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SPECIES VARIATIONS IN THE *O*-METHYLATION OF *n*-BUTYL 4-HYDROXY-3,5-DIIODOBENZOATE

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Abstract—The metabolism and excretion of orally administered or injected *n*-butyl 4-hydroxy-3,5-di[^{125}I]iodobenzoate has been studied in man, the rhesus, cynomolgus, squirrel and capuchin monkeys and the rat and rabbit. These species excreted most (about 80 per cent of dose) of the radioactivity in the urine over a period of 3 days except in the case of the squirrel and capuchin monkeys and the rat which excreted considerable amounts (17–30 per cent of dose) in the faeces. Four metabolites were characterized by thin-layer chromatography, gas–liquid chromatography–mass spectral analysis and by reverse isotope dilution and these were: 4-hydroxy-3,5-diiodobenzoic acid and its glycine conjugate and a product of *O*-methylation, namely, 3,5-diiodoanisic acid and its glycine derivative. There is a marked species difference in the excretion of 3,5-diiodoanisic acid for this metabolite was found only in the urine of the human subjects and the new and old World primate species and not in the rat and rabbit which excreted only 4-hydroxy-3,5-diiodobenzoic acid and its glycine conjugate. The excretion of the *O*-methylation product, 3,5-diiodoanisic acid, may be a further example of a metabolic reaction which is restricted in its occurrence to man and sub-human primates.

n-BUTYL 4-HYDROXY-3,5-DIIODOBENZOATE has antithyroid properties[1] and it has been examined clinically in patients with thyrotoxicosis.[2] In a metabolic study of the drug in one normal volunteer and in patients with thyroid disorder, the expected hydrolysis product of the ester, 4-hydroxy-3,5-diiodobenzoic acid, was isolated from the urine. In addition to this, Maclagan and Wilkinson[3] isolated a second metabolite which was identified as 3,5-diiodoanisic acid (3,5-diiodo-4-methoxybenzoic acid) which in some cases accounted for about a half of the iodine excreted. This second metabolite appears to be a methylation product of 4-hydroxy-3,5-diiodobenzoic acid and was, in fact, the first reported instance of the methylation *in vivo* of a phenolic hydroxyl group. *O*-Methylation has, subsequently, been found to be an important metabolic reaction of many phenols, especially certain di- and tri-hydric phenols.

Maclagan and Wilkinson[3] also reported that only 4-hydroxy-3,5-diiodobenzoic acid could be isolated from the urine of rats and rabbits dosed with the butyl ester and the methylated acid appeared to be absent. This suggested a species difference in the metabolism of the drug. We have now examined the fate of the drug in man, four subhuman primates, that is the rhesus, squirrel, capuchin and cynomolgus monkeys, and two non-primate species namely the rabbit and rat. 3,5-Diiodoanisic acid was found in the urine of man and the monkeys but not in the urine of the rabbit and rat.

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MATERIALS AND METHODS

Chemicals. *n*-Butyl 4-hydroxy-3,5-diiodobenzoate, m.p. 86–88°, was synthesized according to Maclagan and Wilkinson,[3] 4-hydroxy-3,5-diiodobenzoic acid, m.p. 277–279°, according to Blatt[4] and 3,5-diiodo-4-methoxybenzoic acid, m.p. 261–262°, according to Wheeler and Liddle.[5]

4-Hydroxy-3,5-diiodohippuric acid. A solution of 4-hydroxy-3,5-diiodobenzoic acid (1 g) in dry dioxan (50 ml) was treated with tri-*n*-butylamine (1·04 g or 1·4 ml) followed by ethyl chloroformate (0·61 g or 0·5 ml). The mixture was kept at 10° for 1 hr and then a solution of glycine (0·42 g) in N-NaOH (5·6 ml) was added. The mixture was kept at room temperature for 18 hr and then evaporated to small bulk in a rotary evaporator. The syrupy residue was dissolved in water (20 ml) and the solution was brought to pH 9·5 with N-NaOH and extracted with ether (2 × 30 ml) to remove tributylamine. The aqueous residue was brought to pH 3 with 2 N-HCl and extracted with ether (2 × 30 ml). The ether extracts were evaporated to dryness and the residue was crystallized first from aqueous ethanol and then from benzene-hexane (yield 0·48 g, 41 per cent). 4-Hydroxy-3,5-diiodohippuric acid formed white crystals, m.p. 218–221°. (Found: C, 24·4; H, 1·5; N, 3·0 per cent. $C_9H_7I_2NO_4$ requires C, 24·2; H, 1·6; N, 3·1 per cent). In aqueous ethanol, the compound gave a red colour with diazotized *p*-nitroaniline indicating a phenolic group and an orange colour when heated with 1% *p*-dimethylaminobenzaldehyde in acetic anhydride, indicating a hippuric acid residue.

3,5-Diiodo-4-methoxyhippuric acid (*3,5-diiodoanisuric acid*). This compound was prepared from 3,5-diiodoanisic acid and glycine following the method of Hofmann[6] for glycochenodeoxycholate. 3,5-Diiodoanisuric acid formed white needles, m.p. 156–157°, from aqueous ethanol (Found: C, 26·3; H, 2·2; N, 3·1 per cent. $C_{10}H_9I_2NO_4$ requires C, 26·05; H, 2·0; N, 3·0 per cent). It gave an orange colour when heated with 1% *p*-dimethylaminobenzaldehyde in acetic anhydride.

The synthesis of *n*-butyl 4-hydroxy-3,5-di[^{125}I]iodobenzoate was carried out exactly as described by Wilkinson[7] for the preparation of the ^{131}I-labelled compound. Sodium [^{125}I]iodide containing 500 μCi and 0·97 g (0·005 mole) of *n*-butyl 4-hydroxybenzoate were used. The yield of radioactive compound was 1·65 g, 73 per cent (sp. act. 230 μCi/g). After recrystallization from aqueous ethanol, the radioactive compound had m.p. 86–88° (Sheahan *et al.*[1] give m.p. 90° for the non-radioactive compound). When chromatographed on thin-layer Silica gel, the compound gave a single radioactive peak (Table 1).

Animals. The animals used in this work were obtained from dealers in the London area. They were maintained on appropriate diets. During experiments each animal was kept in a separate and suitable metabolism cage which allowed the separate collection of urine and faeces. The mode of administration of the compound in the various species is described in Table 2. To reduce contamination of the urine by food, the animals were not allowed access to food for the first day after dosing.

Determination of ^{125}I. Urine (0·1–0·5 ml) was added to 20 ml of a dioxan-based scintillation fluid[8] and the ^{125}I counted in a Packard tri-carb liquid scintillation spectrometer (model 3214). Efficiency (52 per cent) was determined for all samples by the internal counting technique and values were corrected for the decay of ^{125}I. Faeces were homogenized in 10 vol. of water and portions (0·5 ml) of the homogenate counted as above.

TABLE 1. THIN-LAYER CHROMATOGRAPHY AND COLOUR REACTIONS OF 4-HYDROXY-3,5-DIIODOBENZOIC ACID AND ITS DERIVATIVES

Compound	Solvent	R_f A	R_f B
Butyl 4-hydroxy-3,5-diiodobenzoate		0·73	0·73
4-Hydroxy-3,5-diiodobenzoic acid		0·48	0·30
4-Hydroxy-3,5-diiodohippuric acid		0·04	0·30
3,5-Diiodo-4-methoxybenzoic acid (3,5-diiodoanisic acid)		0·64	0·61
3,5-Diiodo-4-methoxyhippuric acid (3,5-diiodoanisuric acid)		0·11	0·46

Compounds were chromatographed on Silica gel HF (Merck & Co., Darmstadt, Germany) 0·25 mm on glass 20 × 5 cm. Solvents: A, Hexane–dioxan–acetic acid (10:1:2 v/v); B, butan-1-ol–propan-1-ol–NH_3 solution (sp. gr. 0·88) (4:1:1 v/v). The chromatograms were run for approx. 30 min in A and 2 hr in B. These compounds appeared as dark spots under u.v. light (254 nm; Hanovia chromatolite lamp). The glycine conjugates showed up as bright orange spots after spraying with 1% (w/v) *p*-dimethylaminobenzaldehyde in acetic anhydride and heating at 100° for 5 min. The phenolic compounds gave a red colour on spraying with diazotized *p*-nitroaniline.

Chromatography. R_f-values and detection methods for the compounds used are shown in Table 1. For chromatography the urine (2–200 ml) was adjusted to pH 2 with 15% v/v HCl and then extracted with ethyl acetate (10 vol.). The extract was assayed for ^{125}I to check on the efficiency (85–100 per cent of the radioactivity present) of extraction. It was then taken to dryness at reduced pressure in a rotary evaporator. The residue dissolved in ethanol (0·1–0·5 ml) was chromatographed on thin-layer plates prepared with Silica gel HF, 0·25 mm thick on a glass support and the chromatograms developed with solvents A or B (Table 1.) The plates were then allowed to dry and the position of reference compounds was determined using the appropriate detection method. The plates were scanned for radioactivity using a Packard radiochromatogram scanner and then carefully divided into 0·5 cm sections from which the Silica was removed and transferred to glass vials each containing ethanol (0·5 ml). Scintillation fluid (20 ml) was added and the ^{125}I counted.

Gas–liquid chromatography–mass spectrometry. An LKB 9000 instrument was used. An ethanolic solution (0·1 ml) of 4-hydroxy-3,5-diiodobenzoic acid or 3,5-diiodoanisic acid (1 mg/ml) was treated with *N,O*-bis(trimethylsilyl)-acetamide (Regisil, Regis Chemical Co., Chicago) at room temperature for 24 hr. The product was injected into the column which consisted of 1% W 98 on Diatoport S (100–200 mesh) operated at 140° with helium carrier gas. The retention times of the trimethylsilyl derivatives of 4-hydroxy-3,5-diiodobenzoic acid and 3,5-diiodoanisic acid were 11·8 and 5·5 min, respectively. Mass spectral scans of the compounds were taken as they emerged from the column. For urine, the alcoholic solution of the extract obtained by ethyl acetate extraction (see above) was treated with Regisil as before and then submitted to gas–liquid chromatography–mass spectrometry.

Reverse isotope dilution for 3,5-*diiodoanisic acid.* 3,5-Diiodoanisic acid (0·5–1 g) was added to a sample of urine containing 0·1–1 μCi and dissolved by adding an appropriate amount of 5% w/v NaOH. The solution was then adjusted to pH 2 with 10 M-HCl and extracted twice with 5 vol. of ether. The extract was evaporated to dryness and the residue recrystallized from aqueous ethanol to constant specific activity.

TABLE 2. EXCRETION OF RADIOACTIVITY FOLLOWING THE ADMINISTRATION OF BUTYL 4-HYDROXY-3,5-DI[^{125}I]IODOBENZOATE TO VARIOUS SPECIES

Species (no. and sex)	Dose		Route of administration	Dose excreted (%)				
				Urine			Faeces	
	mg/kg	μCi		1	2	3 (days)	3 (days)	Total
Man (2M)	2·8	6	p.o.	51, 51	74, 74	84, 80	2·5—	87, 80
Rhesus monkey (2F)	5·0	11	p.o.	69, 59	80, 72	86, 77	0·4, 3·5	86, 81
Cynomolgus monkey (1F)	10·0	5	i.m.	43	49	—	6	55
Squirrel monkey (2F)	10·0	5	i.m.	20, 20	31, 31	—, 36	23, 25	54, 61
Capuchin monkey (1F)	5·0	5	i.m.	28	43	—	30	73
Rabbit (2F)	10·0	14	p.o.	53, 25	58, 91	62, 92	5, 3	67, 95
Rat (3F)	2·0	3	p.o.	61 (57–64)	78 (75–80)	80 (76–85)	17 (6–25)	97 (95–99)

Individual results are given, except in the case of the rat (three animals) when the mean is given with ranges in parentheses. Human subjects took the dose in gelatin capsules. For the rhesus monkey, the compound dissolved in ethanol was injected into a piece of banana which was then fed to the animal. The cynomolgus, squirrel and capuchin monkeys were given the compound (dissolved in 75% aqueous propan-1,2-diol) by intramuscular injection and the rat and rabbit (dissolved in propan-1,2-diol) by stomach tube. M = male; F = female; p.o. = oral; i.m. = intramuscular; — = not determined.

RESULTS

Excretion of ^{125}I. The excretion of ^{125}I after the administration of butyl 4-hydroxy-3,5-di[^{125}I]iodobenzoate in the seven species examined is shown in Table 2. The differing dose levels used were influenced by the decay of ^{125}I, the higher dose levels (10 mg/kg) being used in the latter part of the investigation in order to ensure sufficient radioactivity for measurement. The compound was given orally to man, rhesus monkey, rabbit and rat but by intramuscular injection to the other monkeys as a matter of convenience. In man, rhesus and cynomolgus monkeys and rabbit, the compound seemed to be well absorbed since the faecal excretion was low (< 5 per cent), but in the squirrel and capuchin monkeys and the rat there was an appreciable excretion (up to 25–30 per cent) of the ^{125}I in the faeces. In man about half the dose was excreted in the urine in 24 hr and just over 80 per cent in 3 days. Similar values were also found for the rhesus monkey, rabbit and rat. In the squirrel and capuchin monkeys excretion appeared to be slower and despite giving the compound intramuscularly, an appreciable amount was excreted in the faeces possibly through excretion in the bile.

In the rat there was also a relatively high faecal excretion (17 per cent). In this case the faecal excretion could be due to incomplete absorption or to excretion in the bile after absorption. Two rats were therefore bile duct-cannulated[9] and then injected intraperitoneally with *n*-butyl 4-hydroxy-3,5-di[^{125}I]iodobenzoate (50 mg/kg and 2 μCi in 0·3 ml propylene glycol). The bile was collected for 24 hr and its ^{125}I content determined. These two rats excreted 27 and 26 per cent of the dose of ^{125}I in the bile and 37 and 44 per cent in the urine in the 24 hr period.

Identification of urinary metabolites. Thin-layer chromatograms of urine extracts from animals receiving radioactive butyl 4-hydroxy-3,5-diiodobenzoate were scanned for radioactivity. The extracts from man, rhesus monkey, cynomolgus monkey and capuchin monkey showed three peaks in solvent A and two in B, from the squirrel monkey four peaks in A and three in B, and from the rabbit and rat two peaks in A and one in B. The probable identity of these peaks are shown in Table 3.

The first group of animals including man (Table 3) were excreting 4-hydroxy-3,5-diiodobenzoic acid and its glycine conjugate and 3,5-diiodoanisic acid. The rat and

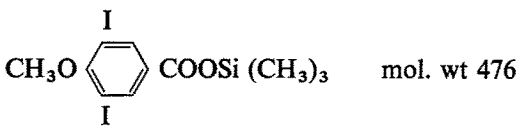
TABLE 3. THE R_F-VALUES OF RADIOACTIVE PEAKS FOUND IN URINE EXTRACTS FROM VARIOUS SPECIES

Urine extracts from	R_f-values of radioactive peaks in solvent A	R_f-values of radioactive peaks in solvent B	Identity
Man	0·05	0·30*	4-Hydroxy-3,5-diiodohippuric acid
Rhesus monkey	0·50	0·30*	4-Hydroxy-3,5-diiodobenzoic acid
Cynomolgus monkey	0·64	0·60	3,5-Diiodoanisic acid
Capuchin monkey			
Squirrel monkey	0·05	0·30*	4-Hydroxy-3,5-diiodohippuric acid
	0·12	0·45	3,5-Diiodoanisuric acid
	0·50	0·30*	4-Hydroxy-3,5-diiodobenzoic acid
	0·65	0·60	3,5-Diiodoanisic acid
Rat	0·05	0·30*	4-Hydroxy-3,5-diiodohippuric acid
Rabbit	0·50	0·30*	4-Hydroxy-3,5-diiodobenzoic acid

* In this solvent 4-hydroxy-3,5-diiodo-hippuric and -benzoic acids are not separated (see Table 1).

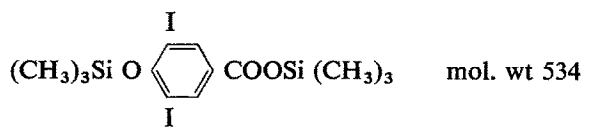
rabbit, however, were excreting only two compounds, namely 4-hydroxy-3,5-diiodobenzoic acid and its glycine conjugate and there was no evidence for the presence of the methylated acids.

The presence of 3,5-diiodoanisic acid in the urine of human subjects and the cynomolgus monkey was confirmed by gas–liquid chromatography–mass spectrometry. The retention times of the trimethylsilyl derivatives of authentic 4-hydroxy-3,5-diiodobenzoic acid and its 4-*O*-methyl derivative were found to be 5·5 and 11·8 min, respectively. Extracts from human and capuchin monkey urine similarly treated also gave peaks of retention times of 5·5 and 11·8 min. The peak of retention time 5·5 min gave a molecular ion at m/e 476 and prominent fragments at 461, 417, 387 and 329. Its mass spectrum was identical with that of the trimethylsilyl derivative of authentic 3,5-diiodoanisic acid below.



mol. wt 476

The peak of retention time 11·8 min gave a molecular ion at m/e 534 and prominent fragments at 520, 519, 477, 475, 461, 417, 416 and 386. The mass spectrum was identical with that of the trimethylsilyl derivative of authentic 4-hydroxy-3,5-diiodobenzoic acid below.



mol. wt 534

Quantitative aspects of the metabolites. The output in the urine of the metabolites of butyl 4-hydroxy-3,5-diiodobenzoate is shown in Table 4. The unchanged butyl ester was not detected in the urine of any of the animals examined. In the two human subjects, the ester was excreted mainly as 4-hydroxy-3, 5-diiodohippuric acid (45 and 60 per cent of the dose), with smaller amounts of free 4-hydroxy-3,5-diiodobenzoic acid (20 and 5 per cent) and of its *O*-methylated derivative, 3,5-diiodoanisic acid (21 and 10 per cent). Very similar results were obtained with the two rhesus monkeys (Table 4). With the cynomolgus monkey, the recovery of ^{125}I in the urine was much less (43 per cent of dose) than with the rhesus monkey, but in this case the main metabolite was 4-hydroxy-3, 5-diiodobenzoic acid (29 per cent) with small amounts of the methyl ether (4 per cent) and the glycine conjugate (2 per cent). Again with the capuchin monkey, the recovery of ^{125}I in the urine was lower (43 per cent) than with the rhesus monkey, but the main metabolite was now 4-hydroxy-3,5-diiodohippuric acid (18 per cent) with appreciable amounts of 3,5-diiodoanisic acid (16 per cent) and small amounts of 4-hydroxy-3,5-diiodobenzoic acid (5 per cent).

The two squirrel monkeys were the only animals tested which excreted 3,5-diiodoanisuric acid (13 and 19 per cent of the dose) as well as 3,5-diiodoanisic acid (6 and 4 per cent). They also excreted small amounts of 4-hydroxy-3,5-diiodobenzoic acid (6 and 3 per cent) and its glycine conjugate (3 and 5 per cent). In the squirrel monkey, the urinary metabolites were therefore largely methylated. Both the squirrel and capuchin monkeys, which are New World monkeys, excreted appreciable amounts

TABLE 4. URINARY METABOLITES OF BUTYL 4-HYDROXY-3,5-DI[^{125}I]IODOBENZOATE IN VARIOUS SPECIES

Metabolite sought	Dose found in the urine (%)						
	Man	Rhesus monkey	Cynomolgus monkey*	Squirrel monkey	Capuchin monkey*	Rabbit	Rat*
Butyl 4-hydroxy-3,5-diiodobenzoate	0, 0	0, 0	0	0, 0	0	0, 0	0, 0
4-Hydroxy-3,5-diiodobenzoic acid	20, 5·4	38, 17	29	6·2, 2·8	4·9	33, 22	63 (55–70)
4-Hydroxy-3,5-diiodohippuric acid	45, 60	38, 50	2	3, 5	18	14, 63	6·5 (3·1–7·0)
3,5-Diiodoanisic acid	21, 9·8	11, 10	4·4	6·1, 3·6	16	<1, <1	<1
3,5-Diiodoanisuric acid	0, 0	0, 0	0	13, 19	0	0, 0	0
Sum of above metabolites	86, 75	87, 77	35	28, 30	39	47, 85	70 (69–72)
Radioactivity in the urine	83, 81	86, 81	43	31, 35	43	62, 93	80 (70–85)

* The urine of the cynomolgus monkey, capuchin monkey and rat appeared to contain an additional radioactive metabolite of unknown nature amounting to 8, 4 and 2 per cent of the dose, respectively.

Dose and route of administration as in Table 2. Individual results are given except in the case of the rat (three animals) where mean values are given with ranges in parentheses. 0 = Not detected. The values given were obtained by quantitative radiochromatogram scanning. 3,5-Diiodo-4-methoxybenzoic acid was determined by reverse isotope dilution.

(20–30 per cent of dose) of the administered ^{125}I in the faeces (Table 2) but the nature of this radioactivity was not examined.

The rat and rabbit excreted in the urine only 4-hydroxy-3,5-diiodobenzoic acid and its glycine conjugate, the rat excreting mainly the free acid (63 per cent) and a little of the conjugate (6 per cent) and the two rabbits appreciable amounts of the free acid (33 and 22 per cent) and the conjugate (14 and 63 per cent). Neither of these species excreted detectable amounts of the methylated acids.

DISCUSSION

The elimination of radioactivity from a small dose (2–10 mg/kg) of *n*-butyl 4-hydroxy-3,5-di[^{125}I]iodobenzoate is relatively slow. In man, the rhesus monkey, the rabbit and rat about half the oral dose of ^{125}I is eliminated in the urine in one day, but appreciable amounts are still being excreted on the second and third day after dosing (Table 2). This relatively slow elimination could be due to one or more causes including slow absorption, slow metabolism, tissue binding and enterohepatic circulation. The drug was administered intramuscularly to the cynomolgus, squirrel and capuchin monkeys and again the excretion was relatively slow and in the case of the squirrel and capuchin monkeys, there was an appreciable faecal excretion of ^{125}I (Table 2). This would suggest that the drug or its metabolites was being excreted in the bile in these monkeys and therefore could be undergoing enterohepatic circulation. This is supported by the finding that, in bile duct-cannulated rats some 27 per cent of the ^{125}I of an injected dose of the drug was excreted in the bile in 24 hr.

TABLE 5. SPECIES VARIATIONS IN THE METABOLISM OF 4-HYDROXY-3,5-DIIODOBENZOIC ACID

Species (no.)	^{125}I excreted in urine (dose %)	^{125}I excreted in urine (%)	
		Glycine conjugation	*O*-methylation
Homo sapiens (2)	84, 80	54, 74	25, 13
Macaca mulatta (2)	86, 87	44, 62	13, 13
M. fascicularis (*irus*) (1)	49	5	10
Saimiri sciureus (2)	31, 31	52*, 69*	61†, 65†
Cebus albifrons (1)	43	42	37
Lepus cuniculus (2)	58, 91	23, 68	0, 0
Rattus norvegicus (3)	80 (70–85)	11 (10–13)	0

* Sum of two glycine conjugates (see Table 4).
† Sum of two *O*-methyl compounds (see Table 4).

The drug is apparently metabolized initially by ester hydrolysis to 4-hydroxy-3,5-diiodobenzoic acid which is then conjugated with glycine or methylated. The conjugation with glycine occurs in all the species examined, although to a lesser extent in the cynomolgus monkey and the rat than in the other species (Table 5), but only in one species, the squirrel monkey (*Saimiri sciureus*), does glycine conjugation and methylation occur in the same molecule to produce 3,5-diiodo-4-methoxyhippuric acid (3,5-diiodoanisuric acid; see Table 4). The significant species difference found was in the excretion of 3,5-diiodoanisic acid. This was found in man and the four subhuman primates but not in the two non-primate species, the rat and the rabbit (Table 5). These results confirm the earlier work of Maclagan and Wilkinson[3] who found

diiodoanisic acid in human urine but not in rat or rabbit urine following the administration of the drug. However, it has been shown by Tomita *et al.*[10] that, rat and rabbit liver and kidney preparations *in vitro* can methylate 4-hydroxy-3,5-diiodobenzoic acid. The enzyme responsible, iodophenol *O*-methyltransferase, utilizes *S*-adenosylmethionine as a source of methyl groups but is apparently a different enzyme from catechol *O*-methyltransferase. It is possible that, in the whole rat and rabbit, iodophenol *O*-methyltransferase is inactive or that 4-hydroxy-3,5-diiodobenzoic acid does not penetrate to the sites where the enzyme occurs. Another possible explanation of the difference between the *in vivo* and *in vitro* findings in these two species is that methylation does occur but before the methylated acid is excreted it is demethylated. The species differences (Table 5) in the excretion of 3,5-diiodoanisic acid following the administration of butyl 4-hydroxy-3,5-diiodobenzoate could be due to the extent to which methylation and subsequent demethylation occur in various species.

It would appear that in the excretion of 3,5-diiodoanisic acid in the urine, the four species of monkeys representing both Old and New World monkeys are like man. This is another instance of a metabolic reaction occurring in man and also in subhuman primates, but not in non-primate species. Other instances observed in this Laboratory of similarities between man and monkeys in the metabolism of foreign compounds are the aromatization of quinic acid,[11] the glutamine conjugation of phenylacetic acid[12] and N^1-glucuronide formation with sulphadimethoxine.[13]

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