11 per cent in bile. Although a similar percentage of the dose (69.4 per cent) was excreted by the hepatectomized rats into urine, excretion was at a slower rate. Decarboxylation of injected [^{14}C]DOPA within 24 hr appeared to be as great in the hepatectomized rats as in the controls, but metabolism of 3,4-dihydroxyphenylethylamine (dopamine, DA) to norepinephrine was less. In the operated animals most of the DA was metabolized to 3,4-dihydroxyphenylacetic acid or to homovanillic acid. Little radioactivity was present in tissues at 24 hr after injection of L-DOPA into control rats or into hepatectomized rats; however, some radioactivity appeared to be bound to protein in some tissues in both groups of rats.

In the present experiments, a pharmacologic dose of radiolabeled 3,4-dihydroxyphenylalanine (DOPA) was given to control rats and to rats after complete hepatectomy, and a study was made of the excretion of metabolites during the following 24 hr. Because the liver has been shown to be very active in the metabolism of DOPA in *in vitro* experiments [1], it was expected that metabolism would be much slower in the hepatectomized animals. However, the enzymes metabolizing DOPA are present in most tissues [2], and it was found that the hepatectomized rat metabolized DOPA with surprising efficiency. These studies have clinical relevance because L-DOPA has been given to patients with hepatic insufficiency[3], and the doses that have been used are quite similar to those given to patients without liver impairment.

METHODS

Materials. The sources of materials and radiolabeled compounds have been described [4]. Glusulase [100,000 units of β-glucuronidase (EC 3.2.1.31) and 50,000 units of arylsulfatase (EC 3.1.6.1) per ml] was obtained from Endo Laboratories, Inc., Garden City, NY.

Animals. Rats (average wt 300 g) were hepatectomized and control rats underwent laparotomy as described previously [4]. At the time of surgery the bile ducts of control rats were cannulated, and cystostomy was done on all rats for collection of urine and bile.

Three hours after surgery, rats were injected via the tail vein with L-[3-^{14}C]DOPA (20 μCi, 3.3 mg), and bile and urine were collected continuously. Rats which survived for 24 hr were killed by aortic exsanguination under ether anesthesia, and the following tissues were removed and frozen on pulverized dry ice: brain, heart, kidney, adrenal, pancreas, gastrointestinal tract (with contents), liver, muscle (from hind limbs), lung, spleen and testes.

Chemical separations. Extracts of bile and of urine were prepared by the addition of 5 vol. of 0.4 M perchloric acid and centrifugation. The perchlorates were removed from aliquots of these extracts by adjustment to pH 5 on ice with KOH and further centrifugation. Metabolites were then separated by alumina and cation exchange chromatography using Dowex 50 as described [4].

It has been shown previously [5, 6] that amine conjugates are adsorbed on Dowex 50 at pH 2 and subsequently are eluted in a fraction which had previously been considered to contain only 3-methoxy, 4-hydroxyphenylalanine (3-*O*-methyldopa). Therefore, separation procedures were done on extracts of urine and bile with and without preincubation with Glusulase (0.1 ml in 0.1 M citrate buffer, pH 5.4). The method used and the compounds found in each fraction are shown in Table 1.

Further separations of acid and alcohol metabolites in the effluents from the Dowex columns were accomplished by paper chromatographic methods. Acids and alcohols were extracted from portions of the effluents, after acidification to pH 1, by shaking three times with double volumes of ethyl acetate. The ethyl acetate extracts were combined and reduced to a small volume. Subsequent chromatography was done in a nitrogen atmosphere. Separation of the catechol acids and alcohols was done by cochromatography with reference compounds using Whatman 81 paper and the solvent system *n*-butanol–pyridine–water (14:3:3) [7]. Marker compounds were located by spraying with diazotized *p*-

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Table 1. Separation of L-DOPA and its metabolites in urine and in bile*

Fraction	Metabolites present	
	Without Glusulase preincubation	With Glusulase preincubation
Alumina effluent	Noncatechols (*O*-methylated compounds and catechols which have been conjugated at a phenolic group)	Noncatechols (*O*-methylated compounds)
Alumina eluate	Free catechols	Total catechols
Alumina effluent fractionation		
Dowex 50 effluent	Free *O*-methylated acids and alcohols (HVA, VLA, MHPE, MHPG and VMA)†; conjugates of acidic and neutral catechols and guaiacols	Total *O*-methylated acids and alcohols
Dowex 50, eluate 1 (20 ml 0.1 M K^+ acetate, pH 6.5)	Free 3-*O*-methyldopa; conjugated amines	Total 3-*O*-methyldopa
Dowex 50, eluate 2 (10 ml 2 N HCl)	Free NMN	Total NMN
Dowex 50, eluate 3 (25 ml 2 N HCl)	Free 3-MT	Total 3-MT
Alumina eluate fractionation		
Dowex 50 effluent	Free catechol acids and alcohols (DOPAC, DHPE, DOMA, DHPG))	Total catechol acids and alcohols
Dowex 50, eluate 1 (20 ml 0.1 M K^+ acetate, pH 6.5)	Free DOPA	Total DOPA
Dowex 50, eluate 2 (15 ml 1 N HCl)	Free NE	Total NE
Dowex 50, eluate 3 (25 ml 2 N HCl)	Free DA	Total DA

*Perchloric acid extracts of urine and of bile were incubated in the presence or absence of Glusulase. Metabolites in the extracts were then separated by adsorption of catechols on alumina at pH 8.4, followed by elution with acid. Compounds in the alumina effluents and eluates were further fractionated by adsorption at pH 2 of amino acids and amines on the cation-exchange resin Dowex 50, followed by subsequent differential elution.

† Abbreviations used in Tables 1, 3 and 4: HVA, homovanillic acid; VLA, vanillactic acid; MHPE, 3-methoxy,4-hydroxyphenylethanol; MHPG, 3-methoxy,4-hydroxyphenylglycol; VMA, vanilmandelic acid; 3-*O*-methyldopa, 3-methoxy,4-hydroxyphenylalanine; NMN, normetanephrine; 3-MT, 3-methoxytyramine or 3-methoxy, 4-hydroxyphenylethylamine; DOPAC, 3, 4-dihydroxyphenylacetic acid; DHPE, 3, 4-dihydroxyphenylethanol; DOMA, 3,4-dihydroxymandelic acid; DHPG, 3,4-dihydroxyphenylglycol; DOPA, 3,4-dihydroxyphenylalanine; NE, norepinephrine; and DA, dopamine.

nitroaniline. In butanol–pyridine–water, the following R_f values were obtained: 3,4-dihydroxymandelic acid (DOMA), 0.29; 3, 4-dihydroxyphenylglycol (DHPG), 0.67; 3, 4-dihydroxyphenylacetic acid (DOPAC), 0.77; and 3, 4-dihydroxyphenylethanol (DHPE), 0.88. For separation of the *O*-methylated acids and alcohols it was necessary to do separations in *n*-butanol–acetic acid–water (BAA) (12:3:5) and also in isopropanol–ammonia–water (IPA) (20:1:2). In BAA, the following R_f values were obtained: 3-methoxy,4-hydroxyphenylacetic acid (HVA) and 3-methoxy, 4-hydroxyphenylethanol (MHPE), 0.81; vanillactic acid (VLA), 0.75; 3-methoxy, 4-hydroxyphenylglycol (MHPG) and vanilmandelic acid (VMA), 0.69. In IPA, the R_f values were: VMA, 0.9; HVA and VLA, 0.16; MHPG, 0.57; and MHPE, 0.77. Thus, the amount of [^{14}C]VLA was calculated from the BAA separation, the amounts of [^{14}C]MHPG, [^{14}C]VMA and [^{14}C]MHPE from the IPA separation, and the amount of [^{14}C]HVA from the difference between the radioactivity in the [HVA + VLA] peak obtained in the IPA separation and the [^{14}C]VLA obtained from the BAA chromatogram.

Radiolabeled metabolites in bile were separated most efficiently by paper chromatographic separations of methanol–acetone extracts of bile (prepared by the addition of 4 vol. of a mixture (1:1) of methanol and acetone) as described previously [1]. Aliquots of the extracts were chromatographed by descent in *n*-butanol–acetic acid–water or in *n*-butanol–pyridine–water in a nitrogen atmosphere. Radioactive areas were located by scanning strips with a 4Π gas-flow strip scanner, and these were then cut out and eluted with 0.1 N acetic acid. These eluates were dried *in vacuo*. The residues were dissolved in small volumes of appropriate buffers and incubated at 37° for 18 hr with bacterial glucuronidase at pH 6.5 (0.07 M phosphate buffer) or with Glusulase at pH 5.4 (0.1 M citrate buffer) at a final concentration of 1000 units β-glucuronadase/ml. After incubation, protein was precipitated by addition of methanol–acetone and centrifugation. The supernatant fraction was concentrated and the products of enzyme reaction were identified by cochromatography with authentic standards in the above solvents or in toluene–methanol–ethyl acetate–water (1:1:1:1).

Tissues were homogenized in 5 vol. of 0.4 N perchloric acid and proteins were precipitated by centrifugation. In three experiments, tissues were homogenized in 4 vol. of ice-cold 6% trichloroacetic acid. The precipitated protein was washed successively by resuspension and recentrifugation four times in 5% trichloroacetic acid, four times in 80% methanol and four times in ethanol–ether (1:1, by vol.) [8]. The resulting washed protein precipitate was air dried, and then dried in a desiccator to constant weight.

Portions (30–50 mg) of the protein precipitate were hydrolyzed by heating at 110° in 7 ml of 6 N HCl *in vacuo* for 22 hr. After boiling, the extract was lyophilized and the residue was dissolved in pH 2.2 buffer and applied to an amino acid analyzer.

Measurements of radioactivity. Radioactivity was measured in aliquots of urine, bile, homogenates of tissues, protein-free extracts of tissues and in fractions isolated by column chromatography, after their addition to 10 ml Instagel (Packard Instrument Co., Downers Grove, IL). Radioactivity in washed protein precipitates (100 mg) was measured after addition of 0.4 ml water, 2.0 ml Soluene 350 and 10 ml Dimilume (Packard Instrument Co.). Corrections for quench were made by using external standards.

The radioactivity in the effluent from the amino acid analyzer was measured in a flow cell, packed with anthracene, in a liquid scintillation counter.

The distribution of radioactivity on paper chromatograms was determined using a 4 Π strip scanner, or by cutting up the chromatograms into 1 cm pieces, adding 2 ml of 0.02 N acetic acid and 10 ml of Instagel, and measuring radioactivity in the liquid scintillation counter.

Recoveries. The recoveries of standard compounds through the column extractions have been reported [4]. The recoveries of most standard compounds were the same whether or not the extracts had been preincubated with Glusulase. However, the catechol acids and alcohols were poorly and variably recovered through Glusulase incubations: these recoveries varied between 20 and 50 per cent through Glusulase and the extraction procedures. In addition, the paper chromatograms of the catechol acid and alcohols after incubation of tissue extracts with Glusulase frequently showed radioactivity at the solvent front or at the origin, and this did not correspond to any known authentic marker.

RESULTS

Excretion of total radioactivity in urine and in bile. Cumulative excretion of radioactivity in urine of control rats accounted for 59.15 per cent of the dose in 24 hr following L-[^{14}C]DOPA injection. Most of this was excreted in the first 3 hr (Table 2). Radioactivity excreted in bile in 24 hr accounted for 11.38 per cent of the dose. The amount of radioactivity excreted in urine in the first 3 hr by the hepatectomized rats was not significantly different from that by control rats, but more ^{14}C was excreted by the hepatectomized rats in the later urine specimens. The total amount of radioactivity excreted in urine in 24 hr was not different in the two groups of rats.

Metabolites of L-DOPA in urine. The major metabolites of L-[^{14}C]DOPA in urine of control rats were [^{14}C]HVA and [^{14}C]dopamine ([^{14}C]DA); smaller amounts of [^{14}C]DOPAC, 3-methoxy[^{14}C]tyramine ([^{14}C]3-MT), [^{14}C]VLA and [^{14}C]3-*O*-methyldopa were present (Table 3). Unmetabolized [^{14}C]DOPA in urine accounted for only 1.23 per cent of the dose. Known metabolites of norepinephrine (NE) accounted for 2.54 per cent of the dose. Approximately 5.35 per cent of the dose was present as known conjugated compounds (DOPA, DA, 3-MT, DOPAC and NMN).

More [^{14}C]DOPAC and [^{14}C]HVA, and less [^{14}C]NMN, were excreted in urine of hepatectomized rats. Known metabolites of NE accounted for 1.78 per cent of the dose. Conjugated compounds in urine of hepatectomized rats accounted for 7.28 per cent of the dose.

Complete analyses with Glusulase preincubation were done only on a "combined" urine (consisting of a mixture of 1/10 volume of each urine specimen) for each animal. However, analyses done on individual urines without Glusulase preincubation revealed some differences between control and hepatectomized rats in the excretion of certain metabolites with time. More free DOPA, DA and DOPAC were excreted in urine of the hepatectomized rats at the later time intervals (Table 4). The fraction containing *O*-methylated acids and alcohols together with conjugates (Table 1), which was mainly HVA both in control rats and in hepatectomized rats (78–84 per cent of the fraction in the combined urines), was less in the 0–3 hr urine and greater in later urines in the hepatectomized rats than in

Table 2. Excretion of radioactivity in urine and in bile of control and hepatectomized rats after injection of 20 μCi (3 mg) of L-[^{14}C]dopa

Hours after injection	Radioactivity in urine*		Radioactivity in bile*
	Control rats (N = 6)	Hepatectomized rats (N = 6)	Control rats (N = 6)
0–3	47.55 ± 4.37†	36.76 ± 7.56	8.96 ± 0.70
3–6	7.06 ± 1.00	18.26 ± 2.06‡	1.17 ± 0.07
6–24	4.54 ± 0.14	14.39 ± 1.76‡§	1.26 ± 0.08
0–24	59.15 ± 4.73	69.40 ± 6.49	11.38 ± 0.80

* Data as per cent of injected dose.
† Values are expressed as means ± S.E.M.
‡ $P < 0.001$ for differences from control rats.
§ Four of the six rats lived for only 20–22 hr after injection of L-DOPA.

Table 3. Metabolites in urine of rats excreted during 24 hr after injection of L-[^{14}C]DOPA*

Metabolite	^{14}C (per cent of dose)	
	Control rats	Hepatectomized rats
DOPA, free	1.23 ± 0.09	1.65 ± 0.25
DOPA, total	1.35 ± 0.17	1.83 ± 0.21
3-*O*-Methyldopa, total	2.68 ± 0.31	1.96 ± 0.19
DA, free	16.13 ± 1.92	19.30 ± 2.65
DA, total	18.35 ± 2.03	23.76 ± 2.65
3-MT, free	1.09 ± 0.14	1.21 ± 0.23
3-MT, total	2.81 ± 0.37	2.54 ± 0.26
DOPAC, free	4.97 ± 0.38	9.06 ± 0.78†
DOPAC, total	5.06 ± 0.39	9.35 ± 0.75†
HVA, total	19.74 ± 1.92	27.65 ± 2.45‡
MHPE, total	1.75 ± 0.35	1.90 ± 0.22
VLA, total	3.12 ± 0.34	3.02 ± 0.24
NE, total	Trace	Trace
NMN, free	0.26 ± 0.03	0.12 ± 0.007†
NMN, total	1.64 ± 0.19	1.14 ± 0.07†
MHPG, total	0.64 ± 0.09	0.52 ± 0.09

* Values are expressed as means ± S.E.M. of six determinations in each group.
† $P < 0.001$, differences are statistically significant.
‡ $P < 0.05$, differences are statistically significant.

the controls. Less NMN was excreted in urine of the hepatectomized rats during the early time intervals.

Metabolites in bile. Four metabolites of [^{14}C]DOPA were present in bile. These were identified as the glucuronides of DA (0.88 per cent of the dose, S.E.M. = 0.16), of 3-MT (2.25 per cent of the dose, S.E.M. = 0.34), of *N*-acetyldopamine (1.73 per cent of the dose (S.E.M. = 0.13) and of *N*-acetyl-3-methoxytyramine (5.12 per cent of the dose, S.E.M. = 0.41).

Radioactivity in tissues at 24 hr after ^{14}[C]DOPA injection. At 24 hr after injection of L-[^{14}C]DOPA, only small amounts of radioactivity were detected in most tissues except the adrenals and the kidneys (Table 5). Total radioactivity in all the tissues that were mea-

Table 4. Urinary excretion of key metabolites of L-DOPA at different times after injection of L-DOPA to control and hepatectomized rats*

Hours after injection	^{14}C (per cent of dose)	
	Control rats	Hepatectomized rats
DOPA (free) in urine		
0–3	1.03 ± 0.08	1.08 ± 0.26
3–6	0.12 ± 0.02	0.32 ± 0.04†
6–24	0.08 ± 0.006	0.25 ± 0.02†
DA (free) in urine		
0–3	15.29 ± 1.92	16.61 ± 3.19
3–6	0.71 ± 0.17	1.52 ± 0.45
6–24	0.13 ± 0.02	0.28 ± 0.05‡
DOPAC (free) in urine		
0–3	4.31 ± 0.35	6.05 ± 1.01
3–6	0.38 ± 0.06	2.30 ± 0.49‡
6–24	0.28 ± 0.02	0.71 ± 0.14‡
O-methylated acids and alcohols + conjugates		
0–3	18.90 ± 1.98	8.83 ± 2.33§
3–6	3.99 ± 0.46	10.55 ± 1.60§
6–24	2.88 ± 0.21	10.38 ± 1.56†
NMN (free) in urine		
0–3	0.19 ± 0.03	0.08 ± 0.009§
3–6	0.04 ± 0.007	0.02 ± 0.002§
6–24	0.02 ± 0.005	0.01 ± 0.002

* Values are expressed as means ± S.E.M. for six determinations in each group.
† $P < 0.001$, differences are statistically significant.
‡ $P < 0.05$, differences are statistically significant.
§ $P < 0.01$, differences are statistically significant.

Table 5. Total ^{14}C in tissues at 24 hr after injection of L-[^{14}C]DOPA to control and hepatectomized rats*

Tissue	Total ^{14}C (10^3 dis./min/g)	
	Control (6)	Hepatectomized (2)
Kidney	68.52 ± 7.12	172.29†, 269.70†
Adrenals	33.69 ± 5.15 (5)	22.34, 30.03
Spleen	8.80 ± 5.62	20.95‡, 26.21‡
Pancreas	8.71 ± 1.10	34.01†, 35.13†
Liver	6.98 ± 1.41	
Lung	5.15 ± 0.84	20.29†, 19.55†
GI tract	3.96 ± 0.73	10.75†, 11.97†
Heart	3.32 ± 0.29	8.35†, 10.37†
Muscle	3.01 ± 0.67	7.68†, 7.82†
Plasma	2.58 ± 0.50	11.52†, 14.68†
Testes	2.09 ± 0.61	7.08†, 9.12†
Brain	1.73 ± 0.23	6.17†, 6.58†
Erythrocytes	1.45 ± 0.84	7.36†, 9.25†

* Values are expressed as means ± S.D.; numbers in parentheses indicate the number of rats.
† Data outside mean ± 3 S.D. for control rats.
‡ Data outside mean ± 2 S.D. for control rats.

sured accounted for an average of 2.35 per cent of the injected dose (S.E.M. = 0.14). The main depots of radioactivity were muscle, which did not have a high concentration of radioactivity, but because of its large bulk (45 per cent of the body wt) contained a total of 1.37 per cent of the dose (S.E.M. = 0.11). Radioactivity in kidney accounted for 0.46 per cent of the dose (S.E.M. = 0.02), in liver for 0.21 per cent (S.E.M. = 0.010) and in the gastrointestinal tract for 0.10 per cent (S.E.M. = 0.01).

All of the tissues of two hepatectomized rats except the adrenals contained two to three times as much radioactivity as did those of the controls. Total radioactivity in the tissues that were analyzed accounted for 6.17 and 6.93 per cent of the dose. Of this total, 3.67 and 3.78 per cent was in muscle, 1.52 and 1.98 per cent was in kidney and 0.26 and 0.28 per cent was in the gastrointestinal tract.

Further separations were not done of metabolites in extracts of tissues because it was found that in many of the tissues much of the radioactivity precipitated with the proteins in perchloric acid extracts. In kidney, spleen, lung and liver of control animals only 36.7 per cent (S.E.M. = 3.2), 44.7 per cent (S.E.M. = 6.6), 41.2 per cent (S.E.M. = 3.0) and 40.0 per cent (S.E.M. = 3.9), respectively, of the ^{14}C in the homogenate was recovered in the perchloric acid extracts of the tissues. In the two hepatectomized rats, the corresponding amounts were: kidney 41.5 per cent, spleen 38.6 per cent and lung 38.0 per cent. No differences were apparent in the fraction of total radioactivity precipitating with the proteins in tissues of hepatectomized and control rats.

The poor recovery of radioactivity into protein-free extracts was investigated further. Trichloroacetic acid extracts of kidneys were prepared, and the precipitates were extensively washed. Radioactivity in the isolated protein was 16,800 and 19,400 dis./min/100 mg in the kidneys of two control rats and 36,000 dis./min/100 mg in the kidneys of a hepatectomized rat. It was calculated that radioactivity incorporated into protein in kidney accounted for 0.2 and 0.3 per cent of the dose in the control rats and 0.7 per cent of the dose in the hepatectomized rat.

After acid hydrolysis of the protein, a single peak of radioactivity was found in the effluent from the amino acid analyzer. This emerged after 154 min, well separated from DOPA and 3-*O*-methyldopa, which elute after 240 and 260 min, respectively. When L-[^{14}C]DOPA (280,000 dis./min, 2μmoles) was added to the acid extract of the protein before boiling, 96 per cent of the radioactivity was recovered in the DOPA peak in the subsequent amino acid analysis, indicating that the radioactivity incorporated into protein was not an artifact due to breakdown of DOPA during the hydrolysis procedure.

DISCUSSION

In our recent experiments [4, 9], we could not much decarboxylation of injected L-[^{14}C]DOPA within the 24 hr of the experiments by the hepatectomized rats as by the controls. However, metabolism of DA to NE by β-hydroxylation was less in the operated animals, and more DA was metabolized by monoamine oxidase [MAO, amine oxidase (flavin containing) EC 1.4.3.4] and catechol methyltransferase (EC 2.1.1.6) to form DOPAC and HVA.

In our recent experiments (4, 9], we could not demonstrate changes in the concentrations of DOPA or DA in tissues after hepatectomy. Thus, differences we observe in the present study in the metabolism of DA by control and by hepatectomized rats are not likely to be due to differing specific activities of the amine in the two groups of animals.

We have interpreted the increased formation of DOPAC and decreased formation of NE after hepatectomy as indicating that uptake or retention of DA by vesicles was impaired [4]. Then DA in the cytosol could be metabolized by mitochondrial MAO to form DOPAC. However, DOPAC can also be formed from DOPA by transamination [10]. There is no indication that this pathway is increased after hepatectomy because formation of VLA, another product of transamination, was not increased.

The urinary excretion of larger amounts of the fraction containing free *O*-methylated acids and alcohols and conjugates by hepatectomized rats at later times after L-DOPA injection was of interest. In the combined urine this fraction comprised largely HVA. It is probable that this HVA was formed from DOPAC by direct *O*-methylation, because we found abundant formation of DOPAC in tissues of hepatectomized rats at 20 min after DOPA injection [4]. It has been shown in intact rabbits that circulating DOPAC is *O*-methylated [11].

Although the hepatectomized rat decarboxylated approximately the same amount of [^{14}C]DOPA within 24 hr as did the controls, this does not necessarily mean that the liver is unimportant in the economy of L-DOPA in the intact animal [12]. Thus, the metabolism of L-DOPA was considerably slower in hepatectomized rats. In addition, the 11.4 per cent of the dose excreted in bile was clearly a product of hepatic metabolism. Also, in recent experiments we found that the liver was

the most active of tissues in the metabolism of L-DOPA because it had the lowest percentage of free unmetabolized DOPA [4]. In addition, uptake by the liver accounted for a considerable percentage of the dose of L-DOPA [4]. Although it is clear that much metabolism of the DOPA that is administered orally is degraded in the gut [6], and by the gut flora [13], our present and past experiments [1] indicate that hepatic metabolism makes a significant contribution to the metabolism of L-DOPA that is absorbed into the blood. Clearly, in the absence of the liver, compensatory increases in the metabolism of L-DOPA by other tissues would be expected because the enzyme aromatic L-amino acid decarboxylase (EC 4.1.1.28) is present in most tissues [2].

In the control rats, total radioactivity in bile, urine and tissues accounted for a total of 73 per cent of the dose of L-[^{14}C]DOPA at 24 hr after its injection. In hepatectomized rats, radioactivity in urine and in tissues accounted for a similar percentage (76 per cent). This leaves a significant percentage of the dose unaccounted for.

That radioactivity is bound in protein at 24 hr after L-[^{14}C]DOPA injection is an important finding. In previous experiments in which analyses were done of tissues removed from rats killed 20 min after injection of L-[^{14}C]DOPA, radioactivity was well extracted by perchloric acid, and between 78 and 95 per cent of the radioactivity was found in the supernatant fractions of extracts of all the tissues of both control and hepatectomized rats. However, in erythrocytes from rats killed 20 min after L-[^{14}C] DOPA injection, only 66.6 per cent (S.E.M. = 2.1) and 44.5 per cent (S.E.M. = 3.0) of the total radioactivity was present in the perchloric acid supernatant fraction in control and in hepatectomized rats, respectively [4]. Association of radioactivity with proteins of red blood cells after injection of L-[^{14}C]DOPA has also been noted in the isolated perfused rat liver system [11].

Scheulen *et al.* [14] have shown *in vitro* irreversible binding of DOPA and DOPA metabolites to protein by preparations of human and rat liver microsomes. It was shown that addition of systems which generated superoxide increased this binding and they postulated that the heme protein cytochrome P-450 was involved, although this compound was not always necessary. It was suggested that *O*-semiquinones, which are oxidation products of catechols, were the moieties which were bound. *In vivo* association of radioactive metabolites of DOPA with proteins does not appear to have been demonstrated previously. It is possible that binding of DOPA metabolites to protein could give rise to compounds with antigenic properties and could account for some of the adverse effects (agranulocytosis, hemolytic anemia) occasionally associated with treatment by this drug [15, 16]. Further experiments are needed to determine the nature of this binding.

Acknowledgements—We are greatly indebted to Mr. Robert J. Fountain and Mr. Curtis Grabau for their excellent technical assistance. This work was supported in part by the National Institutes of Health Research Grant NS 9143.

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