Effect of Diet on Fecal Excretion and Gastrointestinal Tract Distribution of Unmetabolized Benzo(a)pyrene and 3-Methylcholanthrene When These Compounds Are Administered Orally to Hamsters

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ABSTRACT

3-Methylcholanthrene (3MC) administered p.o. has induced tumors of the hamster gastrointestinal tract (GIT), including the large intestine. This process may depend on the concentration of unchanged hydrocarbon in the GIT contents. Benzo(a)pyrene (BP) ingestion could be involved in human GIT carcinogenesis. Accordingly, male Syrian golden hamsters were fed diets containing BP or 3MC for 10 days. Feces collected during the last two to three days of feeding were analyzed for the unchanged hydrocarbons by KOH:methanol digestion, during the last two to three days of feeding were analyzed for the unchanged hydrocarbons by KOH:methanol digestion, and ultraviolet spectrophotometry. With a semisynthetic diet containing 5% Alphacel, 6% corn oil, and 100 µg BP per g, fecal BP excretion was 0.45% of the dose. Variation of the corn oil content had little effect. Fecal BP excretion was increased 13 times (to 6% of the dose) when 5% wheat bran was used in place of Alphacel and 4.5 times when a commercial diet was used. This suggests that bran adsorbed or sequestered the BP. Water content of the large-intestine contents was increased when the bran diet was fed. Both these factors could affect mucosal exposure to BP. For 3MC, fecal excretion of unchanged hydrocarbon was 14 times greater than for BP under similar conditions. The GIT contents of hamsters fed BP or 3MC showed hydrocarbon concentrations in the order: stomach > lower large intestine > other sections.

INTRODUCTION

A factor in the etiology of human colorectal cancer could be cancer initiators, derived from food, that pass unabsorbed into the colonic contents and act there on the mucosa. BP and other PAH's occur in charcoal-broiled and smoked foods at levels up to 60 µg/kg (6, 7, 14, 20). This occurrence has been associated with a high incidence of stomach cancer in Iceland and Eastern Europe (6) and, more speculatively, with colon cancer (7, 15, 25). Human colonic mucosa can produce DNA-bound BP metabolites from BP (2).

When 3MC was gavaged to Syrian golden hamsters, it induced tumors of the forestomach and small and large intestines (5, 8). To understand the induction of these tumors, it is important to know how much of the ingested 3MC reaches each part of the GIT. When 3MC was injected into mice and rats, the bile and feces contained, in addition to unchanged 3MC, the 2-hydroxy and 2-keto derivatives, in which 3MC is substituted on the ethylene bridge (9, 17). The 1-hydroxy compound is converted into a metabolically active dihydrodiol (18). Although these hydroxy and keto derivatives are carcinogenic (9, 16), unchanged 3MC is likely to be the major carcinogen in the colonic contents when 3MC is administered p.o. Similar metabolites are not possible for BP (24), and the major fecal metabolites in rats fed BP (7) are not carcinogenic or only weakly so.

In view of these considerations, we decided as a first approach to study the excretion of unchanged BP and 3MC in hamster feces and their distribution in hamster GIT contents. BP was selected because of its environmental importance. The hamster was used because it is the only rodent in which a PAH has induced tumors of the large intestine (5, 8, 12, 13, 19). Animals were treated with PAH's in the diet for 7 to 10 days before samples were collected to give adequate time to reach steady-state PAH concentrations in the feces and GIT contents. Initial studies were done with a commercial diet. Later, semisynthetic diets were used to study the effect of varying the dietary composition, including the addition of wheat bran.

MATERIALS AND METHODS

Preparation of Diets. BP (98%) was obtained from Aldrich Chemical Co. (Milwaukee, Wis.) and 3MC from Eastman Organic Chemicals (Rochester, N.Y.). The commercial diet was pelleted or powdered Wayne Lab-Blox (Allied Mills, Chicago, III.), containing 24% protein, 4% fat, and 3.2% fiber. The composition of the semisynthetic diets is shown in Table 1. PAH-containing diets were prepared in a chemical hood and pelleted because powdered diets would present a hazard. To prepare semisynthetic diets, a 10-mg/ml solution of BP or 3MC in corn oil was diluted suitably with corn oil, mixed with casein, and then blended with the other components. The dry food was mixed on a roller for 2 hr, stirred with an equal weight of water containing 1% gelatin (Knox type A unflavored gelatin; Lipton Inc., Englewood, N.J.), poured into 2-cm-deep trays, allowed to set, and cut into 3- to 5-cm squares. The pellets were dried for 2 days and stored at 6°C.

BP was mixed with the commercial diet by 2 methods. In Method 1, a BP solution in 150 ml acetone was pipetted onto 1 kg pelleted diet contained in a glass bottle, with occasional gentle shaking. The pellets were dried overnight on trays. In Method 2, a BP solution in 20 ml corn oil was mixed with 1 kg powdered diet, stirred with gelatin solution, and pelleted as for the semisynthetic diets. Method 1 was used in the earlier experiments where BP concentration was varied. Method 2 was used in the later experiments where the BP concentration was constant.
from the Eppley Institute breeding farm and treated similarly to feces collected. In most experiments, 2 food samples were collected from untreated hamsters, and the feces was allowed to dry over CaSO4, reweighed to give "dry weight" (in the hamsters were killed with CO2, and the GIT was removed. Analyzed for the PAH. Male MRC-Wistar rats were obtained or 10. Food consumption was measured for the period when for the PAH. The hamsters were weighed on Days 0, 7, and 9 was collected and stored at —15° each day, combined as 1 sample, dried in a vacuum dessicator, weighed, and analyzed for the PAH. The hamsters were weighed on Days 0, 7, and 9 to 10 a.m. and 3 to 4 p.m. each day. Before the hamsters were replaced in the metabolism cages, food in the cheek pouches was removed with a spatula. Feces from each group was applied over 20 ml of eluate had a high absorption around 380 nm. BP was eluted in the next 80 ml of hexane. This was concentrated to 5 to 10 ml, and BP was determined by measuring (A at 383 nm) — (A at 420 nm). An A of 1.0 corresponded to 10.8 μg BP per ml. The full UV spectrum was recorded to ensure that BP was measured.

**Determination of BP: Method 1.** Those samples that yielded <4 μg BP per 2-g sample by Method 1 or showed UV spectra not typical of BP were then submitted to reverse-phase paper chromatography (modified from Ref. 11). Whatman No. 1 paper (23 x 23 cm) was dipped in dimethylformamide and allowed to dry for 10 min. The sample from Method 1 was applied over 20 min as a 20-cm strip. Ascending paper chromatography in the machined direction was performed with 3,3,5-trimethylpentane ("iso-octane;" Phillips Petroleum Co., Bartlesville, Okla.). The BP band (Rf, 0.2 to 0.4) was visualized by its blue fluorescence under 254-nm UV, cut out, and eluted 3 times with 3 ml of 95: pc ethanol. The combined eluate was concentrated to 3 ml under a stream of nitrogen and estimated for BP by the A at 383 nm, using a Carey-15 spectrophotometer with a 0.01-A unit scale. In a typical analysis, we obtained an A of 0.041 in 3.0 ml solution, corresponding to 1.33 μg BP.

**Determination of 3MC.** Method 1 for BP was used, except that the Florisil column was eluted with 25 ml hexane, which was discarded, and then with 125 ml hexane, which contained the 3MC. This was determined by its UV absorption at 298 nm, where an A of 1.00 was given by 3.43 μg 3MC per ml.

**Expression of Results.** BP results were expressed as μg/g dried feces, GIT contents, or food. The percentage of fecal BP excretion was calculated as follows.

% of excretion = \[ \frac{\text{BP in feces (\mu g/g) \times fecal weight (g)}}{\text{BP in food (\mu g/g) \times food eaten (g)}} \times 100 \]

BP in food refers to the concentration added. Feces and food weights are those for the 2- or 3-day feces collection period. Feces excreted in the feeding cages was not corrected for. BP dose/hamster/day was given by (BP concentration in food) \times (food consumption/day). 3MC results were expressed similarly. All results are given as mean ± S.E. Differences between mean values were considered significant when p < 0.05.

**RESULTS**

**Checks on the Methods**

Twenty-two analyses of semisynthetic diets to which were added 6.25 to 25 μg BP per g showed BP recoveries of 86 ± 3%. In 5 analyses of commercial diet to which was added 6.25 to 100 μg BP per g, the BP recovery was 88 ± 3%. No corrections were made for these recoveries. BP was not detected in samples of commercial diet and semisynthetic diet containing 5% bran. The detection limit was 0.2 μg BP per g.
when Method 2 was applied to the analysis of feces. When 2-g samples of feces from hamsters fed commercial diet were mixed with 4 to 75 μg BP, The BP recovery by Method 1 was 84 ± 2% (6 samples). When 4 similar 2-g feces samples were mixed with 2.22 μg BP, the recovery by Method 2 was 52 ± 5%. The 3MC recovery from 2 g feces spiked with 50 μg 3MC was 92 ± 1% (2 samples).

Analysis of Feces for BP

Use of Semisynthetic Diets without Roughage or with Alphacel. With a semisynthetic diet containing 1% corn oil and no roughage, 0.18% of the BP dose of 100 μg/g diet was excreted in the feces (Table 2, Group 1). Groups 2 to 6 of Table 2 show the variations in this value when the corn oil was increased to 30% and when 5% Alphacel was included in the diet.

Use of Commercial Diet. Groups 7 to 13 of Table 2 show the results when BP concentration in the commercial diet was lowered from 400 to 6.25 μg/g diet. When commercial diet prepared by Method 2 was fed, the percentage of fecal BP excretion did not significantly differ from that for the same diet prepared by Method 1 (see Groups 9 and 15). Two adult male MRC-Wistar rats were fed commercial diet with 100 μg BP per g, prepared by Method 1, for 10 days. BP in the combined feces collected on Days 8 to 10 was 2.7% of the dose (12.4 μg BP per g feces), similar to the results for hamsters (cf. Table 2, Group 9). Three groups of 3 hamsters maintained on commercial diet were given i.p. injections of 1.0 mg BP in 0.5 ml olive oil. BP excretion in the feces collected for 3 days after the injection was 0.029 ± 0.003% of the dose, with 0.39 ± 0.05 μg/g feces.

Use of Semisynthetic Diets Containing Wheat Bran. Semisynthetic diets with 5 to 15% wheat bran were administered. In Group 17 of Table 2, with 5% bran, fecal BP excretion was 6% of the dose, the highest value in this table.

Table 2

<table>
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<tr>
<th>Group</th>
<th>Diet¹</th>
<th>Diet components²</th>
<th>BP in food (μg/g)</th>
<th>No. of groups³</th>
<th>Food eaten (g/hamster/day)</th>
<th>Feces dry weight (g/hamster/day)</th>
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¹ See Table 1 for Diets 1 to 9; Comm.-1 and Comm.-2, commercial diets prepared by Methods 1 and 2, respectively.
² Other components are listed in Table 1.
³ Values were calculated separately for each group of hamsters.

Studies on GIT Contents

The wet:dry weight ratio of the large-intestine contents was greater for a bran diet than for an Alphacel diet, with p < 0.01 for the lower one-third of the large intestine (Table 3). The GIT contents were analyzed after BP-containing diets were fed (Table 4). With both semisynthetic and commercial diets, the highest BP concentrations occurred in the stomach contents. With commercial diet, BP concentration fell to a minimum in the small intestine and rose in the lower large intestine nearly to the level in the stomach. BP concentration in the lower large intestine was 9 times greater with commercial diet than with the semisynthetic diet.

Similar experiments were performed with 3MC given in a semisynthetic diet, but dry weights were also recorded. The wet:dry weight ratio was highest in the upper small intestine (Table 4, Column 4). The 3MC concentration in μg/g wet weight showed a pattern similar to that for BP given in the same diet, except that PAH concentration was smaller in the stomach and greater in the lower large intestine than for BP. The 3MC concentration in μg/g dry weight varied much less than that based on the wet weight, was highest in the stomach, and was identical in the lower large intestine and feces. Fecal 3MC...
excretion was 6.2 ± 1.4% of the dose, 14 times higher than for BP administered similarly (Table 2, Group 5).

**DISCUSSION**

The fecal excretion of 0.029% of a BP dose injected i.p. into hamsters was less than the 0.3 to 1.0% values found in similar studies on rats and mice (10). When BP was fed in commercial diets, fecal BP excretion was 3.8% (Table 2, Group 9) or 2.0% (Table 2, Group 5). The percentage of fecal excretion of BP remained relatively constant as its concentration in commercial diet was lowered to 0.40 ± 0.14 g for hamsters fed 3MC in Semisynthetic diet and 0.70 ± 0.02 g for hamsters fed BP in commercial diet.

The increased percentage of fecal BP excretion when bran was used could have occurred without a change in fecal BP concentration, if the bran had increased the fecal weight. In fact, fecal dry weight (Table 2, Column 9) was similar for the bran and Alphacel diets (except with the 15% bran diet), and the use of bran did raise BP concentration in the dried feces; e.g., in Group 17, the fecal concentration was 86 µg/g, 86% greater with a bran diet than with an Alphacel diet (Table 3). This suggests that bran could have increased fecal BP excretion by raising the fecal wet weight while BP concentration on a wet weight basis remained constant. Hence, bran could affect exposure of the large-intestine mucosa to PAH's in the contents, both by absorbing the PAH's (as discussed earlier) and by diluting the PAH's with water. The second factor fits in with the view of Burkitt (3) that human consumption of a high-fiber diet dilutes colonic carcinogens and hence reduces the risk of developing colon cancer. These factors may help explain the finding (4) that arylhydrocarbon hydroxylase induction in the rat small intestine by dietary BP was reduced when 10% wheat bran was included in the diet. The increased percentage of fecal BP excretion when bran was used could have occurred without a change in fecal BP concentration, if the bran had increased the fecal weight. In fact, fecal dry weight (Table 2, Column 9) was similar for the bran and Alphacel diets (except with the 15% bran diet), and the use of bran did raise BP concentration in the dried feces; e.g., in Group 17, the fecal concentration was 86 µg/g, 86% of that in the diet.

The wet: dry weight ratio in the lower large intestine was greater with a bran diet than with an Alphacel diet (Table 3). This suggests that bran could have increased fecal BP excretion by raising the fecal wet weight while BP concentration on a wet weight basis remained constant. Hence, bran could affect exposure of the large-intestine mucosa to PAH's in the contents, both by absorbing the PAH's (as discussed earlier) and by diluting the PAH's with water. The second factor fits in with the view of Burkitt (3) that human consumption of a high-fiber diet dilutes colonic carcinogens and hence reduces the risk of developing colon cancer. These factors may help explain the finding (4) that arylhydrocarbon hydroxylase induction in the rat small intestine by dietary BP was reduced when 10% wheat bran was included in the diet.

In the GIT contents, PAH levels as µg/g wet weight generally followed the order: stomach > large intestine > small intestine and cecum (Table 4). The high gastric levels were consistent with findings that p.o. administration of BP induced arylhydro-
carbon hydroxylase and tumors in the rodent forestomach (12, 13, 19). PAH concentration in the small intestine and feces was lower, presumably because most of the PAH was absorbed in the small intestine and the contents were diluted by bile and pancreatic juice. In the large-intestine contents, the high PAH concentration on a wet-weight basis was mainly due to absorption of water, as shown by the relatively constant 3MC concentration on a wet-weight basis. Alternatively, BP absorbed by bran might be metabolized more efficiently. This suggests that 3MC is more likely than BP to induce large-intestine tumors after p.o. administration to hamsters (BP has not been thus tested).

After repeated gavage of 3MC to hamsters, Della Porta et al. (5) obtained GIT tumors in the order cecum > small intestine > large intestine, whereas Homburger et al. (8) obtained tumors in 2 hamster lines sensitive to colon carcinogenesis in the order large intestine > small intestine > stomach (the order differed in other lines). Our 3MC analyses of the GIT contents may explain why Homburger et al. (8) obtained a high incidence of large-intestine tumors, although the experiments differed, especially in the method by which 3MC was administered (diet versus gavage). The results support the suggestion that p.o. ingestion of PAH's might be a factor in the induction of human gastric and colon cancer. Finally, the results suggest that adding bran to the diet would make BP less available for reaction with the colonic mucosa, if bran absorbs BP in the colonic contents. Alternatively, BP absorbed by bran might be released in the colon and become more available for reaction with the mucosa. This question has yet to be resolved.

REFERENCES

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