Metabolism and Caloric Utilization of Orally Administered Maltitol-^{14}C in Rat, Dog, and Man

Hans H. Rennhard* and Joseph R. Bianchine[1]

Investigation of the metabolism of orally administered maltitol (4-*O*-α-D-glucopyranosyl-D-sorbitol), by a radiotracer study in man, rat, and dog, indicates that it is readily metabolized. Caloric utilization of approximately 90% was established in two species (rat and man). The value is based on the recovery of $^{14}CO_2$ from expired breath, and corroborated by matching plasma and correspondingly low feces and urine levels. The radiorespirometric patterns suggest that a portion of the maltitol is utilized rapidly following ingestion, probably via hydrolysis in the stomach and absorption of the hydrolysis products, and via absorption of intact maltitol from the small intestine and subsequent splitting by tissue enzymes, while the principal utilization pathway involves fermentation by the large intestinal microflora to volatile fatty acids, which are subsequently absorbed and metabolized. Radiolabeled volatile fatty acids were shown to be present in both rat and human fecal samples.

Because of their sweet taste and desirable physical properties polyhydric alcohols have been investigated and, in certain instances, actually used as replacements for sucrose in special dietary foods, particularly foods for the diabetic and those with limited cariogenic potential. Investigations of the mammalian metabolism of sorbitol (Adcock and Gray, 1957) and xylitol (Asano et al., 1973) show that these polyols are efficiently utilized by man so their caloric value approaches that of digestible carbohydrates. Mannitol, due to its dose related absorption rate, is utilized to varying degrees depending upon the amount ingested (Nasrallah and Iber, 1969). Considerable recent interest has developed in another polyol, maltitol (4-α-D-glucopyranosyl-D-sorbitol), because of its aftertaste-free sweet taste approaching that of sugar and ready availability by hydrogenation of maltose. Earlier studies on the metabolism of maltitol in the mouse (Kamoi et al., 1972) and the rat (Oku et al., 1971; Hosoya, 1975) suggest a low level of caloric utilization. However, maltitol has also been reported to produce hyperglycemia in the mouse similar to that caused by an equal amount of sucrose or glucose but at slower rate (Lederer et al., 1974). In this paper we report detailed metabolism investigations in animals and man using maltitol-*U*-^{14}C.

METHODS

Preparation of Radiolabeled Maltitol. Maltose-*U*-^{14}C (Amersham/Searle Corp., Arlington Heights, Ill.) was reduced with sodium borohydride according to the procedure reported for the synthesis of D-sorbitol-*1*-^{14}C (Karr et al., 1970). Recovery of label was quantitative. The identity of the maltitol was confirmed by GLC analysis of its *O*-trimethylsilyl derivative (Laine et al., 1972). The maltitol-*U*-^{14}C showed the same retention time relative to sucrose, used as internal standard, as commercial maltitol (Hayashibara Co., Ltd., Okayama, Japan). The absence of residual reducing sugars was demonstrated by a ferricyanide ultramicro method (Park and Johnson, 1949), and a sorbitol level of <0.25% was shown by GLC assay (Reid et al., 1970).

Radiometric Methods. All radioactive samples were measured in a Nuclear-Chicago Mark I (refrigerated Models 6860 or 6873) liquid scintillation spectrometer. Quenching corrections were made by the internal standard procedure employing toluene-^{14}C. The fluor of the scintillator solutions was composed of 0.3% 2,5-diphenyloxazole (PPO) and 0.1% *p*-bis(*O*-methylstyryl)-benzene (bis-MSB), dissolved in either toluene (Toluene scintillator), a mixture of 37% Triton X-100 and 63% toluene (Triton X-100 scintillator), or a mixture of 30% ethanol and 70% toluene ("30/70" scintillator).

Assay Methods for Breath $^{14}CO_2$, Urine, Plasma, and Feces. Expired carbon dioxide was trapped either with 10% (w/v) sodium hydroxide, using a 0.2-ml aliquot in 15 ml of 30/70 scintillator solution, or with 1 M Hyamine hydroxide (Packard Instrument Co., Downers Grove, Ill.), using a 0.5 to 1 ml aliquot in 15 ml of toluene scintillator solution.

Urine was centrifuged if necessary, using 0.5 to 2 ml aliquots in 15 ml of Triton X-100 scintillator solution. Plasma (or serum) was assayed by digesting 0.5 ml in 1.25 ml of NCS Solubilizer (Nuclear-Chicago), followed by addition of 15 ml of Triton X-100 scintillator solution.

Feces were assayed by either of two methods. *Procedure A (Extraction Method, Used for Rat Feces Only).* Feces were extracted three times with 60 ml of water by homogenizing in a Virtis homogenizer, followed by centrifugation (15–20 min at 15000 rpm). The aqueous extracts were combined and diluted to 200 ml. Aliquots (0.2 ml) were assayed in 15 ml of 30/70 scintillator solution. The remaining extracts were lyophilized.

Procedure B (Combustion Method). Feces were homogenized in a Waring Blendor with sufficient water to yield a thin suspension, adjusted to pH 9 with sodium hydroxide to avoid loss of volatile fatty acids, and lyophilized. Triplicate 100–150-mg aliquots of the fluffy fecal solids were pressed into pellets for ease of handling, weighed, and burned in an atmosphere of oxygen in a Schöniger combustion flask containing 10 ml of a 1:2 mixture of ethanolamine–cellosolve at −70 °C (Kalberer and Rutschmann, 1961). Three aliquots of 0.1–3 ml of the absorption fluid were diluted with 15 ml of 30/70 scintillator solution. An established recovery of 90% was used in the calculations of the fecal activity.

All radioassays were performed at least in duplicate. All samples were counted twice 10 min or longer in order to accumulate a minimum of 10000 counts.

Gel permeation chromatography was performed on a 1.9 × 80 cm column packed with Sephadex G-15. The compounds were eluted with water at a flow rate of 15 to 18 ml/h. Usually 3-ml fractions were collected by drop counting (50 drops) with an automatic fraction collector.

Food Chemicals Research and Development Department, Central Research, Pfizer Inc., Groton, Connecticut 06340 (H.H.R.), and the Department of Clinical Pharmacology, Johns Hopkins University, Baltimore, Maryland 21218 (J.R.B.).

[1]Present address: Department of Pharmacology, The Ohio State University, Columbus, Ohio 43210.

The radioactivity of the fractions was monitored using Triton X-100 scintillator solution.

METABOLISM EXPERIMENTS

Studies in Rats. Five albino Charles River rats of both sexes weighing 95–200 g were dosed with 1 ml of the radioactive maltitol solution by intubation (125–260 mg/kg, 49.7 μCi). After dosing, one of the animals was immediately placed in an Aerospace Industries Rat Restrainer and Breath Collecting Chamber (Model CR-150); the other four were placed in individual metabolism cages designed to separate urine and feces. The entire $^{14}CO_2$ collection train consisted of: (1) Drierite and Ascarite towers to remove atmospheric moisture and carbon dioxide; (2) flowmeter; (3) rat breath collection chamber; (4) two parallel gas wash bottles to collect expired CO_2, containing 200 ml of 10% (w/v) aqueous sodium hydroxide; the air stream was diverted to a parallel bottle containing fresh sodium hydroxide solution at the end of each hour; (5) a back up gas wash bottle filled with 100 ml of 10% NaOH to check for complete absorption; (6) three-necked safety bottle with air bleed needle valve to adjust the air flow; (7) pump to pull air through the system at a constant rate.

The breath collection chamber permitted the separate collection of urine and feces during the course of the experiment.

Exhaled carbon dioxide was collected over a period of 14 h from rat 1. Urine and feces were collected in all cases, either over a period of 48 h (rats 4 and 5) or 72 h (rats 1, 2, and 3). Feces of rats 3–5 were assayed by the extraction method (procedure A). Feces of rats 1 and 2 were analyzed by the combustion method (procedure B).

To investigate the nature of the radioactive components in the feces an 0.8-g aliquot of the lyophilized feces extract from rat 3 (0–24 h) with an activity of 1.8 μCi was steam distilled. The 15 20-ml fractions collected were assayed for radioactivity. Lyophilized feces extract (280 mg) from rat 4 (0–24 h) with an activity of 1.9 μCi was subjected to Sephadex G-15 gel permeation chromatography. Fractions (100, 2.6 ml) were collected, and 0.1-ml aliquots used for radioactivity monitoring. Recovery of radioactivity was 1.88 μCi (100%). Fractions 42–62 were combined and lyophilized. An aliquot (93.8 mg) was silylated with Tri-Sil Z (*N*-trimethylsilylimidazole, Pierce Chemical Co., Rockford, Ill.) and analyzed by GLC, revealing the presence of a small amount of maltitol, verified by co-chromatography with commercial maltitol.

Studies in Dogs. Two male beagle dogs were each given a solution containing 51.2 μCi (45 mg) of maltitol-U-^{14}C and 3 g of carrier maltitol by stomach tube. Blood samples (5 ml) were collected at 1, 2, 3, 4, 5, 6, 7, 8, 24, and 32 h post dose. Plasma was obtained by centrifugation of the heparinized blood at 1500 rpm for 20 min. Two 0.5-ml aliquots were used for radioassay. Urine was collected at daily intervals for 2 days. Two 0.2-ml samples were used for radioassay. In order to determine the radioactive species in the plasma, the plasma sample with the highest radioactivity was chromatographed on Sephadex G-15 and 80 fractions were collected.

Studies in Man. Four healthy men (P.D., F.G., F.L., M.V.), 21 to 32 years of age, who were fully informed of the nature and possible risks of the study, were given a daily dose of 10 g of maltitol for a period of 7 days. The volunteers were dosed 30 min after having a standard breakfast with 10 g of maltitol containing 79.95 μCi of maltitol-U-^{14}C in the form of a 20% solution. Expired breath, blood, urine, and feces were collected and prepared for radioassay as follows.

(a) *Expired Breath.* Ten liters of expired breath was collected at 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, and 24 h in a 30-l. rubber balloon following passage through a dry ice trap. The collection was timed with a stop watch. (The time aliquot was used to calculate the total radioactive carbon dioxide exhalation for the preceding time interval.) The contents of the balloons were passed slowly through 15 ml of a solution of 1 M Hyamine hydroxide in methanol. The resulting solutions were tightly capped and stored in a freezer until assayed. For radioassay 0.5 ml of the 0–10-h samples and 1.0 ml of the 12–24-h samples were diluted with 15 ml of toluene scintillator solution.

(b) Blood specimens were obtained by venipuncture at 1, 2, 4, 8, 12, 16, and 24 h and then daily through day 7. After clotting, the serum was separated by centrifugation, frozen immediately, and stored in a freezer until radioassayed. Three serum samples with a high radioactivity count (M.V., 1 and 2 h; P.D., 2 h) were combined and centrifuged 20 min at 20 000 rpm. The clear supernatant (13 ml, 12.9 nCi) was applied to a Sephadex G-15 gel chromatography column.

(c) Urine was collected for 0–6, 6–12, and 12–24 h and then daily through day 7. All urine samples were kept frozen until analyzed. At that time, volumes and pH values were determined. Lyophilized urine solids (1.0 g) (M.V., 0–6 h) were dissolved in 9 ml of water and centrifuged, and the clear supernatant chromatographed on Sephadex G-15. For positive identification of the suspected maltitol peak, the chromatography was repeated with a urine sample fortified with radioactive maltitol.

(d) Feces were collected daily for 7 days post-dose in polyethylene bags and frozen immediately. The frozen specimens were weighed and processed by procedure B. To investigate the possible volatile radioactive components in the feces 10 g of lyophilized feces (F.G., day 2) was suspended in 80 ml of water and acidified with 8 ml of 6 N sulfuric acid. After addition of 34 g of anhydrous magnesium sulfate, the flask was immersed in a preheated oil bath at 145 °C and steam distilled. Steam distillate (200 ml) was collected and assayed. The experiment was repeated with feces from M.V., day 2.

RESULTS

Studies with Rats. The recovery of radioactivity following oral administration of maltitol-U-^{14}C to rats is shown in Table I, and the radiorespirometric pattern is represented in Figure 1. The steam distillation of the fecal extract showed that approximately 13% of the total fecal radioactivity was due to volatile fatty acids. A small quantity of maltitol was shown to be present in the feces by the gel chromatography isolation–GLC identification experiment.

Studies in Dogs. Table II shows the radiolabel concentrations in plasma (as maltitol equivalent) and urine (as percent of dose administered) after oral administration of maltitol-U-^{14}C to dogs. The Sephadex G-15 gel permeation chromatogram indicated that the radioactive species was mostly bicarbonate. Additional amounts of radiolabel were found to be incorporated in material of considerably higher molecular weight (lipids and polypeptides?). Maltitol could not be identified with certainty.

Studies in Man. Table III summarizes the ranges and averages of the excretion and serum levels of radioactivity following oral administration of maltitol-U-^{14}C to four human volunteers, and Figure 2 shows the radiorespirometric pattern representing ranges and averages of $^{14}CO_2$ recoveries. The gel permeation chromatogram of the early (0–6 h) urine sample showed two peaks. The first one, representing 82% of the total urine radioactivity, was identified as maltitol, and the second one, representing 6%

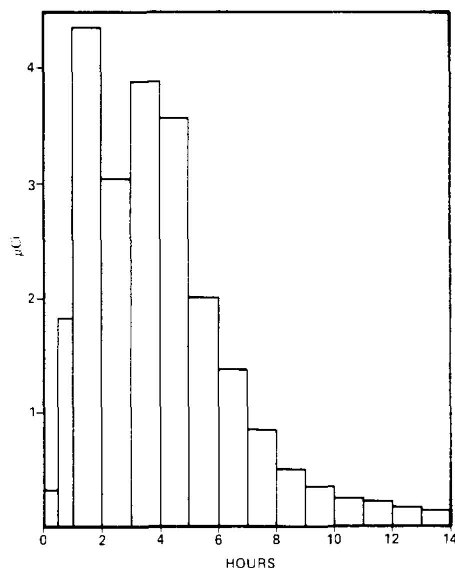

Figure 1. Radiorespirometric pattern of rat no. 1. Radioactivity recovered from expired breath following administration of single oral dose of maltitol-U-^{14}C (49.7 μCi).

Table I. Recovery of Radioactivity following Oral Administration of Maltitol-U-^{14}C to Rats

Rat no.	Sex	Wt, g	Interval, h	% of admin. radioact. recovd: $^{14}CO_2$	Urine	Feces[a]
1	M	100	0–14	45.5	8.65	9.76
			14–24		0.35	2.27
			24–48		0.26	0.93
			48–72		0.14	0.26
			0–72		9.40	13.22
2	M	200	0–24		3.59	8.23
			24–48		0.30	3.06
			48–72		0.14	0.26
			0–72		4.03	11.55
3	M	123	0–24		3.60	3.82
			24–48		0.26	0.48
			48–72		0.08	0.08
			0–72		3.94	4.38
4	F	148	0–24		4.13	4.11
			24–48		0.21	0.85
			0–48		4.34	4.96
5	F	163	0–24		3.66	3.08
			24–48		0.24	1.21
			0–48		3.90	4.29
			Caloric utilization:[b]	~76%		
			Total ^{14}C recovery:[c]	~98%		

[a] The presence of labeled insoluble metabolites of bile acids and pigments probably accounts, in part, for the higher radioactivity values found for whole feces (rats 1 and 2) vs. aqueous extract (rats 3–5). [b] Based on recovery of $^{14}CO_2$ of rat no. 1 and catabolic transformation factor of 0.6 for readily absorbed compounds such as acetate (Shreeve et al., 1959; Hellman et al., 1951) and glucose (Adcock and Gray, 1957; Domingues et al., 1959). [c] Caloric utilization + urine + feces of rat no. 1.

of the total urine radioactivity, was identified as urea. Gel chromatography of the serum sample indicated the presence of radioactive maltitol and bicarbonate, amounting to about 83% of the total serum radioactivity. The steam distillation of the two feces samples showed the presence of residual volatile fatty acids (3.5 and 7.6% of the total fecal radioactivity).

Table II. Radioactivity Levels of Plasma (Expressed as Maltitol Equivalent) and Urine (Expressed as Percent) after Oral Administration of Maltitol-U-^{14}C to Dogs

Time, h	Plasma levels, μg/ml, for dog no. (wt, kg): 30524 (12.4)	30592 (11.0)
1	192	341
2	353	382
3	304	263
4	223	194
5	160	135
6	94	104
7	70	83
8	58	69
24	43	54
32	31	40
Eq for regression line	$y = 1.204 - 0.143x$	$y = 1.146 - 0.140x$
Half-life, h	2.1	2.1

Time, h	Urine levels, %, for dog no. (wt, kg): 30524 (12.4)	30592 (11.0)
0–24	6.8	3.6
24–48	1.0	0.2
0–48	7.8	3.8

Table III. Recovery of Radioactivity after Oral Administration of Maltitol-U-^{14}C to Four Humans

	0–24 h	24–48 h	48–72 h	72–96 h	96–168 h	0–168 h
Urine[a]						
Range	2.3–2.5	0.6–0.8	0.1–0.3	0.1–0.2	0.1	
Av	2.4	0.7	0.2	0.1	0.1	3.6
Serum levels[b]						
Range[c]	36–142	29–50	26–42	25–43	20–32	
Av	66	38	35	31	25	
Feces[a]						
Range	0–2.9	0.7–5.3	0.1–4.0	0.1–3.6	0–0.3	
Av	0.7	2.1	1.1	1.0	0.1	4.9
Breath $^{14}CO_2$[a]						
Range	38–59					
Av	52.6					
Caloric utilization[d]						
Range 63–98%						
Av 88%						
Total recovery (caloric utilization + feces + urine)						
Range 72–100%						
Av 96%						

[a] In percent of dose administered. [b] As maltitol equivalent (micrograms per milliliter). [c] Lowest and highest value found at any time in any of the subjects. [d] See footnote b, Table I.

DISCUSSION

Figure 1 shows the radiorespirometric pattern of the rat after oral administration of a single dose of maltitol-U-^{14}C. The rapid (1–2 h) and appreciable appearance of $^{14}CO_2$ in the breath indicates that a portion of the maltitol is hydrolyzed in the stomach and the resulting components, glucose and sorbitol, are absorbed and catabolically utilized. Another portion of the maltitol, for which an intestinal splitting enzyme does not exist (Dahlqvist and Telenius, 1965), is apparently absorbed intact from the small intestine and then split by tissue enzymes in analogy

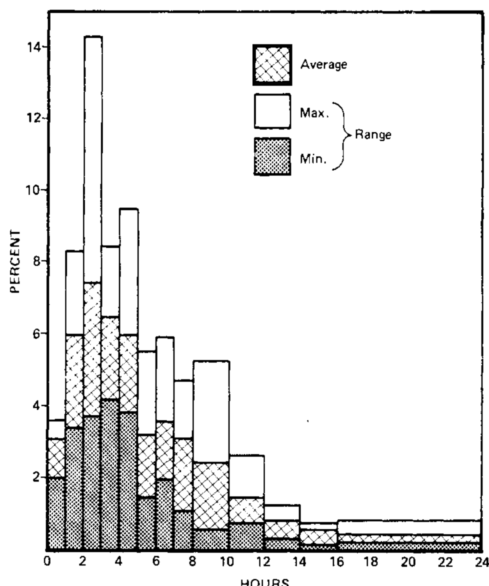

Figure 2. Radiorespirometric pattern (range and average) of four human volunteers. Radioactivity recovered from expired breath following administration of single oral dose of maltitol-U-^{14}C (79.95 μCi).

to intravenously administered maltose (Young and Weser, 1971, 1974; Yoshimura et al., 1973). The observation of urinary maltitol in the present study with human subjects corroborates this view.

The broad peak at 3 to 7 h in the radiorespirometric pattern indicates that a substantial amount of label is absorbed from the lower intestinal tract. Radiotracer studies with ^{14}C-labeled cellulose in rats (Johnson et al., 1960; Conrad et al., 1958) demonstrated that up to 50% of an ingested dose is metabolized to carbon dioxide with lesser amounts excreted in urine and retained in the body. The digestion of cellulose was greatly reduced after administration of oxytetracycline, showing that the gastrointestinal micropopulation, which is antibiotic sensitive, was very essential for cellulose metabolism. Cecectomized rats were virtually unable to digest cellulose, which indicated that the cecum was the site of cellulose degradation in the rat. Bacteria are the microorganisms responsible for the interconversion of cellulose and it appears that short-chain fatty acids are the end products. It is, therefore, reasonable to conclude that the $^{14}CO_2$ expired after about 2 h following the administration of maltitol results from its microbial fermentation in the cecum of the rat, followed by absorption and metabolism of the resulting fermentation products. Radioactive volatile fatty acids were, in addition to small amounts of maltitol, shown to be present in the fecal samples.

Application of an established catabolic transformation factor for glucose and acetate to the observed $^{14}CO_2$ recovery (see footnote b in Table I) indicates that rat no. 1 utilized maltitol to the extent of about 76%. However, considering the low fecal recoveries of rats no. 3 to 5 the value is probably closer to 90%. These results are in sharp contrast to those of the rat metabolism study reported by Hosoya (1975), who claims that only 1.2% of the orally administered radioactive maltitol was recovered as $^{14}CO_2$ over a period of 24 h. The total urinary excretion of the radioactivity (5%) in the Japanese experiment agrees with our results. No adequate explanation for the discrepancy in $^{14}CO_2$ recovery values can be given but it should be pointed out that in the Japanese study most of the radioactivity (63%) was found in the cecum and large intestine 24 h after administration of the radioactive dose. Unfortunately, the experiment was not continued for a longer period to determine the ultimate fate of material present in the gut. It has been our experience with a variety of food ingredients and drugs that passage through the rat GI system takes approximately 12 h for most of the material. This transit time is substantiated by our observed $^{14}CO_2$ recovery pattern which dropped to a very low value after 10 h (see Figure 1). Further $^{14}CO_2$ exhaled probably is more representative of general carbon pool turnover than of direct maltitol utilization. The urine and fecal recoveries (Table I) also support a transit time of less than 24 h for most of the material. The main purpose of the study in dogs (Table II) was the determination of plasma radioactivity levels over an extended period of time following oral administration of a single dose of maltitol-U-^{14}C, since this cannot be done with rats. The relatively high plasma levels attained in the 2 to 6 h interval serve as positive qualitative proof of maltitol metabolism. (Sephadex G-15 chromatography of the plasma revealed that the radioactive species is mainly bicarbonate.) Urine radioactivity levels were similar to those found in the rat.

The results from the human study with maltitol-U-^{14}C closely parallel the metabolism in the rat. An average of 17% of the total $^{14}CO_2$ recovered was exhaled within the first 2 h, a period when most of the maltitol-U-^{14}C should still be in the stomach (Van Liere and Northup, 1943), or partly in the upper intestine (Hunt and Stubbs, 1975), and 43% of the total $^{14}CO_2$ was exhaled within 4 h (Figure 2). This suggests that, as in the case of the rat, an appreciable utilization pathway for maltitol must be by hydrolysis to glucose and sorbitol in the stomach and subsequent absorption of these components, as well as by absorption of intact maltitol from the small intestine and hydrolysis by tissue enzymes. Nevertheless, the recovery of 49% of the total $^{14}CO_2$ in the 4–16 h interval, and especially the second peak at 7 h, as well as the presence of volatile fatty acids in the fecal samples indicate that the large intestinal microbial fermentation pathway is also operative. The total recovery of $^{14}CO_2$ suggests a caloric utilization of maltitol in man of approximately 90%. This value is substantiated by the low (5%) radioactivity levels found in the feces and by the presence of appreciable quantities of radioactive metabolites in the blood and urine 7 days after administration.

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Metabolism of Bromobenzenes in the Rabbit

L. O. Ruzo, S. Safe,* and O. Hutzinger

A series of brominated (Br_1–Br_5) benzenes was administered to rabbits by intraperitoneal injection and mass spectrometric analysis revealed the presence of oxygenated bromobenzene metabolites in the urine and feces. The metabolites were isolated and their structures determined. At least two major metabolites were identified from each bromobenzene (Br_1–Br_3) fed. Tetrabromo- and pentabromobenzene yielded only trace amounts of metabolite in the urine and none was detected in the feces. The compounds obtained from 1,4-dibromo- and 1,3,5-tribromobenzene showed migration of bromine atoms, suggesting the possibility of arene oxide intermediates.

Halogenated aromatic compounds are among the most persistent and ubiquitous environmental pollutants. The metabolic degradations of DDT (Feil et al., 1973, 1975), DDD (Reif et al., 1974), DDE (Sundstrom et al., 1975), 2,4-D (Feung et al., 1975), and polychlorinated biphenyls (Safe et al., 1975) have recently been reported and in all cases hydroxylated metabolites have been identified. Mechanistic studies suggest that many halogenated aromatics are metabolized via arene oxide intermediates which can rearrange into the phenolic products. The rearrangement is often accompanied by a 1,2-H (or ^2H) or substituent (e.g., Cl, Br, CH_3) shift from the site of hydroxylation to the adjacent carbon atom and is referred to as the NIH shift (Daly et al., 1972).

Brominated benzenes are industrial compounds produced in North America by several companies (Great Lakes Chemical Co., Dow Chemical, and Mallinckrodt Chemical Works) and are used as fuel additives and top cylinder compounds. In addition to the potential environmental hazard resulting from their entrance into the ecosystem they are interesting as analogues of other compounds already identified as pollutants such as the brominated biphenyls (Ruzo and Zabik, 1975). The metabolites of bromobenzene have previously been reported and dihydrodiols (Azouz et al., 1953), mercapturic acids (Knight and Young, 1958), and bromophenols (Tomaszewski et al., 1975) have been identified. This paper describes the isolation and structure of several brominated benzene metabolites in rabbit urine.

MATERIALS AND METHODS

Bromobenzenes. Bromobenzene, 1,2-, 1,3-, and 1,4-dibromobenzene, 1,2,4- and 1,3,5-tribromobenzene, and 1,2,4,5-tetrabromobenzene were obtained from ICN-K&K Pharmaceuticals, Plainview, N.Y. Pentabromobenzene was kindly supplied by Dr. Sundstrom, University of Amsterdam, Netherlands.

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Bromophenols. 2-, 3-, and 4-bromophenol, 2,4- and 2,6-dibromophenol, 2,4,6-tribromophenol, and pentabromophenol were obtained from ICN as well.

Preparation of Bromophenol Standards. Nitration of 1,2-dibromobenzene, followed by reduction with Zn–acetic acid, yielded a mixture of two dibromoanilines. Diazotization of the mixture followed by treatment with 50% copper(II) sulfate (aqueous) (Goto et al., 1974) gave two dibromophenols. The mixture was separated by thin-layer chromatography (1:3, benzene–petroleum ether). The mass spectra of the two major products (R_f 0.16 and 0.34) exhibited molecular ions at m/e 250. A summary of their nuclear magnetic resonance spectra is given in Table I and the structures are assigned as 3,4-dibromophenol (R_f 0.16) and 2,3-dibromophenol (R_f 0.34).

Nitration, reduction, and diazotization of 1,2,4-tribromobenzene followed by treatment with aqueous copper(II) sulfate gave a single major product isolated by TLC. Its mass spectrum showed a molecular ion at m/e 328 and the spectroscopic data were consistent with the structure of 2,4,5-tribromophenol (Table I). Diazotization of 2,5-dibromoaniline, after treatment with aqueous copper(II) sulfate, yielded 2,5-dibromophenol (m/e 250).

Administration of Substrates. The bromobenzene (600 mg) was dissolved in corn oil and equally administered to two albino rabbits (~50 mg/kg) by intraperitoneal injection. Urine and feces were collected for 10 days after administration of the substrate and stored at −25°C prior to use.

Extraction and Analysis. The urine samples were acidified to pH 5 with acetic acid, cooled, and extracted with an equal volume of ether. The aqueous layer was then diluted with sufficient concentrated sulfuric acid to make a 6 *N* acid solution and then heated for 2 hr at 100°C. The solution was then diluted with an equal volume of distilled water and extracted with ether. Both ether extracts were kept separate and were dried over anhydrous sodium sulfate, concentrated, and purified by preparative thin-layer chromatography (TLC) on silica gel HF_{254} (Merck) using benzene as the eluting solvent. Bands with R_f values similar to corresponding bromophenol standards were removed from the plates and extracted with ether to give