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The Reduction of *N*-Hydroxy-4-acetylaminobiphenyl by the Intestinal Microflora of the Rat[1]

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SUMMARY

The role of the intestinal flora in the conversion of *N*-hydroxy-4-acetyl-aminobiphenyl (N-OH-AABP) to 4-acetylaminobiphenyl has been examined. This reaction, which reverses the metabolic activation of the parent carcinogen, can be demonstrated in cultures of some bacteria indigenous to the intestinal microflora. These include cultures of *Clostridium* sp., *Clostridium perfringens*, *Peptostreptococcus productus* I, and *Bacteroides fragilis* ss. *thetaiotaomicron* and ss. *vulgatus*. In contrast, cultures of *Lactobacillus plantarum* and *Escherichia coli* show little or no capacity for this reaction. The reduction of N-OH-AABP is also carried out by homogenates of liver, kidney, and brain. On a weight basis, the cecal flora is considerably more active in reducing N-OH-AABP than are homogenates of tissues of the gastrointestinal tract. The cecal flora also has a greater activity for reducing N-OH-AABP than the stomach flora, an observation which may relate to the induction of tumors in the forestomach but not in the cecum of rats fed this compound.

The products of the metabolism of N-OH-AABP have been compared in germ-free and conventional animals. Glucuronide conjugates of N-OH-AABP are found in the cecal contents and feces only of the germ-free rats, while 4-acetylaminobiphenyl is found in the feces only of conventional rats. These results suggest that the flora, by hydrolyzing glucuronides and reducing N-OH-AABP, may influence the level of metabolites of 4-acetylaminobiphenyl which are critical for carcinogenesis.

INTRODUCTION

N-Hydroxylation is the obligate initial step in the sequence of reactions required to form the electrophilic metabolites which are the ultimate carcinogens of various aromatic amines and amides (11, 13). However, the demonstration of the *N*-dehydroxylation of N-OH-FAA[2] (6, 10, 27) in preparations from various organs of the rat indicates that a metabolic pathway exists for the elimination of activated intermediates required for carcinogenesis by aromatic amines. Organs capable of this reaction might thus be protected from tumor formation if they have sufficient capacity to reverse the metabolic activation of amide carcinogens at a step prior to the formation of the ultimate carcinogen.

Williams *et al.* (27) have called attention to the capacity of the intestinal microflora to carry out the *N*-dehydroxylation of N-OH-FAA and have specifically demonstrated this reaction in a strain of *E. coli* (27). In these studies it was also noted that there were changes in the flora after prolonged feeding of N-OH-FAA (27), an observation which may explain the increased excretion of N-OH-FAA in rats under these circumstances (12, 15, 23). An analogous alteration in the excretion of N-OH-AABP has been noted after prolonged feeding of AABP and N-OH-AABP (15).

Although previous experimental results suggest a role for the intestinal microflora in the metabolism of N-OH-FAA, the bacteriology in these early studies was confined to the aerobic flora (27). Since it is known that the anaerobic flora outnumber the aerobic flora by approximately a thousand to one (16), a more complete study of the flora in the metabolism of *N*-hydroxylated amides has been undertaken, using N-OH-AABP as the model compound. In these studies, particular attention has been given to the utilization of bacteriological methods that assure the survival of the predominant anaerobic flora of the gastrointestinal tract by satisfying their physiological requirements. In addition to reporting the metabolism of N-OH-AABP by isolated constituents of the flora, the relative importance of the flora in the metabolism of N-OH-AABP in the rat is assessed by comparing the metabolism of this compound in germ-free and conventional rats.

MATERIALS AND METHODS

Materials. 4-Nitrobiphenyl (Aldrich Chemical Co., Cedar Knolls, N. J., m.p. 113-114°) was recrystallized from hot ethanol; crude N-OH-ABP (m.p. 150-151°) was synthesized by the reduction of 4-nitrobiphenyl with aluminum-amalgam, according to the method of Bell *et al.* (2), and acetylated with acetic anhydride (7). The *O*-acetyl group was hydrolyzed with ammonium hydroxide (7). Further purification of N-OH-AABP was accomplished by precipitation under slightly acid conditions according to the method of Poirier *et al.* (18). The final product had a

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[2] The abbreviations used are: N-OH-FAA, *N*-hydroxy-*N*-2-fluorenylacetamide; AABP, 4-acetylaminobiphenyl; N-OH-AABP, *N*-hydroxy-*N*-4-acetylaminobiphenyl; N-OH-ABP, *N*-hydroxy-*N*-4-aminobipheyl; ABP, 4-aminobiphenyl.

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melting point of 146–147° [literature, 144–146° (15)] and a molar extinction coefficient (λ 279 nm) in ethanol of 24,400 [literature, 23,500 (8)].

Additional evidence supporting the purity of N-OH-AABP was obtained from thin-layer chromatography and gas-liquid chromatography. Thin-layer chromatography of synthetic N-OH-AABP on Silica Gel G plates (Analtech; Newark, Del.) was carried out according to the method of Radomski *et al.* (19). After development with chloroform-methanol (20:1 v/v), only a single spot was revealed under UV (R_F, 0.4 for N-OH-AABP and 0.6 for AABP). Furthermore, the spot yielded a blue color when sprayed sequentially with 0.5 N NaOH and 2 N phenol reagent (Folin-Ciocalteu) or, alternatively, a yellow color after spraying with *p*-dimethylaminobenzaldehyde (19). A sample of N-OH-AABP was silylated as described below and produced only a single peak on gas-liquid chromatography.

[^{14}C]-N-OH-AABP was synthesized from 100 mg of 4-nitrobiphenyl to which had been added 100 μCi of 4-[^{14}C]nitrobiphenyl [3.54 μCi/mmole, uniformly labeled in the aromatic ring (California Bionuclear, Sun Valley, Calif.)]. The crude [^{14}C]N-OH-ABP synthesized in this manner was added to an additional 20 mg of carrier N-OH-ABP and acetylated. The specific activity of the final product was 3.8×10^5 dpm/mg. The [^{14}C]-N-OH-AABP migrated as a single radioactive spot with the mobility of authentic N-OH-AABP in the thin-layer chromatographic system mentioned previously.

AABP (m.p. 169 171°) was synthesized from ABP (Aldrich), using acetic anhydride (14), and yielded a single compound when analyzed by the thin-layer chromatographic method mentioned above and the gas-liquid chromatographic method mentioned below.

An authentic sample of 3-hydroxy-4-acetylaminobiphenyl was kindly provided by Dr. J. A. Miller of the McArdle Institute of the University of Wisconsin, Madison, Wis. However, less than 5% of the uncharacterized radioactive material obtained from metabolic studies in either germ-free or conventional rats had chromatographic properties of this authentic compound.

The Analysis of AABP and N-OH-AABP by Gas-Liquid Chromatography. Because the method previously used to quantify N-OH-FAA (27) proved to be unsatisfactory, AABP and N-OH-AABP have been analyzed by gas-liquid chromatographic methods. AABP and N-OH-AABP were extracted from tissue homogenates, bacterial media, or cecal contents, and conditions were chosen selectively to silylate the *N*-hydroxyl compound in the presence of AABP. For this procedure the biological material (tissue homogenates, bacterial media, or cecal contents) was extracted with ether and the extract was evaporated to dryness at room temperature under a stream of nitrogen. The residue was dissolved in 175 μl of benzene and 25 μl of *N,O*-bis(trimethylsilyl)acetamide (Pierce Chemical Co., Rockland, Ill.) was added. After a period of 5 min at room temperature, silylation had occurred with greater than 97% of the N-OH-AABP but with less than 1% of the AABP. Both AABP and the silyl derivative of N-OH-AABP were then chromatographed on a 7620A Hewlett-Packard research chromatograph with 6-ft coiled glass columns; the injection port and the detector were maintained at 250°. With 3% OV-1 on Chromosorb Q, the column was maintained at 190° for 4 min and then programmed to increase at 10°/min to 260°. For 3% OV-17 on Chromosorb Q, the column was isothermal at 220° for 4 min and then was programmed to increase at 10°/min to 270°. *p*-Phenylphenol and/or 4-nitrobiphenyl were added to the samples just prior to the extraction with ether to serve as internal standards. The retention times of ABP, *p*-phenylphenol, 4-nitrobiphenyl, AABP, and N-OH-AABP (or their derivatives) on 3% OV-1 were 3.3, 3.5, 6.0, 9.4, and 10.0 min, respectively. The retention times on 3% OV-17 were 2.4, 1.9, 3.7, 9.1, and 7.4 min, respectively. Recoveries of AABP and N-OH-AABP by these methods were greater than 90%.

Preparation of Stomach and Cecal Contents for *in Vitro* Metabolism Studies. The gut of either germ-free or concentional Sprague-Dawley rats was exposed by laparotomy by aseptic techniques. A portion of the cecum (weighing approximately 1 g) was clamped, removed with its contents, and placed immediately in a preweighed sterile culture tube containing glass beads and 4.0 ml of prereduced anaerobically sterilized buffer. The buffer was 0.1 M potassium phosphate, pH 7.4, which was prepared in an anerobic atmosphere provided by a V.P.I. anaerobic culture system (Bellco Glass Co., Vineland, N. J.). The anaerobic atmosphere consisted of a gas mixture (10% carbon dioxide, 10% hydrogen, and 80% nitrogen or argon) made oxygen-free by sequential passage through a Deoxo-palladium catalyst (Engelhard Industries, East Newark, N.J.) and a copper combustion furnace heated to 400°. The culture tube was closed with a rubber stopper and reweighed; the contents of the tube were suspended with a Vortex Genie Mixer (Fisher Scientific Co., Boston, Mass.). Additional prereduced anaerobically sterilized buffer was then added to make a 1:10 w/v dilution of the cecal contents. Both the cecum and its contents were cultured because it is known that certain anaerobes adhere to the intestinal mucosa (22). Stomach contents were prepared by the same methods except that dilutions of 1:5 and 1:10 were prepared.

Bacterial Isolation, Storage, and Identification. All bacteria were isolated from the cecum of conventional Sprague-Dawley rats and were stored, identified (with the collaboration of Dr. W. E. C. Moore and Dr. L. V. Holdeman), and incubated as described previously (26).

The Incubation of Tissue Homogenates. Homogenates of stomach, small intestine, colon, and cecum (rinsed of bacterial contents with 0.1 M potassium phosphate buffer at pH 7.4), and of liver, kidney, and brain were prepared from the organs of Sprague-Dawley rats in 10 volumes of 0.25 M sucrose (w/v) by means of a glass and Teflon homogenizer. Reaction mixtures contained 2 μmoles NADP, 20 μmoles glucose 6-phosphate (Sigma Chemical Co. St. Louis, Mo.), 10 μmoles $MgCl_2$, 2 units of glucose 6-phosphate dehydrogenase (Boehringer Biochem, New York, N. Y.), 2.2 μmoles of N-OH-AABP (added in 150 μl ethanol), 150 μmoles of potassium phosphate buffer at pH 7.2, 300 μmoles KF, and tissue homogenate in a total volume of 3.0 ml. The flasks were incubated under nitrogen at 37° with

shaking for 30 min. The reaction was terminated by extracting the incubation mixture with 4 volumes of diethyl ether. Under these conditions, active tissues converted N-OH-AABP to AABP at a constant rate for periods of at least 30 min with tissue additions of between 20 and 150 mg (calculated in terms of initial wet weight). The addition of glucose 6-phosphate dehydrogenase was required for reduction of N-OH-AABP only in incubations with brain homogenate.

The Feeding of N-OH-AABP to Germ-free and Conventional Rats. Germ-free or conventional male Sprague-Dawley rats weighing 180 to 220 g were purchased from Charles River Breeding Laboratories, Wilmington, Mass. The feeding of [^{14}C]-N-OH-AABP to germ-free animals was conducted in germ-free facilities in this laboratory as outlined previously (26). Experiments on conventional animals were performed in metabolism cages outside of the isolators.

Acetone solutions of [^{14}C]-N-OH-AABP (1.6 mg, 5.3×10^5 dpm) were sterilized by filtration (0.2-μm Fluoropore filter, Millipore Filter Corp., Bedford, Mass.) and mixed with 10 g of ground diet that had previously been autoclaved. The diet remained at room temperature for 1 hr to allow the acetone to evaporate before it was presented to the animals.

The diet containing [^{14}C]-N-OH-AABP was added to the cages at 4 p.m. and was invariably consumed by the following morning. The rats were subsequently allowed access *ad libitum* to their standard diet. Urine and feces passed during the 24 hr following presentation of the compound were collected, after which the rats were sacrificed and the cecal contents removed for analysis. Samples of urine and feces were collected at room temperature and stored at −20° prior to analysis; cecal contents were extracted immediately.

Analysis of Metabolites of N-OH-AABP. An aliquot of each urine sample was assayed for total radioactivity. Another aliquot was extracted twice with ether, and the extracts were pooled, dried, and assayed for radioactivity. To a 5-ml aliquot of the urine which had been extracted with ether was added 0.5 ml of 1 M sodium acetate buffer at pH 6.0. The mixture was incubated with 60 units of β-glucuronidase per ml from *E. coli* (Sigma Chemical Co., St. Louis, Mo.) for 1 h at 37°. After the incubation was completed, the urine was again extracted with ether and the radioactivity was determined to assess the quantity of glucuronides in urine.

Radioactive N-OH-AABP and AABP in ether extracts were separated on Silica Gel G plates as described above; the areas of the plates identified by authentic compounds (and adjacent areas) were scraped into counting vials and assayed for radioactivity by scintillation spectrometry.

Cecal contents and feces were extracted 3 times with 80% ethanol (25). The extracts were pooled, concentrated on a rotary evaporator at 40°, and the material in the reduced volume was analyzed according to the procedures described for urine. An estimate was made of the radioactivity remaining in the residue of the feces and cecal contents after the ethanol extraction. The residue was dissolved in Soluene-100 (Packard Instrument Corp., Downers Grove, Ill.) and assayed for radioactivity; less than 4% of the administered radioactivity remained in feces and less than 6% in cecal contents.

Samples containing radioactivity were assayed in a Packard liquid scintillation spectrometer. Ten ml of Aquasol (New England Nuclear Corp., Boston, Mass.) were used as the scintillation mixture for aqueous samples. Samples soluble in organic solvents and material removed from silica gel plates were assayed in a scintillation fluid mixture which contained 4.2% Liquiflor (New England Nuclear Corp.) in toluene and methanol (45:1 v/v). Efficiency of counting was determined for each sample with a ^{14}C internal standard. Efficiencies varied between 62 and 88% for samples assayed in Aquasol and the toluene:methanol mixture, while efficiency was between 28 and 42% for samples solubilized with Soluene-100.

In another experiment, 4 germ-free and 4 conventional rats were allowed to eat 40 mg of N-OH-AABP in a single 24-hr period. Urine and feces were collected for the 24 hr of ingestion of the compound and for the 2 subsequent 24-hr periods. After treatment of the urine with β-glucuronidase (25), aliquots of the urine were extracted with benzene, and the solvent was removed. The dried residue was selectively silylated and analyzed by gas-liquid chromatography; the amounts of N-OH-AABP and AABP were quantified on the basis of added 4-nitrobiphenyl.

The identity of N-OH-AABP and AABP obtained from urine in this manner was verified by gas-liquid chromatography-mass spectroscopy, using an integrated LKB (Rockville, Md.) 9000 system. Conditions for obtaining these spectra were an ionizing energy of 70 eV, an emission current of 200 μa, and an accelerating voltage of 3.5 kV. We thank Dr. James Orr of the Department of Biological Chemistry and the Laboratory of Human Reproduction and Reproductive Biology of Harvard Medical School for consultation in performing this analysis.

RESULTS

Characterization of the Reduction of N-OH-AABP by Rat Cecal Contents. Some of the conditions required for the reduction of N-OH-AABP by rat cecal contents are demonstrated in Chart 1. Cecal contents from conventional rats reduce 82% of the added N-OH-AABP in 6 hr when incubated under anaerobic conditions. The reduction of N-OH-AABP is considerably diminished if the cecal contents are autoclaved, suggesting that a major part of this activity is enzymic. The enzymic activity appears to be of bacterial origin, since cecal contents of germ-free rats have greatly diminished capacity to reduce N-OH-AABP. The reduction of N-OH-AABP is also greatly decreased when the incubation is performed aerobically. When the incubation of germ-free or autoclaved cecal contents is continued for 22 hr (either aerobically or anaerobically under sterile conditions), approximately 50% reduction of N-OH-AABP occurs. This reduction can be attributed to the organic consituents of cecal contents, since ashed cecal contents

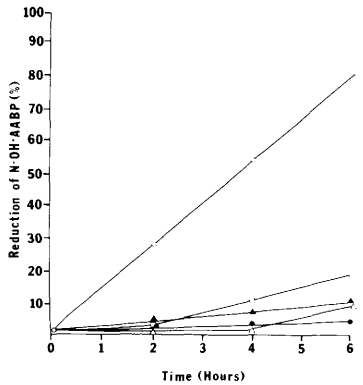

Chart 1. Time course of the reduction of N-OH-AABP by rat cecal contents. Ninety mg of rat cecal contents (diluted in 1 ml of 0.1 M potassium phosphate buffer at pH 7.4) were added to 8.1 ml of anaerobic, sterile phosphate buffer, and 450 μg of N-OH-AABP were added in 0.125 ml ethanol. The reaction mixture was incubated at 37° under sterile conditions. Cecal contents of conventional rats were incubated anaerobically (○) or in air (□). Autoclaved cecal contents of conventional rats also were incubated anaerobically (△) or in air (●). Cecal contents of germ-free rats (▲) gave the same results, whether or not they were autoclaved or incubated aerobically or anaerobically.

show only a 1.5% reduction of N-OH-AABP in 22 hr under the same conditions.

The Reduction of N-OH-AABP by Isolated Bacteria. Several different genera of bacteria isolated from the cecum of conventional rats were tested *in vitro* for their capacity to reduce N-OH-AABP. The concentration of N-OH-AABP used was 50 μg/ml, since bacterial growth of some of the test strains was inhibited at higher concentrations. The results (Table 1) indicate that N-OH-AABP is reduced by the anaerobes *Clostridium* sp., *P. productus* I, and *B. fragilis* ss. *thetaiotaomicron* and ss. *vulgatus*, while the facultative anaerobes *L. plantarum* and *E. coli* exhibit activity that is indistinguishable from that observed in the N_2C growth medium (26) itself.

The Reduction of N-OH-AABP in Preparations from Various Sites in the Conventional Rat. Homogenates of brain, liver, and kidney are capable of reducing N-OH-AABP to AABP or ABP (Table 2); but activity in homogenates of stomach, small intestine, colon, and cecum was not observed (activity as little as 5 μg/100 mg/30 min would have been detected under the conditions used). Homogenates prepared from the organs of germ-free rats showed the same results as those made from the organs of conventional rats. N-OH-AABP is more rapidly reduced on a weight basis by cecal contents than by cecal homogenates or stomach contents. In these experiments there were 3 ×

Table 2

The reduction of N-OH-AABP by homogenates of various organs and by intestinal contents of the conventional rat

Incubation of tissue homogenates, as described in "Materials and Methods," was for 30 min using the equivalent of 100 mg (wet weight) of tissue.

Biological material	Conversion of N-OH-AABP to AABP (μg/100 mg/30 min)
Liver homogenate	105 (75–140)[a]
Kidney homogenate	65 (40–95)
Brain homogenate	150 (120–180)[b]
Stomach contents	2
Cecal contents[c]	30

[a] Results for tissue homogenates are the average of 3 experiments, with range shown in parenthesis. For stomach contents, the results are the average value from experiments performed using 100 and 200 mg of stomach contents under conditions described in Fig. 1.

[b] Includes conversion to ABP, which was approximately 10% in brain but less than 5% in homogenates of other tissues.

[c] The value for cecal contents is obtained from the data of Fig. 1.

Table 1

Time course of the reduction of N-OH-AABP by selected intestinal bacteria

Eight to 9 ml of N_2C broth were inoculated with an aliquot of an 18-hr broth culture to give a concentration of approximately 10^6 bacteria per ml. N-OH-AABP dissolved in ethanol (4 mg/ml) was added to the bacterial suspension to give a final concentration of 50 μg/ml. The results are of typical experiments.

Inoculum	Conversion of N-OH-AABP to AABP (%)			Viable bacteria[a] (log_{10})/ml			
	24 hr	48 hr	72 hr	0	24 hr	48 hr	72 hr
L. plantarum	5	10	23	5.6	6.1	6.2	6.2
B. fragilis ss. *theta.*	18	24	40	5.7	6.0	6.3	6.3
B. fragilis ss. *vulgatus*	10	60	60	5.0	9.0	7.5	5.8
Clostridium sp.	44	95	100	6.8	7.9	7.7	7.7
P. productus I	22	72	90	4.5	6.1	7.9	7.9
E. coli	4	6		6.1[b]	8.5	8.4	8.1
C. perfringens[c]	30	54		5.0	7.7	4.3	3.3
Autoclaved cultures	6	16	22				
No bacterial inoculum	8	18	20				

[a] Measured by colony count using the roll tube method unless otherwise noted.

[b] Measured on nutrient agar (Difco) plates.

[c] *C. perfringens* (No. 8) isolated from human feces (17).

10^9 bacteria per g in stomach contents while cecal contents contained 9×10^9 bacteria per g.

The Metabolism of N-OH-AABP by Germ-free and Conventional Rats. [^{14}C]-N-OH-AABP was fed to germ-free and conventional rats, and the distribution of radioactive compounds in urine, feces, and cecal contents was compared (Table 3). Radioactive material tends to reach higher levels in the urine of conventional rats and in the cecal contents and feces of germ-free rats. Some of the radioactive constituents in the feces and cecal contents of conventional rats are extracted by ether, but the radioactive constituents found at these sites in the germ-free rat are not extracted by ether. When the aqueous extracts of germ-free cecal contents and feces are treated with β-glucuronidase, ether-extractable material is liberated, suggesting that these radioactive metabolites have been conjugated with glucuronic acid. No additional ether-soluble radioactive metabolites are liberated when aqueous extracts of the feces and cecal contents of the conventional rats are treated with β-glucuronidase. Additional ether-soluble radioactivity is liberated in almost equal amounts by β-glucuronidase from the urines of germ-free and conventional rats. Thus the major metabolic differences in the 2 kinds of rats can be attributed to the presence of β-glucuronidase activity derived from the flora (5) in conventional rats. The results are consistent with the diminished β-glucuronidase activity in the intestinal contents of germ-free rats (25) and in rats treated with antibiotics (3).

Conjugated N-OH-AABP, found in the feces and cecal contents of germ-free rats, is not found at these sites in conventional rats. On the other hand, AABP is present in the feces of conventional rats but is absent from these sites in germ-free rats. There is little difference in the urinary metabolism of N-OH-AABP in the germ-free and conventional rats. Similar results with regard to urinary metabolites were found in another experiment in which 40 mg of N-OH-AABP were fed to germ-free and conventional rats. In this experiment urine collections were continued for 72 hr, but little additional conjugate of N-OH-AABP was found after 24 hr.

DISCUSSION

Differences in the distribution of the metabolites of N-OH-AABP in germ-free and conventional rats can be explained on the basis of bacterial metabolism of the glucuronide of N-OH-AABP which is excreted in the bile (8). In the germ-free rat, in which there is no bacterial β-glucuronidase, the poorly absorbed glucuronide (3) survives within the gastrointestinal tract and can be recovered in the cecal contents and feces. In the conventional rat, however, the glucuronide is hydrolyzed and the released N-OH-AABP is reduced by the flora so that only AABP is recovered in the feces. A more rapid intestinal transit of compounds in the conventional rat might explain the failure to recover AABP in cecal contents at the time of sacrifice.

The reductive capacity of the flora for N-OH-AABP differs in various parts of the gastrointestinal tract, and this might influence the location of tumors in rats fed this compound. Tissue preparations from all levels of the gastrointestinal tract have the capacity to convert N-OH-AABP to a putative ultimate carcinogen by means of arylhydroxamic acid acyltransferase (1, 9) and lack the

Table 3

Distribution of metabolites in urine, feces, and cecal contents of germ-free and conventional rats fed [^{14}C]N-OH-AABP

Each of 5 germ-free and 5 conventional rats was fed 1.6 mg of [^{14}C]N-OH-AABP (530,000 dpm) in its diet. Urine and feces were collected for 24 hr, after which the rats were sacrificed and the cecal contents of 4 were removed. Sample preparation is described in "Materials and Methods."

Location of metabolites	Radioactivity recovered[a]				
		Ether extractable			
	Total	Directly[b]	After β-glucuronidase[c]	Unconjugated[d] AABP	Conjugated[e] N-OH-AABP
Conventional rats					
Cecal contents	10 (5.9–17)[f]	4.5 (1.9–8.7)	0	0	0
Feces	8.9 (3.6–14)	5.1 (2.8–7.1)	0	2.0 (1.1–2.6)	0
Urine	26 (20–30)	2.2 (1.1–4.5)	4.0 (2.4–4.9)	0	1.4 (0.9–1.6)
Germ-free rats					
Cecal contents	27 (22–32)	0	4.4 (2.3–6.2)	0	2.4 (1.5–3.3)
Feces	13 (9–20)	0	2.7 (2.0–4.7)	0	1.9 (0.9–3.0)
Urine	16 (13–20)	1.3 (0.8–2.6)	3.7 (1.6–6.1)	0	2.5 (1.4–3.9)

[a] Expressed as a percentage of the dose administered.

[b] Radioactivity extracted by ether without prior treatment of the aqueous extract with β-glucuronidase.

[c] Radioactivity extracted by ether after treatment of the aqueous extract with β-glucuronidase.

[d] Unconjugated AABP was extracted by ether without prior treatment with β-glucuronidase; no additional radioactive material identified as AABP was extracted after treatment with β-glucuronidase.

[e] Negligible radioactive material identified as N-OH-AABP was extracted into ether unless there had been prior treatment with β-glucuronidase.

[f] Results are average values; range is in parentheses. A zero indicates a recovery of less than 0.5%.

capacity to remove N-OH-AABP by reduction. It might be expected therefore that all organs of the gastrointestinal tract would be equally susceptible to carcinogenesis by N-OH-AABP. However, rats fed N-OH-AABP develop tumors of the forestomach but apparently not at other locations in the gastrointestinal tract (15), suggesting that the critical level of N-OH-AABP for carcinogenesis is only achieved in the forestomach. This explanation is in accord with the observation that the stomach flora and its major component, lactobacillus (20, 24), are only weakly capable of reducing N-OH-AABP, while the cecal flora and its component anaerobic bacteria have strong reductive activity toward N-OH-AABP. Thus reductive capacity of the flora may protect the lower bowel from carcinogenesis by this compound. This speculation is in accord with the absence of tumor development in tissues such as liver and kidney which have high arylhydroxamic acid acyltransferase (1, 9) or sulfotransferase (4) activity but which are also active in reducing N-OH-AABP.

The available evidence suggests that the flora may be capable of exerting a protective effect against carcinogenesis by reducing N-OH-AABP or, alternatively, by eliminating the glucuronide of N-OH-AABP, a compound which has been shown to have properties of the ultimate carcinogen (8). On the other hand, hydrolysis of the glucuronide of N-OH-AABP by the flora may accentuate the carcinogenic process elsewhere in the body by making it possible to reabsorb N-OH-AABP or its reduced product AABP and hence to recycle these compounds for reactivation. Recently, attention has been directed at the possible role of the flora in tumor formation by other carcinogens. Studies have indicated that azoxymethane and 1,2-dimethylhydrazine may cause different tumor patterns in germ-free and conventional rats (21). However, until such studies are undertaken with AABP or N-OH-AABP, it will not be clear whether bacterial metabolism of these compounds will have any influence on the carcinogenic process.

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