Activation of Carcinogens and Mutagens by Rat Colon Mucosa

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ABSTRACT

Colon mucosal cells can catalyze the activation of precarcinogens to mutagenic metabolites without the intermedacy of intestinal bacteria as shown in a mutagenesis assay system composed of Salmonella typhimurium strain TA100 and the 9000 x g supernatant fraction of rat colon mucosal cells. Pretreatment of rats with β-naphthoflavone increased the activation of 2-aminoanthracene 10- to 20-fold and the activation of benzo(a)pyrene 4-fold. Pretreatment of rats with Aroclor 1254 doubled the activation of 2-aminoanthracene over control but had no effect on the activation of benzo(a)pyrene. The activation of 2-aminoanthracene and benzo(a)pyrene by liver was induced significantly by pretreatment with β-naphthoflavone and Aroclor 1254. Phenobarbital/hydrocortisone pretreatment did not increase the activation by the colon system of any precarcinogen tested but did increase the activation of 2-aminoanthracene, cyclophosphamide, and isophosphamide by the liver system. The activation of precarcinogens in the bacterial test system is directly correlated with the activities of the pretreated colon and liver preparations toward several drug and polycyclic hydrocarbon substrates assayed in vitro.

INTRODUCTION

Considerable attention has been given in recent years to the etiology of colon cancer. Epidemiological studies (23, 36-39) have indicated that diet is associated with colon cancer incidence, and it has been suggested (13) that the fecal bacteria, especially the anaerobes, may activate or partially activate dietary precarcinogens directly, although this postulate has been questioned (11). Alternately, it has been suggested that fecal flora may function primarily in the release of activated carcinogens from conjugates formed in the liver. In this proposal the liver is cited as the primary site of activation of colonic precarcinogens (32) and of conjugation with glucuronic acid. Fecal bacterial enzymes such as β-glucuronidase are implicated in the subsequent release of activated metabolites in the large bowel (33). Most investigations of carcinoen activation in the colon have, however, focused on the involvement of fecal bacteria in one way or another.

Weisburger (33), however, pointed out that some activation of carcinoogens takes place in the lower gut as indicated by studies involving surgical isolation of parts of the colon by Wittig et al. (35) and by colon transposition studies by Gennaro et al. (12). The proposal that the colon might have a system capable of activating carcinoens has received support from the demonstration of drug and polycyclic hydrocarbon hydroxylation activity in the forestomach (30) and small intestine (21, 31). In addition, Stohs et al. (28, 29) prepared microsomes from the small intestine mucosa and demonstrated in vitro the metabolism of benzo(a)pyrene, 7-ethoxyresorufin, 7-ethoxycooumarin, and biphenyl by this microsomal system. Recently, Fang and Strobel (9) reported the presence of a drug and carcinoen metabolism system in microsomes isolated from the mucosal layer of the colon. These authors demonstrated that the colon system activities, like those of the liver system, were dependent on cytochrome P-450 or P-448. This report utilizes the Ames test system (1, 3) with known precarcinogens [benzo(a)pyrene and 2-aminoanthracene] and anticancer drugs (cyclophosphamide and isophosphamide) to examine the potential of the rat colon microsomes to metabolize carcinoens and chemotherapeutic agents to their active forms. This paper demonstrates that the colon system is able to activate compounds without the intermediacy of intestinal bacteria.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (80 to 100 g) were purchased from Flow Laboratories, Dublin, Va., and were housed in wire-bottomed cages during this study. They were maintained on Purina Laboratory Chow and water ad libitum during all phases of pretreatment unless otherwise stated. The phenobarbital/hydrocortisone-pretreated rats received i.p. 75 mg phenobarbital sodium in 0.9% NaCl solution per kg body weight once daily on the fifth, fourth, and third days before sacrifice and phenobarbital sodium (75 mg/kg body weight) plus hydrocortisone-21-sodium succinate (50 mg/kg body weight in 0.9% NaCl solution twice daily for the 2 days immediately preceding sacrifice). β-Naphthoflavone-pretreated rats received 1 i.p. injection of β-naphthoflavone (80 mg/kg body weight) in corn oil each day for 3 days and were fasted overnight before sacrifice. Aroclor-pretreated rats received 1 i.p. injection of Aroclor 1254 (500 mg/kg body weight; Monsanto Co., St. Louis, Mo.) 3 days before sacrifice. The drug metabolism activities of 0.9% NaCl solution- and corn oil-treated animals were not significantly different from the activities of untreated animals. Fasting the rats overnight did not affect the colon microsomal drug metabolism activities. Control and pretreated rats were sacrificed by decapitation between 8 and 9:30 a.m. The colon was cut from the end of the cecum to the end of the rectum, flushed with cold 1.14% (w/v) KCl, and everted into cold 1.14% (w/v) KCl solution. The colon mucosal cells were scraped from the everted colon with the edge of a glass slide into cold Tris-HCl buffer.
(10 mM, pH 7.4) containing 0.14 mM KCl/10 mM EDTA/1 mM dithiothreitol. The mucosal cells were washed 3 times in the Tris-HCl buffer solution and recovered by low-speed centrifugation (2500 rpm in a Sorvall SS-34 rotor). The washed cells were then resuspended in Tris-HCl buffer solution and made 0.25 mM in phenylmethylsulfonyl fluoride immediately prior to cell breakage. The cells were homogenized in a Teflon/glass homogenizer and centrifuged at 9000 x g for 10 min. The 9000 x g supernatant fraction containing the microsomal and soluble enzymes was used at once or frozen at −70°C. Liver fractions were prepared in the same way.

Assays. Protein was determined according to the procedure of Lowry et al. (14) with bovine serum albumin as a standard. NADPH-cytochrome P-450 (cytochrome c) reductase activity was measured by a modification of the method of Phillips and Langdon (22) at 30°C in 1.0-mL reaction mixtures containing 0.3 mM potassium phosphate buffer (pH 7.7) as the rate of reduction of cytochrome c with an extinction coefficient of 21 cm/mM at 550 nm (34).

The rate of hydroxylation of benzphetamine or ethylmorphine was estimated in 1.5-mL reaction mixtures containing 150 μmol potassium phosphate buffer (pH 7.7), 0.15 μmol NADPH, 0.5 to 1.0 mg microsomal protein, and substrates as previously described (15) by formaldehyde liberation according to the method of Nash (18) as modified by Cochin and Axelrod (6). Benzo(a)pyrene hydroxylation activity was measured in 1.0-mL reaction mixtures containing similar quantities of cofactors by fluorometric estimation of 3-hydroxy and other products according to the method of Nash (18) as modified by Cochin and Axelrod (6). Benzo(a)pyrene hydroxylation activity was measured in 1.5-mL reaction mixtures containing similar cofactor concentrations with p-nitroanisole and p-nitrophenol as substrates.

The rate of dealkylation was determined by formation of the product p-nitrophenol judged colorimetrically according to the method of Netter and Seidel (20). The statistical significance of enzymatic activities was determined with Student's t test.

Mutagen and Carcinogen Activation. The ability of colon mucosal preparations to activate carcinogens and mutagens was assayed according to the procedure of Ames et al. (1–3) with the histidine auxotroph S. typhimurium TA100 and various mutagens and carcinogens. Rat colon 9000 x g supernatant fractions prepared as described contained bacteria that overgrew the histidine auxotroph used in the activation studies. Therefore in preparation of 9000 x g supernatant fractions (S9) to be used in activation studies, all wash solutions, buffers, vessels, and surgical equipment were sterilized before use. The colons were washed in successive rinses of sterile KCl solution and subsequently were handled under sterile conditions as described previously. With these precautions the S9 fractions were without bacterial contaminants. One ml of a carcinogen activation mixture was prepared by mixing 0.3 mL of the S9 fraction with 0.7 mL of cofactor supplement mixture that contained 5.7 mM KCl, 11.4 mM MgCl₂, and 0.14 mM sodium phosphate buffer (pH 7.4). The carcinogen or mutagen being tested in 50 μL or less of dimethyl sulfoxide, 0.1 mL of log phase S. typhimurium, and 0.5 mL of the carcinogen activation mixture were mixed with 2.0 mL of warm soft agar (0.6%) and poured onto Vogel agar plates. Each plate contained a final concentration of about 3 mg S9 protein. After resolidification of the top soft agar, the plates were incubated at 37°C for 48 hr. The plates were then scored for his⁺ revertants and compared with controls for which enzyme mixture or carcinogen was omitted.

RESULTS

In Vitro Hydroxylation Activity of Rat Colon and Liver S9 Fractions. The data presented in Table 1 show the ability of

### Table 1

Activities of colon S9 fraction in vitro hydroxylation or reduction assays

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Control Specific activity</th>
<th>Phenobarbital/hydrocortisone Specific activity</th>
<th>β-Naphthoflavone Specific activity</th>
<th>Aroclor 1254 Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzphetamine</td>
<td>0.06 ± 0.02</td>
<td>0.21 ± 0.01</td>
<td>0.09 ± 0.03</td>
<td>0.14 ± 0.01</td>
</tr>
<tr>
<td>Ethylmorphine</td>
<td>0.055 ± 0.005</td>
<td>0.17 ± 0.01</td>
<td>0.11 ± 0.02</td>
<td>0.053 ± 0.005</td>
</tr>
<tr>
<td>p-Nitroanisole</td>
<td>0.043 ± 0.005</td>
<td>0.15 ± 0.01</td>
<td>0.19 ± 0.02</td>
<td>0.12 ± 0.001</td>
</tr>
<tr>
<td>p-Nitrophenetole</td>
<td>0.17 ± 0.02</td>
<td>0.14 ± 0.02</td>
<td>0.47 ± 0.06</td>
<td>0.08 ± 0.02</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>3.9 ± 0.4</td>
<td>7.8 ± 3.5</td>
<td>172.0 ± 11.0</td>
<td>33.0 ± 12.0</td>
</tr>
<tr>
<td>Cytochrome c</td>
<td>17.6 ± 0.71</td>
<td>36.67 ± 4.76</td>
<td>23.33 ± 1.43</td>
<td>21.43 ± 2.86</td>
</tr>
</tbody>
</table>

* a Substrate concentration was 1.0 mM; activities are reported as nmol HCHO per min per mg.
* b Mean ± S.E.
* c Induction over control activity is significant at the 1% level by paired Student's t test.
* d Substrate concentration was 3.3 mM; activities are reported as nmol p-nitrophenol per min per mg.
* e Substrate concentration was 80 μM; activities are reported as pmol 3-hydroxybenzo(a)pyrene per min per mg.
* f Substrate concentration was 40 μM; activities are reported as nmol cytochrome c reduced per min per mg.
rat colon S9 fractions after various pretreatments to hydroxylate in vitro several known substrates of the cytochrome P-450-dependent mixed function oxidase system. The S9 fractions that had been frozen at −70° for up to 6 weeks showed no loss in activity. Demethylation of benzphetamine and ethylmorphine are induced in the colon S9 fraction by pretreatment with phenobarbital/hydrocortisone. p-Nitroanisole demethylation is induced by both phenobarbital/hydrocortisone and β-naphthoflavone pretreatment. Benzo(a)pyrene hydroxylation is induced by pretreatment with β-naphthoflavone. Aroclor 1254 pretreatment appears to induce only benzphetamine demethylation and benzo(a)pyrene hydroxylation in the rat colon S9 fraction.

The hydroxylation activity of rat liver S9 fractions after various pretreatments is shown in Table 2. In marked contrast to the colon S9 activities, Aroclor 1254 induces hydroxylation activity for all substrates with the exception of ethylmorphine by liver S9 fractions that were isolated from the same rats as the corresponding colon fractions. On the other hand the induction of liver S9 fraction hydroxylation activities by β-naphthoflavone and phenobarbital/hydrocortisone is similar to that observed in the colon fractions (Table 1). Thus Aroclor 1254 appears to be a good inducer of the liver drug metabolism system but not of the colon system.

**Activation of Carcinogens by Colon and Liver S9 Fractions.** The S9 fractions isolated from liver or colon and characterized for in vitro hydroxylation activity (Tables 1 and 2) were examined for their ability to activate the precarcinogens benzo(a)pyrene and 2-aminoanthracene (1) to mutagenic metabolites in the S. typhimurium TA100 system. The activation of benzo(a)pyrene at 2 concentrations by colon and liver is shown in Table 3. The addition of control (uninduced) S9 fraction from colon or liver to the test system caused approximately a doubling of the number of revertants over the spontaneous rate (150 revertants/plate). Phenobarbital/hydrocortisone pretreatment did not induce a higher rate of activation of benzo(a)pyrene in either the colon or the liver. Pretreatment with β-naphthoflavone, however, induced a 4-fold increase in the activation of benzo(a)pyrene by both the colon and liver systems. Increasing the amount of precarcinogen in this series had no effect in the colon system and very little effect in the liver system, suggesting that 25 μg of benzo(a)pyrene are

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Control</th>
<th>Phenobarbital/hydrocortisone</th>
<th>β-Naphthoflavone</th>
<th>Aroclor 1254</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzphetamine</td>
<td>2.82 ± 0.10</td>
<td>5.77 ± 0.42</td>
<td>1.74 ± 0.13</td>
<td>6.65 ± 0.28</td>
</tr>
<tr>
<td>Ethylmorphine</td>
<td>2.54 ± 0.44</td>
<td>3.75 ± 0.16</td>
<td>1.69 ± 0.09</td>
<td>3.43 ± 0.20</td>
</tr>
<tr>
<td>p-Nitroanisole</td>
<td>0.53 ± 0.04</td>
<td>2.10 ± 0.08</td>
<td>0.84 ± 0.05</td>
<td>2.33 ± 0.19</td>
</tr>
<tr>
<td>p-Nitrophenetole</td>
<td>1.21 ± 0.12</td>
<td>2.33 ± 0.22</td>
<td>4.69 ± 0.19</td>
<td>10.18 ± 0.51</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>132.0 ± 9.0</td>
<td>200.0 ± 25.0</td>
<td>720.0 ± 30.0</td>
<td>570.0 ± 30.0</td>
</tr>
<tr>
<td>Cytochrome c</td>
<td>37.14 ± 4.76</td>
<td>120.48 ± 13.33</td>
<td>45.71 ± 7.14</td>
<td>52.38 ± 17.62</td>
</tr>
</tbody>
</table>
| a Substrate concentration was 1.0 mM; activities are reported as nmol HCHO per min per mg.  
| b Mean ± S.E.        |         |                             |                  |
| c Substrate concentration was 3.3 mM; activities are reported as nmol p-nitrophenol per min per mg.  
| d Substrate concentration was 80 μM; activities are reported as nmol 3-hydroxybenzo(a)pyrene per min per mg.  
| e Substrate concentration was 40 μM; activities are reported as nmol cytochrome c reduced per min per mg.  
| f Induction over control activity is significant at the 1% level by paired Student's t test. |

**Activation of benzo(a)pyrene by colon and liver S9 fractions**

Liver and colon 9000 × g supernatant fractions were prepared from control rats or rats pretreated with phenobarbital/hydrocortisone, β-naphthoflavone, or Aroclor 1254. These fractions were tested in an Ames mutagenesis assay (1, 3) system with S. typhimurium TA100 for their ability to activate the precarcinogen benzo(a)pyrene. The results are reported as revertants/mg S9 protein. Each value has been corrected for spontaneous revertants occurring in the absence of either the precarcinogen or the S9 fraction.

<table>
<thead>
<tr>
<th>Pretreatment group</th>
<th>25 μg</th>
<th>50 μg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Colon</td>
<td>Liver</td>
</tr>
<tr>
<td>Control</td>
<td>63 ± 2.7(^a)</td>
<td>103 ± 6.5</td>
</tr>
<tr>
<td>Phenobarbital/hydrocortisone</td>
<td>65 ± 2.5</td>
<td>75 ± 1.7</td>
</tr>
<tr>
<td>β-Naphthoflavone</td>
<td>239 ± 13.5(^b)</td>
<td>476 ± 14.2</td>
</tr>
<tr>
<td>Aroclor 1254</td>
<td>72 ± 2.2</td>
<td>497 ± 45.0</td>
</tr>
</tbody>
</table>

\(^a\) Mean ± S.E. for at least 3 separate determinations.  
\(^b\) Significantly increased over control group, \(p < 0.01\).
near or at a saturating level for these systems. Pretreatment with Aroclor 1254 had no effect on the activation of benzo(a)pyrene by the colon system but did increase the activation of benzo(a)pyrene by the liver system [4-fold increase at 25 \( \mu \)g benzo(a)pyrene]. The lower degree (double the control rate) of induction of activation by Aroclor 1254 at a dosage level of 50 \( \mu \)g of benzo(a)pyrene may be attributable to inhibitory effects of other benzo(a)pyrene metabolites formed by Aroclor 1254-inducible alternate pathways. The Aroclor 1254-induced increase in the activation of benzo(a)pyrene to mutagenic metabolites by the liver system but not the colon system is consistent with the lower degree of effectiveness of Aroclor 1254 as an inducer of colon S9 fraction hydroxylation activities (Table 1) versus its effect on the liver system (Table 2).

The activation of 2-aminoanthracene by colon and liver is shown in Table 4. Phenobarbital/hydrocortisone pretreatment does not enhance the activation of 2-aminoanthracene by the colon system at either dosage level of 2-aminoanthracene, but it does enhance activation by the liver system at 6 \( \mu \)g of 2-aminoanthracene. \( \beta \)-Naphthoflavone pretreatment increases activation by the colon system 10-fold at the low dosage level and almost 20-fold at the high dosage level of 2-aminoanthracene. The liver system responds similarly to pretreatment with \( \beta \)-naphthoflavone, although not so spectacularly. Pretreatment with Aroclor 1254 induces the activation of 2-aminoanthracene by both the colon and liver systems. At both dosage levels of the precarcinogen, Aroclor 1254 induces only a doubling of the control level of activation by the colon system. The effect of Aroclor 1254 on the liver system at the higher dosage level, however, is almost 8-fold. These data are also consistent with a greater effect of Aroclor 1254 on liver than on colon.

**Activation of Anticancer Drugs by Colon and Liver.** The activation by colon and liver of the antitumor drug cyclophosphamide (Mead-Johnson Research Center, Evansville, Ind.) to mutagenic metabolites as judged by the bacterial revertant assay is shown in Table 5. At all concentrations of cyclophosphamide tested, the colon systems showed only a doubling of activity over background. None of the inducers tested increased the activity of the colon system above the control value. On the other hand pretreatment with phenobarbital/hydrocortisone induced the activation of cyclophosphamide by the liver system at all concentrations of the drug tested, the greatest activation occurring in the presence of 400 \( \mu \)g cyclophosphamide per plate. In addition, in the presence of 800 \( \mu \)g of cyclophosphamide per plate, the liver S9 fraction from Aroclor 1254-pretreated rats showed a 3-fold increase in activation.

The activation of isophosphamide (Table 6) by liver and colon S9 fractions prepared after various pretreatments showed essentially the same pattern of response as that obtained with cyclophosphamide (Table 5) although at much higher concentrations of the drug. Isophosphamide appears to be a less potent mutagen than is cyclophosphamide. Thus both cyclophosphamide and isophosphamide are activated by the liver but not by the colon drug metabolism system.

### Table 4

**Activation of 2-aminoanthracene by liver and colon S9 fraction**

The experimental details were similar to those described in the legend to Table 3.

<table>
<thead>
<tr>
<th>Pretreatment group</th>
<th>Colon (3 ( \mu )g)</th>
<th>Liver (3 ( \mu )g)</th>
<th>Colon (6 ( \mu )g)</th>
<th>Liver (6 ( \mu )g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>87 ± 1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>138 ± 10.5</td>
<td>87 ± 5.0</td>
<td>145 ± 6.9</td>
</tr>
<tr>
<td>Phenobarbital/hydrocortisone</td>
<td>85 ± 4.9</td>
<td>499 ± 16.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>90 ± 4.9</td>
<td>1237 ± 22.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>( \beta )-Naphthoflavone</td>
<td>867 ± 83.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1223 ± 139.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1713 ± 79.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>667 ± 21.9&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Aroclor 1254</td>
<td>177 ± 4.7&lt;sup&gt;d&lt;/sup&gt;</td>
<td>683 ± 23.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>210 ± 4.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1070 ± 22.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± S.E.  
<sup>b</sup> Significantly increased over control group, \( p < 0.02 \).  
<sup>c</sup> Significantly increased over control group, \( p < 0.003 \).  
<sup>d</sup> Significantly increased over control group, \( p < 0.01 \).

### Table 5

**Activation of cyclophosphamide by colon and liver S9 fractions**

The experimental details were similar to those described in the legend to Table 3.

<table>
<thead>
<tr>
<th>Pretreatment group</th>
<th>200 ( \mu )g</th>
<th>400 ( \mu )g</th>
<th>800 ( \mu )g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon (Control)</td>
<td>110 ± 2.5</td>
<td>78 ± 1.8</td>
<td>95 ± 2.5</td>
</tr>
<tr>
<td>Phenobarbital/hydrocortisone</td>
<td>95 ± 2.6</td>
<td>944 ± 7.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>92 ± 1.2</td>
</tr>
<tr>
<td>( \beta )-Naphthoflavone</td>
<td>113 ± 10.2</td>
<td>52 ± 4.3</td>
<td>102 ± 12.3</td>
</tr>
<tr>
<td>Aroclor 1254</td>
<td>103 ± 8.6</td>
<td>71 ± 0.6</td>
<td>81 ± 0.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± S.E.  
<sup>b</sup> Significantly increased over control group, \( p < 0.001 \).  
<sup>c</sup> Significantly increased over control group, \( p < 0.006 \).
The colonic mucosa of rats catalyzes the activation of precarcinogens to mutagenic metabolites as judged by increases in the number of revertant colonies in the presence of colon mucosal S9 fractions from uninduced rats or rats pretreated with inducers of the microsomal drug metabolism system. Colon mucosal S9 fractions, when added to the test system, caused at least a doubling of the number of spontaneous revertants per plate. Pretreatment of rats with inducers generally increased the number of revertants manyfold over that effected by uninduced colon mucosal S9 fractions. The increase in the number of revertants is, however, dependent both on the inducer used and the precarcinogen tested. While Aroclor 1254 is a good inducer of precarcinogen activation by the liver system, its effect on the colon system is limited to a doubling of the activation of 2-aminoanthracene by control colon. β-Naphthoflavone seems to be the best inducer tested in this series for both colon and liver. On the other hand, β-naphthoflavone induces no increase in the activation by the colon of the antitumor drugs cyclophosphamide or isophosphamide and is a minimally effective inducer of the activation of these compounds by the liver. Pretreatment with phenobarbital/hydrocortisone does increase the activation by the liver system of cyclophosphamide, isophosphamide, and 2-aminoanthracene but not benzo(a)pyrene. Phenobarbital/hydrocortisone pretreatment of the colon system, on the other hand, does not increase the activation of any of the compounds to mutagenic products above the control level.

With the exception of the apparent lack of phenobarbital/hydrocortisone-induction of cyclophosphamide and isophosphamide activation by the colon, induction of in vitro hydroxylation activities and mutagen activation in the bacterial test system are generally well correlated. The elevation of hydroxylation activity of various substrates by the colon system indicates that the inducer is reaching the colon. Induction of colon hydroxylation activities by Aroclor 1254 is significant but low with benzphetamine and benzo(a)pyrene, and no mutagen activation above control is seen with Aroclor 1254-pretreated colon S9. β-Naphthoflavone induction of hydroxylation activities in colon and liver is high and well correlated with an increase in mutagen activation.

As judged by response to inducers, effectiveness of low dosage levels, and number of revertants produced, 2-aminoanthracene is a more efficient mutagen than is benzo(a)pyrene in the colon and liver systems. This conclusion is consistent with that of other investigators (16). Cyclophosphamide and isophosphamide (5) appear not to be substrates for activation by the colon system but are activated by the liver system, although at concentrations higher than those required for 2-aminoanthracene or benzo(a)pyrene.

That cyclophosphamide is not activated by the colon system under the conditions tested is consistent with the relative ineffectiveness of cyclophosphamide as an antitumor agent against colon adenocarcinoma (17). Hepatic activation of cyclophosphamide has been shown (7, 10, 25-27), but the activated metabolites of cyclophosphamide may not act on colon tumors in sufficient quantity to be effective. Additional study of the composition of the colon mucosal drug metabolism system may explain the apparent inability of the colon to activate cyclophosphamide and the inefficacy of cyclophosphamide against colon cancer.

The demonstration in this paper that the mixed-function oxidase system of colon mucosal cells is capable of metabolizing precarcinogens to their active forms may contribute another dimension to the possibilities for colon carcinogenesis. The data in this report indicate that the colonic mucosal layer is itself able to activate some carcinogens without requiring the intermediacy of the colonic bacteria. The colonic mucosa can activate both benzo(a)pyrene and 2-aminoanthracene to mutagenic and, in these 2 cases, carcinogenic metabolites. These data are consistent with the observation by Autrup et al. (4) of activation of carcinogens by cultured human colon. However, the data in this report in no way exclude a significant role for colonic bacteria in colon carcinogenesis. The almost complete dependence of 1,2-dimethylhydrazine-induced colon tumor development on the presence of intestinal bacteria that was shown by Reddy et al. (24) underscores the role of bacteria in colon carcinogenesis. On the other hand the broad substrate specificity shown for the cytochrome P-450-dependent drug metabolism systems for other tissues (8, 15) and the wide substrate specificity already demonstrated for the rat colon microsomal drug metabolism system (9) suggest an important role for this system in carcinogenesis in the colon. A study of the interaction of the activation and metabolism of various carcinogens and...
anticancer drugs mediated by the colon mucosal layer and intestinal bacteria may provide considerable insight into the variability of colon carcinogenesis and the effect of diet on carcinogenesis. Clearly, the ability of the colonic mucosa to activate carcinogens and other compounds present in the diet must be considered in postulates for the development of colon cancer.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Thomas Matney for many helpful discussions, for constant encouragement, and for making his laboratory and expertise available throughout the pursuit of this project. The authors also wish to thank Maryann Butler for her technical aid and advice and Tracy Lanagan for technical assistance.

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