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METABOLISM OF ^{3}H-L-DOPA BY THE RAT GUT *IN VIVO*—EVIDENCE FOR GLUCURONIDE CONJUGATION*†

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Abstract—The metabolism of intravenous ^{3}H-L-dopa by the rat gut *in vivo* has been studied. After a single i.v. bolus of a pharmacologic dose of L-dopa, the rat duodenum accumulated ^{3}H-noncatechol metabolites of dopa at a far greater rate than stomach, spleen or heart. ^{3}H-noncatechols were localized predominantly in the duodenal mucosa and not the muscularis. In the mucosa, ^{3}H-noncatechols accounted for 90 per cent of the total ^{3}H, most of which was in the amino acid noncatechol fraction after chromatographic separation on Alumina and Dowex. Accumulation of noncatechol metabolites in duodenal mucosa was not dependent on the administration of a pharmacologic dose of dopa; after a tracer dose of ^{3}H-L-dopa the per cent of ^{3}H-noncatechols in the amino acid fraction actually increased. The pattern of dopa metabolites in duodenal mucosa was substantially different from a variety of other tissues, but was similar to liver, jejunum and colon. Diversion of the bile by cannulation of the common bile duct prior to the administration of ^{3}H-L-dopa did not change the accumulation or pattern of metabolites in the duodenum. Analysis of the noncatechol amino acid fraction from duodenal mucosa by incubation with Glusulase and β-glucuronidase, thin-layer chromatography, and fluorescent assay revealed that the compounds accumulated by duodenal mucosa were largely glucuronide conjugates of catechols, particularly dopamine. It is concluded that circulating dopa is taken up by the rat intestinal mucosa, decarboxylated, conjugated and stored, largely in the form of glucuronide conjugates. The gut thus makes a major contribution to the over-all metabolism of circulating dopa. Dopa metabolites stored in the gut, moreover, are a potential reservoir of catechols for reutilization by the organism, and this may have important implications for the clinical pharmacology of L-dopa in man.

During the course of experiments on the interaction of L-dopa (3,4-dihydroxyphenylalanine) with the sympathetic nerve endings in the rat [1, 2], it became clear that the uptake and metabolism of dopa varied considerably in different rat tissues [3]. Particularly impressive was the accumulation of dopa metabolites in the duodenum. The present study was undertaken to identify the metabolic pathways involved in the transformation of dopa by the rat duodenum. The major pathways of dopa metabolism are shown in Fig. 1. Evidence is presented that blood-borne dopa is taken up by duodenal mucosa and conjugated with glucuronide. This appears to be a major factor in the over-all metabolism of dopa and has implications for the clinical pharmacology of L-dopa in man.

METHODS

Radioisotopes and counting techniques. The water used in all laboratory procedures was filtered, demineralized (Corning) and glass-distilled (Corning). L-3,4-Dihydroxyphenylalanine-^{3}H (G) (^{3}H-L-dopa), 6–9 Ci/m-mole, was purchased from New England Nuclear Corp. (Boston, Mass.). It was purified prior to use by column chromatography on Alumina. Samples

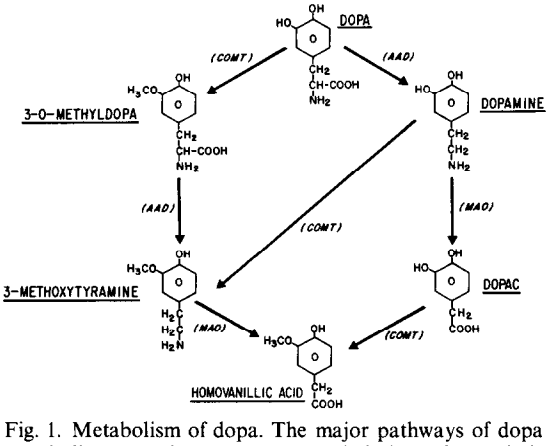

Fig. 1. Metabolism of dopa. The major pathways of dopa metabolism are shown. *meta-O*-methylation of catechols by catechol-*O*-methyltransferase (COMT) results in the formation of the corresponding methoxy (noncatechol) compounds. Decarboxylation by aromatic L-amino acid decarboxylase (AAD) to the corresponding amine and oxidative deamination by monamine oxidase (MAO) to the corresponding acid are also shown. In addition (not shown), glucuronide and sulfate conjugation of the phenolic hydroxyl group can occur, reactions which also result in the formation of noncatechols.

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were counted by liquid scintillation spectrometry in a Nuclear Chicago scintillation counter. Two hundred μl of an aqueous sample was mixed with 4·0 ml ethanol–methanol (3:1) and 10·0 ml toluene liquifluor (New England Nuclear Corp.) scintillator. Efficiency for ^{3}H in this system is 8 per cent. All samples were counted at least ten times the background in cpm to reduce the statistical error of counting.

Animals. Female Sprague–Dawley rats (Charles River Corp., Boston, Mass.) weighing 150–200 g were used in all experiments. They were housed two/cage in a constant temperature animal room and allowed free access to water and Purina laboratory chow. Food was withheld for 24 hr prior to each experiment, and for 48 hr prior to biliary diversion.

L-Dopa was a gift of Dr. W. E. Scott of Roche; it was dissolved in 0·45% NaCl in 0·075 to 0·15 N HCl and injected (25 or 50 mg/kg) via the tail vein in a volume of approximately 1·0 ml. Sufficient ^{3}H-L-dopa was added to the nonradioactive L-dopa so that 400, 800 or 2500 μCi/kg was administered as well. Injections were performed on unanesthetized animals except where biliary diversion was performed prior to administration of dopa.

The animals were killed by a blow at the base of the skull or, when plasma was required, by guillotine. The organs were rapidly removed, weighed and frozen on dry ice (stored at $-20°$) until homogenization in iced 0·4 N perchloric acid (within 4 weeks). The duodenal mucosa was separated from the muscularis by the sharp edge of a glass slide. Specimens of duodenal mucosa so obtained weighed between 100 and 200 mg. In some of the experiments, the glandular portion of the rat stomach was dissected from the non-glandular portion, and the mucosa of the glandular portion stripped from the muscularis as described for the duodenum.

In experiments involving biliary diversion, the animals were anesthetized with nembutal, and a polyethylene catheter inserted into the common bile duct and secured with ligatures. In some experiments, the pylorus and distal duodenum were ligated as well. After the bile was diverted, ^{3}H-L-dopa was administered via tail vein and the bile collected. Bile flow averaged 0·01 ml/min. Bile was frozen in acetone and dry ice and stored at $-20°$. Prior to analysis, it was acidified with perchloric acid to a final concentration of 0·4 N and the resulting extract treated as described for tissue homogenates.

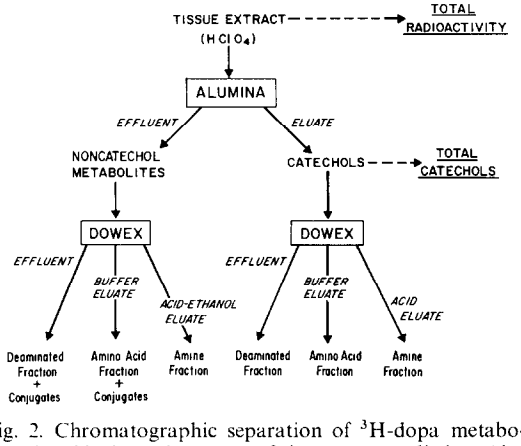

Fig. 2. Chromatographic separation of ^{3}H-dopa metabolites. Perchloric acid extracts of tissues are applied to Alumina columns at pH 8·6. The effluent contains the noncatechols; the catechols are eluted with 0·2 N acetic acid. Both effluent and eluate are applied to Dowex columns at pH 2. The amino acid fraction is eluted with neutral buffer, the amines with strong acid or acid-ethanol. The deaminated compounds (predominantly acids) appear in the Dowex effluent and are calculated by difference. Glucuronide conjugates appear in the Alumina effluent and the neutral buffer eluate of Dowex. Note that the term "noncatechol" as used in this paper includes conjugates that are not retarded on Alumina.

At the time of analysis, the frozen tissues were homogenized in iced 0·4 N perchloric acid in a ground glass homogenizer (Kontes) to extract the catecholamines and precipitate the proteins. After volume adjustment, the precipitated protein was removed by low speed centrifugation, an aliquot removed for counting total radioactivity, and the remainder of the perchloric acid extract subjected to column chromatography as described below.

Column chromatography. The chromatographic separation of dopa metabolites on Alumina and Dowex is shown in Fig. 2. Alumina, at pH 8·6, selectively retards catechols, while the noncatechols (including conjugates) run off in the effluent [4]. Both catechol and noncatechol amino acids, amines and deaminated compounds may be further separated on Dowex, since at pH 2 amino acids and amines are retarded on Dowex-50 and deaminated metabolites escape in the effluent (Fig. 2). Glucuronide conjugates of amino acids, amines and deaminated compounds, which appear in the Alumina effluent, are at least partially absorbed on Dowex and eluted with neutral buffer. This fraction may falsely elevate the noncatechol amino acid fraction which is usually thought to consist of 3-*O*-methyldopa [5].

Both Alumina and Dowex were purified prior to use. Alumina (Woelm neutral) was prepared according to the method of Anton and Sayre [6]: the Alumina was washed repeatedly with hot HCl followed by copious amounts of water (until the pH is over 3·4) and then dried at 200°. The recovery of norepinephrine (NE) was checked with each new batch. Dowex-50 W × 4, 100–200 mesh (Bio Rad) was prepared according to the method of Hirs *et al.* [7]: after thoroughly washing with glass-distilled water, the resin was cycled through the sodium form by washing in 40% sodium hydroxide and converted to the H^+ form by washing in 3 N HCl.

The columns were all glass, with an internal diameter of 0·5 cm and a 50-ml reservoir (Kontes). They were plugged with Pyrex glass wool. The final height of the Alumina column was 4·0 cm and the Dowex column 5·0 cm. The rate of flow was about 20 ml/hr. Four hundred mg dry Alumina was added to both the plugged column and the sample. The column was washed with 0·2 N sodium acetate (pH 8·6), and 10 ml of 2% EDTA and 60 mg sodium *meta*-bisulfite were added to the sample to retard catecholamine oxidation. The sample was gradually titrated up to pH 8·6 with sodium hydroxide, and stirred for 5 min with a motor-driven glass stirring rod. The sample and the Alumina were transferred quantitatively to the column which was then washed with pH 8·6 sodium acetate and distilled water. The Alumina effluent was collected in glass beakers; catechols were eluted with 0·2 N acetic acid. The pH of the effluent (noncatechols) and eluate (catechols) was then

adjusted to 2·0 and the samples were passed over Dowex columns (separate columns for effluent and eluate) which had been washed with 20 ml of 2 N HCl, 5 ml H_2O, 10 ml of 1·0 M sodium acetate (pH 6·0) and 5 ml water [8]. After the sample had run through, the column was washed with water, the amino acids were eluted with 0·5 M, pH 6·5, potassium acetate, the catecholamines with 2 N HCl [2, 8, 9], and the noncatecholamines (Dowex effluent column) with 3 N HCl in 50% ethanol [10]. Recovery of added ^{3}H-dopa averaged 75 per cent, added ^{3}H-dopamine and ^{3}H-normetanephrine 80 per cent, and added ^{3}H-NE 85 per cent. Added ^{3}H-L-dopa contaminated the noncatechol deaminated fraction by 15–20 per cent. Contamination of the other fractions with ^{3}H-L-dopa or any of the fractions with the other compounds was negligible (less than 3·0 per cent). The results reported here are not corrected for recovery or contamination. The total catechol, amino acid and amine fractions were counted directly; the noncatechol and deaminated fractions were calculated by difference (Fig. 2).

Analysis of conjugates. Conjugates of dopa metabolites were identified by incubation of the appropriate sample with Glusulase (Endo, 20,000 units β-glucuronidase, 10,000 sulfatase) or β-glucuronidase (Sigma, 1000 units). Incubations were performed at 37° for 18 hr in the presence of 0·2 ml of 5% ascorbic acid to retard catechol oxidation. The pH of the incubation mixture was 5·4 for Glusulase and 6·5 for β-glucuronidase. The reaction was stopped with 0·4 N $HClO_4$ and the protein removed by centrifugation. The perchloric acid extract was then applied to Alumina and Dowex as described above (see Fig. 2). Samples incubated under identical conditions but without enzymes served as controls. Since Glusulase contains both β-glucuronidase and sulfatase activity, the appropriate conjugate can be identified by comparing the results obtained with Glusulase with those obtained with pure glucuronidase.

Thin-layer chromatography. Specific identification of the tritiated compounds in the column eluates was performed by spotting the appropriate sample on Silica gel plates and running for 4–5 hr in a solvent system of *N*-butanol–acetic acid–water (12:3:5) along with standards of authentic compound. The plates were then dried, sprayed with ninhydrin, dried, and heated for 3–5 min at 100°. The Silica was then scraped off the plate, 0·5 cm at a time, from the origin to the solvent front; each aliquot of Silica was placed in a scintillation vial, mixed with 2·0 ml absolute ethanol, 10 ml liquiflor and counted for ^{3}H. This system allowed adequate separation of dopa, 3-*O*-methyldopa and dopamine.

Assay of O-methyldopa. Authentic 3-*O*-methyldopa was kindly provided by Dr. W. E. Scott of Roche. 3-*O*-methyldopa was determined by fluorescent assay [11]. The neutral buffer eluate was adjusted to pH 7·2, oxidized with iodine and stopped with alkaline-sulfite solution [12]. A heated reversed blank was used [2]. Fluorescence (determined on an Aminco Bowman spectrophotofluorometer at an excitation wave length of 340 and emission wave length of 390 nm) was linear between at least 50 and 400 ng, and 50 ng gave a reading twice blank.

Statistics. Where appropriate, statistical significance was determined by the Student *t*-test for unpaired variables.

RESULTS

Accumulation of noncatechol metabolites of ^{3}H-L-dopa in rat gut. After intravenous administration of ^{3}H-L-dopa, the concentration of noncatechol metabolites of dopa in duodenum and ileum was significantly greater than in other rat tissues (Fig. 3). This impressive difference (10-fold greater in duodenum than heart, stomach or spleen) prompted experiments designed to localize and identify the noncatechol compounds involved. In the experiment shown in Table 1, rats were killed 5 min after the i.v. injection of ^{3}H-L-dopa and the mucosa was separated from the muscularis. The ^{3}H-dopa metabolites in both portions of tissue were then isolated chromatographically as shown in Fig. 2. Significantly more ^{3}H-noncatechols were localized in the mucosa than in the muscularis. The bulk of this was in the neutral buffer eluate (the amino acid fraction) which accounted for 58·7 per cent of the total ^{3}H in the duodenal mucosa (1635 ± 178 nCi/g) as compared with 28·8 per cent in the muscularis (577 ± 74 nCi/g). The difference between mucosa and muscularis is more striking than the figures indicate since muscularis, by this technique, was not completely separated from mucosa.

The pattern of ^{3}H-dopa metabolites in various other tissues is shown in Table 2. Of all the tissues studied, only the liver equaled the duodenal mucosa in the percentage of total radioactivity in the noncatechol amino acid fraction. Similar concentration was not found in the gastric mucosa, where the amino acid noncatechol fraction accounted for only 5 per cent of the total radioactivity. As shown in Table 3, the accumulation of noncatechols in the duodenal

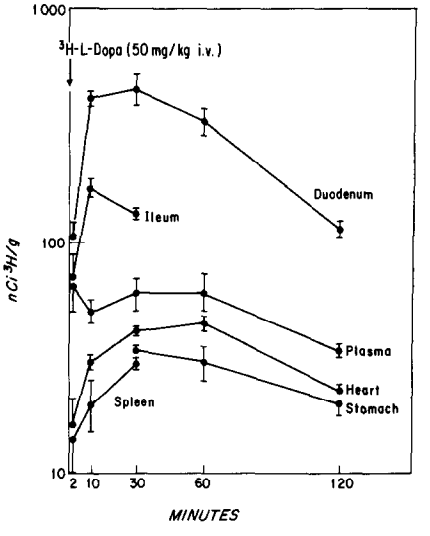

Fig. 3. ^{3}H-noncatechol metabolites in rat tissues after i.v. administration of ^{3}H-L-dopa. Animals were injected with 50 mg/kg of L-dopa containing 400 μCi/kg of ^{3}H-L-dopa and killed (five/group) at 2, 10, 30, 60 and 120 min. Tissues were homogenized in 0·4 N $HClO_4$ as described in Methods, and ^{3}H-noncatechol metabolites calculated from the difference between the total radioactivity and the Alumina eluate. Values for stomach were below 10 nCi/g at 2 and 10 min, and these points are not shown. Duodenal levels were significantly greater ($P < 0·05$ to 0·001) than all other tissues at 10, 30, 60 and 120 min; ^{3}H-noncatechol levels in ileum were greater than all tissues (except duodenum) at 10 and 30 min ($P < 0·001$).

Table 1. ^{3}H-dopa metabolites in rat duodenal mucosa and muscularis*

	Mucosa (N = 11)		Muscularis (N = 10)	
	(nCi/g)	(% ^{3}H)†	(nCi/g)	(% ^{3}H)†
Total ^{3}H	2760·0 ± 264·8	(100%)	2056·7 ± 272·6	(100%)
^{3}H-noncatechols	2473·4 ± 242·4‡	(89·7%)‡	1307·6 ± 145·3	(67·1%)
Amino acid fraction	1635·3 ± 178·5‡	(58·7%)‡	577·4 ± 73·6	(28·8%)
Amine fraction	72·8 ± 24·4	(2·6%)	43·5 ± 16·8	(2·1%)
Deaminated fraction	765·3 ± 100·1	(28·6%)	673·7 ± 77·4	(35·7%)
^{3}H-catechols	297·1 ± 75·8§	(10·3%)§	749·1 ± 188·7	(32·9%)
Amino acid fraction	50·4 ± 22·0	(1·8%)	158·9 ± 42·0	(6·9%)
Amine fraction	27·2 ± 15·0‡	(1·0%)‡	249·7 ± 41·4	(12·1%)
Deaminated fraction	208·9 ± 45·8	(7·6%)	319·25 ± 102·3	(15·5%)

* Rats were killed 5 min after i.v. ^{3}H-L-dopa (25 mg/kg; 800 μCi/kg) and the mucosa and muscularis separated and analyzed as described in Methods. Mucosa differs significantly from muscularis in accumulation of noncatechols, particularly in the amino acid (neutral buffer eluate) fraction. N = number of animals. Values are means ± S.E.M.
† % total ^{3}H/sample.
‡ $P < 0·001$.
§ $P < 0·05$.

mucosa is not the result of administration of a pharmacologic dose. The administration of a tracer dose of ^{3}H-L-dopa (0·02 mg/kg rather than 25 mg/kg) was associated with an even greater percentage of ^{3}H in the noncatechol amino acid fraction (72·7 as compared with 59·1 per cent). Thus, duodenal accumulation of noncatechols cannot be considered an overflow pathway secondary to the administration of a large dose.

The time course of the accumulation of ^{3}H-noncatechols in the amino acid fraction of duodenal mucosa is shown in Fig. 4. Since the plasma levels

Table 2. ^{3}H-dopa metabolites in various tissues*

Tissue	Total ^{3}H (nCi/g)	Noncatechol amino acid fraction (nCi/g)	(% ^{3}H)	Catechol amino acid fraction (nCi/g)	(% ^{3}H)
Duodenal mucosa	2648·5 ± 211·1	1710·5 ± 95·7	(64·9%)	69·7 ± 69·7	(2·8%)
Liver	1823·6 ± 182·3	1142·0 ± 141·0	(61·8%)	22·6 ± 1·7	(1·3%)
Kidney	6706·5 ± 941·4	471·6 ± 12·5	(7·6%)	2081·2 ± 244·4	(31·2%)
Gastric mucosa	1312·9 ± 152·9	59·5 ± 21·2	(5·0%)	587·7 ± 68·3	(44·7%)
Gastric muscularis	1348·8 ± 65·0	56·0 ± 5·8	(4·2%)	660·6 ± 60·0	(48·7%)
Plasma	861·4 ± 44·5	34·0 ± 3·3	(3·9%)	377·2 ± 34·6	(40·8%)
Heart	1028·6 ± 56·8	15·7 ± 2·3	(1·5%)	401·3 ± 66·1	(38·9%)
Diaphragm	1158·1 ± 69·0			474·3 ± 47·4	(40·6%)

* Animals (four to six/group) were injected with ^{3}H-L-dopa (25 mg/kg; 800 μCi/kg) and killed after 5 min. Organs were rapidly removed and analyzed as described in Methods. Values are means ± S.E.M. (% ^{3}H) = % total radioactivity.

Table 3. ^{3}H-dopa metabolites in rat duodenal mucosa after pharmacologic (25 mg/kg) and tracer (0·02 mg/kg) dose of L-dopa*

	25 mg/kg (N = 6)		0·02 mg/kg (N = 7)	
	(nCi/g)	(% ^{3}H)	(nCi/g)	(% ^{3}H)
Total ^{3}H	2693·7 ± 327·3	(100%)	3116·5 ± 322·6	(100%)
^{3}H-noncatechols	2582·6 ± 322·7	(95·7%)	3034·8 ± 316·9	(97·3%)
Amino acid fraction	1626·2 ± 259·0	(59·1%)	2277·9 ± 253·7	(72·7%)†
Amine fraction	40·7 ± 27·0	(1·3%)	193·8 ± 30·5	(6·2%)
Deaminated fraction	915·8 ± 115·1	(35·2%)	563·1 ± 44·1	(18·4%)
^{3}H-catechols	111·1 ± 10·0	(4·3%)	81·7 ± 8·5	(2·7%)
Amino acid fraction			6·13 ± 6·13	(0·1%)
Amine fraction				
Deaminated fraction	111·1 ± 10·0	(4·3%)	75·5 ± 7·4	(2·6%)

* Rats were injected with 800 μCi/kg of ^{3}H-L-dopa containing either 25 mg/kg or 0·02 mg/kg of L-dopa (N = number of animals) and killed after 5 min. The noncatechol amino acid fraction was greater in the tracer group, thus indicating that the accumulation of metabolites in this fraction is not the consequence of a large dose. Note that although the counts are higher in the tracer group, the actual accumulation of compound is less by a factor of 1250.
† $P < 0·02$.

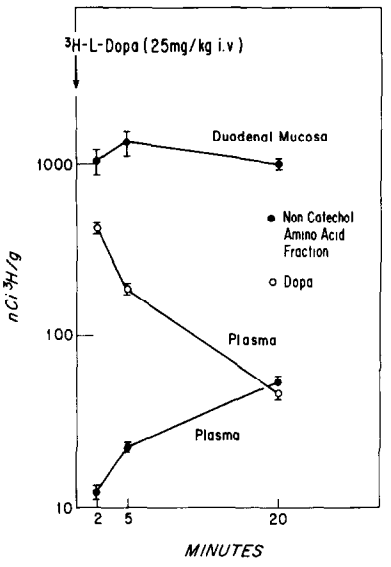

Fig. 4. Relationship between plasma and duodenal levels of metabolites in the noncatechol amino acid fraction. Animals were injected with ^{3}H-L-dopa (25 mg/kg; 800 μCi/kg) and killed (six/group) 2, 5 and 20 min later. The noncatechol amino acid fraction (—●—) in duodenal mucosa and plasma is shown along with the catechol amino acid fraction (dopa) (—○—) in plasma. The catechol amino acid fraction was essentially absent in duodenal mucosa and hence is not shown; in plasma, the catechol amino acid fraction was shown by thin-layer chromatography to consist almost entirely of unmetabolized ^{3}H-L-dopa (see Fig. 5). The pattern shown is consistent with uptake of dopa by the duodenal mucosa with the subsequent formation and storage of the noncatechol compounds that appear in the amino acid fraction.

of this fraction are low in contrast to high initial plasma dopa levels, it appears that the duodenal mucosa takes up dopa and forms and stores the noncatechol metabolites, rather than directly accumulating noncatechol metabolites from the circulation.

Effect of biliary diversion on duodenal accumulation of ^{3}H-L-dopa metabolites. Since liver (Table 2) has high levels of ^{3}H-noncatechols, the possibility of an enterohepatic circulation with duodenal absorption of compounds secreted in bile was investigated. In these experiments, ^{3}H-L-dopa was administered after the common bile duct had been cannulated and the bile flow diverted and collected. The results are shown in Table 4. The accumulation of ^{3}H-noncatechol metabolites was not altered by biliary diversion. Thus, although the bile contained considerable amounts of ^{3}H-dopa metabolites (Table 4), the absorption of compounds secreted in the bile cannot account for the high levels found in duodenal mucosa in the previous experiments.

To rule out gastric or pancreatic secretion of ^{3}H-dopa metabolites with subsequent absorption by the duodenal mucosa, the preceding experiment was repeated with the pylorus and distal duodenum ligated. The pattern of metabolite accumulation was unchanged by this maneuver (Table 5). Furthermore, the small amounts of gastric and duodenal fluid collected after 10 min contained negligible ^{3}H.

Results for jejunum and colon (with the bile diverted) are shown in Table 6. Jejunum was similar to duodenum; colon accumulated less ^{3}H-dopa metabolites than small bowel, but the pattern of metabolites was similar.

Identification of ^{3}H-dopa metabolites in the noncatechol amino acid fraction of gut. As shown in Fig. 1, 3-*O*-methyldopa is a major noncatechol amino acid metabolite of dopa. Analysis of the noncatechol amino acid fraction of duodenum by fluorescent assay revealed no detectable *O*-methyldopa in duodenum, although authentic *O*-methyldopa added to the eluate produced a highly fluorescent derivative.

Since Tyce *et al.* [5] have shown that conjugates of dopa metabolites (which appear in the Alumina effluent) are retarded on Dowex and eluted by neutral buffer, the possibility was investigated that the accumulation of dopa metabolites in the amino acid noncatechol fraction of gut represents conjugates of dopa and dopa metabolites. The noncatechol amino acid fraction of duodenum was incubated with and without Glusulase (glucuronidase and sulfatase) and with and without β-glucuronidase and the incubation mixture repassed over Alumina and Dowex. As shown in Table 7, the percentage of noncatechols dropped from 97 per cent in the samples incubated without enzyme to 40 per cent in the samples incubated with Glusulase, while the amino acid noncate-

Table 4. ^{3}H-dopa metabolites in duodenal mucosa after biliary diversion*

	Duodenal mucosa		Liver		Bile	
	(nCi/g)	(% ^{3}H)	(nCi/g)	(% ^{3}H)	(nCi/g)	(% ^{3}H)
Total ^{3}H	2456·2 ± 263·3	(100%)	1889·9 ± 49·3	(100%)	2603·6 ± 230·9	(100%)
^{3}H-noncatechols	2247·2 ± 291·9	(90·7%)	1856·3 ± 49·1	(98·2%)	245·2 ± 225·7	(94·1%)
Amino acid fraction	1564·7 ± 223·4	(63·0%)	1269·1 ± 48·8	(67·2%)	448·4 ± 47·9	(17·2%)
Amine fraction	17·1 ± 17·1	(0·7%)	54·9 ± 4·9	(2·9%)		
Deaminated fraction	665·5 ± 201·3	(27·0%)	532·3 ± 62·4	(28·1%)	2003·8 ± 183·6	(77·0%)
^{3}H-catechols	209·0 ± 33·6	(9·3%)	33·7 ± 2·1	(1·8%)	151·4 ± 16·3	(5·9%)
Amino acid fraction	23·26 ± 23·26	(0·8%)	12·91 ± 2·4	(0·7%)	104·8 ± 11·6	(4·1%)
Amine fraction	19·84 ± 19·84	(0·8%)	9·7 ± 3·4	(0·5%)		
Deaminated fraction	165·9 ± 41·3	(7·7%)	11·1 ± 1·8	(0·6%)	46·7 ± 5·8	(1·8%)

* Four animals were studied. Under barbiturate anesthesia, the common bile duct was cannulated and ^{3}H-L-dopa (25 mg/kg; 800 μCi/kg) was administered i.v. Bile was collected for 10 min, the animals were killed, and tissues and bile analyzed as described in Methods. The volume of bile collected averaged 100 μl/10 min. Note that diversion of the bile did not change the amount of ^{3}H accumulated by the duodenum or the pattern of metabolites formed (see Tables 1, 2 and 3).

Table 5. ^{3}H-dopa metabolites in duodenal mucosa after diversion of the bile and ligation of the pylorus and duodenum*

	Duodenal mucosa		Liver		Bile	
	(nCi/g)	(% ^{3}H)	(nCi/g)	(% ^{3}H)	(nCi/g)	(% ^{3}H)
Total ^{3}H	2835·7 ± 280·6	(100%)	1770·1 ± 135·3	(100%)	2542·5 ± 240·0	(100%)
^{3}H-noncatechols	2570·2 ± 239·7	(90·9%)	1738·2 ± 132·3	(98·2%)	2376·2 ± 244·4	(93·2%)
Amino acid fraction	1616·5 ± 177·0	(57·1%)	1087·0 ± 111·4	(61·7%)	512·5 ± 69·5	(20·0%)
Amine fraction	171·2 ± 29·7	(6·0%)	87·3 ± 13·4	(5·0%)	62·0 ± 9·4	(2·4%)
Deaminated fraction	782·6 ± 97·0	(27·8%)	563·9 ± 97·6	(31·5%)	1801·8 ± 173·6	(70·8%)
^{3}H-catechols	265·5 ± 79·9	(9·1%)	31·9 ± 3·2	(1·8%)	166·3 ± 16·1	(6·8%)
Amino acid fraction	7·7 ± 7·7	(0·2%)	11·7 ± 1·2	(0·7%)	99·9 ± 14·1	(4·0%)
Amine fraction	109·5 ± 54·1	(3·7%)	6·6 ± 2·5	(0·4%)		
Deaminated fraction	148·2 ± 21·9	(5·2%)	13·6 ± 3·4	(0·8%)	66·4 ± 7·6	(2·7%)

* Experimental design as in Table 4 (four animals) but, in addition to biliary diversion, ligation of the pylorus and distal end of the duodenum was performed before administration of ^{3}H-L-dopa. After 10 min, the animals were killed and tissues were analyzed. Gastric and duodenal fluid was aspirated and analyzed as well, but these fluids contained negligible amounts of ^{3}H. Results do not differ significantly from those presented in Table 4.

Table 6. ^{3}H-dopa metabolites in jejunum and colon*

	Jejunum		Colon	
	(nCi/g)	(% ^{3}H)	(nCi/g)	(% ^{3}H)
Total ^{3}H	9087·9 ± 344·8	(100%)	2028·2 ± 61·7	(100%)
^{3}H-noncatechols	7857·1 ± 217·1	(86·6%)	1548·6 ± 49·0	(76·4%)
Amino acid fraction	4596·2 ± 358·5	(50·6%)	503·3 ± 16·6	(24·9%)
Amine fraction	351·5 ± 43·3	(3·9%)	50·1 ± 4·6	(2·5%)
Deaminated fraction	2909·4 ± 206·5	(32·2%)	995·2 ± 44·6	(49·0%)
^{3}H-catechols	1230·7 ± 190·7	(13·4%)	479·6 ± 20·6	(23·6%)
Amino acid fraction	96·8 ± 9·8	(1·1%)	113·8 ± 11·7	(5·6%)
Amine fraction	229·4 ± 29·8	(2·5%)	114·2 ± 6·9	(5·6%)
Deaminated fraction	904·5 ± 169·2	(9·8%)	251·6 ± 12·12	(2·5%)

* Five animals were studied after biliary diversion under light barbiturate anesthesia. The dose of ^{3}H-L-dopa was 2·5 mCi/kg; 25 mg/kg. Whole gut rather than mucosa was used for these tissues. Total ^{3}H in heart in the experiment was 1686·2 ± 111·9 nCi/g.

Table 7. Noncatechol amino acid fraction in duodenal mucosa—Effect of incubation with Glusulase and β-glucuronidase*

	% ^{3}H			
	pH 5·4 Incubation		pH 6·5 Incubation	
	No enzyme	Glusulase	No enzyme	β-glucuronidase
^{3}H-noncatechols	97·10 ± 0·14	39·64 ± 0·96	97·34 ± 0·18	49·45 ± 0·58
Amino acid fraction	58·62 ± 2·95	0·68 ± 0·03	48·72 ± 2·73	0·44 ± 0·06
Amine fraction	3·80 ± 0·58	3·88 ± 0·34	1·85 ± 0·27	3·29 ± 0·28
Deaminated fraction	25·41 ± 3·55	25·09 ± 0·94	46·77 ± 3·08	45·72 ± 0·83
^{3}H-catechols	2·91 ± 0·14	60·36 ± 0·96	2·66 ± 0·18	50·55 ± 0·58
Amino acid fraction	0·79 ± 0·02	7·08 ± 0·40	0·42 ± 0·06	3·17 ± 0·43
Amine fraction	2·00 ± 0·05	37·49 ± 2·42	1·86 ± 0·21	31·46 ± 1·03
Deaminated fraction	0·14 ± 0·14	15·79 ± 2·36	0·38 ± 0·13	15·92 ± 0·99

* Values are per cent of total ^{3}H ± S.E.M. Under barbiturate anesthesia, five animals were injected with ^{3}H-L-dopa (25 mg/kg; 2·5 mCi/kg) after the common bile duct had been cannulated. Bile was collected for 10 min, the animals were killed and duodenal mucosa was analysed as described in Methods. Total ^{3}H in duodenum was 9422·2 ± 1084·1 nCi/g. Aliquots of amino acid fraction (Alumina effluent, neutral buffer eluate from Dowex, Fig. 2) were then incubated at pH 5·4 with and without Glusulase and at pH 6·5 with and without β-glucuronidase for 18 hr. The specimens were then repassed over Alumina and Dowex as described in Methods. Before incubation, the amino acid noncatechol fraction contained 5339·9 ± 1073·9 nCi/g for duodenal mucosa (58·7 per cent total ^{3}H). All samples incubated with enzymes differed from samples incubated without enzyme ($P < 0·001$) except for noncatechol amines and noncatechol deaminated fraction of duodenal mucosa. Note that incubation without enzyme resulted in a loss of the original amino acid fraction of about 40 per cent. These counts appear in the deaminated fraction and represents either poor recovery of conjugates on Dowex or destruction of the original compounds during the incubation to a derivative not retarded on Dowex. For this reason, the results with enzyme are always compared to similarly incubated samples without enzyme.

Table 8. Noncatechol amino acid fraction—Effect of incubation with Glusulase*

	% ^{3}H			
	No enzyme		With enzyme	
	Noncatechols	Catechols	Noncatechols	Catechols
Bile	98·8 ± 0·3	1·2 ± 0·3	63·2 ± 1·3	36·8 ± 1·3
Jejunum	98·5 ± 0·1	1·5 ± 0·1	45·9 ± 0·7	54·1 ± 0·7
Colon	98·6 ± 0·1	1·4 ± 0·1	60·8 ± 0·5	39·1 ± 0·5
Liver	97·9 ± 0·1	2·1 ± 0·1	45·5 ± 0·2	54·5 ± 0·2

* Five animals were studied as described in Table 7. Values are means ± S.E.M. All samples incubated with enzyme differed significantly from those without ($P < 0.001$). As in duodenum (Table 7), the catecholamine fraction (dopamine) was the predominant catechol in the four tissues studied here, accounting for 20–28 per cent of the total ^{3}H in the incubated samples. In bile and colon, significant amounts of methoxydopamine were released after incubation with Glusulase as indicated by substantial increases in the noncatecholamine fraction of enzyme-incubated sample (from 3 per cent without enzyme to 20 per cent with).

chol fraction fell from 58·6 to 0·7 per cent. Similar results were obtained with β-glucuronidase, as shown in the table. The data in Table 7 indicate that glucuronide conjugates of catechols comprise the amino acid noncatechol fraction in duodenum. Thus, after incubation with Glusulase, the catechol amino acid fraction (dopa) increased from 0·8 to 7·1 per cent, the catecholamine fraction (dopamine) from 2 to 37·5 per cent, and the deaminated catechols (dopac) from 0·1 to 15·8 per cent.

In Table 8, Glusulase incubation studies of the amino acid noncatechol fraction of bile, jejunum, colon and liver are shown. The results are similar to those obtained with duodenum. There was a substantial increase in the catechol fraction in all samples incubated with enzyme (from less than 2 per cent catechols to greater than 35 per cent catechols).

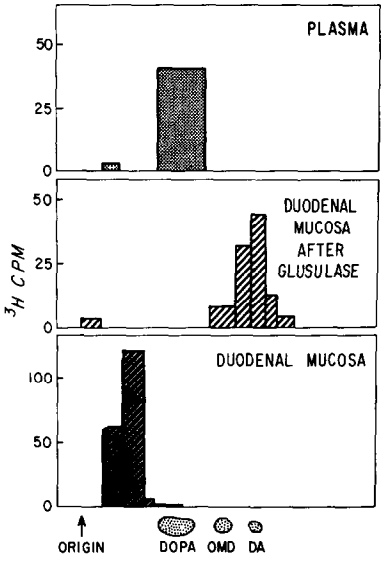

Fig. 5. Thin-layer chromatography of the amino acid noncatechol fraction of duodenal mucosa and the amino acid catechol fraction of plasma. Neutral buffer eluates were spotted on Silica gel plates and run in a solvent system of *n*-butanol–acetic acid–water (12:3:5). Chromatographs of the duodenal mucosa are shown before and after incubation with Glusulase. Note that after incubation with Glusulase most of the ^{3}H travels with authentic dopamine (OMD, 3-*O*-methyldopa; DA, dopamine). In plasma, 5 min after i.v. injection the ^{3}H from the catechol amino acid fraction co-chromatographs with authentic dopa.

Further identification of the amino acid noncatechol fraction of duodenal mucosa by thin-layer chromatography is shown in Fig. 5. Before incubation, all of the tritium was located near the origin; after incubation with Glusulase, most of the ^{3}H traveled with authentic dopamine. In plasma, for comparison, the catechol amino acid fraction co-chromatographed with authentic dopa.

DISCUSSION

These studies demonstrate that the gut is a major site of dopa metabolism in the rat. The amount of dopa taken up and metabolized by duodenum, jejunum, ileum and colon is quantitatively significant when compared with liver and kidney, the two organs usually associated with the metabolism of circulating catechols. The significance of the gut in the metabolism of a variety of diverse drugs and hormones is a subject of increasing interest, and the results reported here are in accord with other recent studies implicating the gut as an important metabolic organ [13].

Duodenum, liver, bile, jejunum, ileum and colon accumulated large amounts of noncatechol metabolites (Tables 2, 4, 6; Fig. 3). The pattern of metabolites in these tissues was substantially different from the pattern in plasma, heart, diaphragm, stomach or kidney. In duodenum, liver and jejunum, most of the noncatechol metabolites were eluted in the neutral buffer eluate (amino acid) fraction. The evidence presented in the paper strongly suggests that the ^{3}H-metabolites in the fraction are glucuronide conjugates of dopamine and other dopa metabolites. As noted by Tyce *et al.* [5] in studies on bile, glucuronide conjugates are adsorbed on Dowex-50 and eluted with neutral buffer in what is usually considered the amino acid fraction in chromatographic separations of dopa metabolites; such conjugates may thus be mistaken for 3-*O*-methyldopa. The studies with glucuronidase (Tables 7 and 8), thin-layer chromatography (Fig. 5), and the results of fluorescent assay for *O*-methyldopa indicate that in the rat gut the amino acid noncatechol fraction is principally composed of glucuronide conjugates of catechols. The generation of metabolites that are retained on Alumina after incubation with glucuronidase suggests (but does not prove) that conjugation with glucuronide occurs on the phenolic hydroxyl groups. The experiments shown in Tables 4 and 5 clearly exclude enterohepatic circulation (duodenal absorption of compounds secreted in bile) as a cause of the duodenal accumulation of conjugates. The high plasma dopa levels and low plasma noncatechol levels (Fig. 4) are consistent with formation of conjugates in the duodenum rather than uptake of circulating conjugates formed elsewhere. The experiment shown in Table 1 indicates that in the duodenum uptake and conjugation of dopa occurs in the mucosa rather than the muscularis. As shown in Table 3 the percentage of ^{3}H in the noncatechol amino acid fraction increased significantly with a low dose of dopa (0·02 μg/kg), as compared with the pharmacologic dose used in all the experiments (25 mg/

kg). This indicates that glucuronide conjugation is not an overflow pathway secondary to a large dose; it suggests, rather, that the conjugation mechanism may be saturable at higher doses.

The fact that the gut forms glucuronide conjugates of estrogens [14] has been known for some time. It is also well recognized that glucuronide conjugates of catecholamines and catecholamine metabolites appear in the urine of rats [15] and man [16–18]. The gut, however, has not been considered a major factor in the metabolism of circulating catechols. Conjugation is usually thought to be of greater importance in the metabolism of ingested catechols [19], and such conjugates are presumed to originate predominantly in liver and kidney. The results described here indicate that circulating dopa is rapidly and effectively taken up and conjugated by the gut mucosa. It is not clear from the present studies whether the uptake process in intestinal mucosa is related to or independent of the conjugation process. Studies in Gunn rats which lack glucuronyltransferase would clearly be of interest in this regard. Nor is it clear whether conjugated dopamine (the principal conjugate) is formed after decarboxylation to free dopamine or whether conjugated dopa is decarboxylated. The demonstration of conjugates of methoxydopamine in colon and bile indicates that at least some transformations occur before conjugation, since *O*-methylation requires an intact catechol group [20]. It thus seems likely that duodenal uptake of dopa is primary. Conjugation may even promote catechol storage by inhibiting further metabolism.

The role of the gut in the metabolism of the endogenous catecholamines, norepinephrine and epinephrine, remains to be explored. Such studies should be performed in animals treated with 6-hydroxydopamine (an agent which destroys the sympathetic nerve endings) so that neuronal uptake of the amine is eliminated.

The uptake and conjugation of circulating dopa by the gut has certain implications for the clinical pharmacology of L-dopa in man. Conjugates stored in the intestinal mucosa are a potential reservoir of dopa metabolites. Although it remains to be shown that ^{3}H-dopa metabolites can be recovered in stool or intestinal contents after intravenous administration of ^{3}H-L-dopa to animals with biliary fistulas, it is conceivable that stored catechol metabolites may gain access to the intestinal lumen as intestinal cells slough or through diffusion. Once inside the bowel lumen, these metabolites would be available to the intestinal microflora which have been shown to cause the deconjugation, *o*-demethylation, *p*-dehydroxylation, and decarboxylation of catechols in mammals [13, 21]. Subsequent reabsorption of dopa metabolites modified by bacteria may have important pharmacologic effects. Sandler *et al.* [19, 22] have speculated, for example, that *m*-hydroxytyrosine derivatives which arise from the *p*-dehydroxylation of dopa may be a significant factor in the effects of L-dopa on the central nervous system. Dopa metabolites originating in intestinal mucosa may be particularly important, since compounds originating in diet or bile are likely to be absorbed higher in the bowel thereby having less access to the gut flora. Compounds from the intestinal mucosa would, theoretically, be available to a greater bacterial population. It has recently been demonstrated that enteric coated L-dopa results in a greater increase in *m*-hydroxytyrosine derivatives than the usual dopa formulation [23], a finding that supports the concept that intestinal origin of substrate further down the gut may be of special significance. The fact that in man sulfate conjugation of dopa metabolites may predominate [24] (rather than glucuronide conjugates as in the rat) does not alter the potential significance of intestinal conjugation for the clinical pharmacology of L-dopa, since the intestinal microflora possess sulfatase as well as glucuronidase [13].

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