1,2-Dimethylhydrazine-induced Alterations in \(N^1\)-Acetylspermidine Levels and Spermidine \(N^1\)-Acetyltransferase Activity in Rat Colonic Mucosa

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ABSTRACT

To determine whether alterations in the “reverse” or “conversion” pathway for putrescine formation were involved in the induction of colonic tumors by 1,2-dimethylhydrazine, male albino rats of the Sherman strain were given injections s.c. of this agent (20 mg/kg body weight/week) or diluent for 5, 10, 15, and/or 26 weeks. Animals were sacrificed at each of these time periods and polyamine levels, including \(N^1\)- and \(N^4\)-acetylspermidine, as well as the activities of ornithine decarboxylase, spermidine \(N^1\)-acetyltransferase and polyamine oxidase were measured and compared in rat proximal and distal colonic mucosa of each group.

The results of these studies demonstrated that: (a) \(N^1\)- and \(N^4\)-acetylspermidine levels were similar in the control and treated proximal colonic segments at all time periods examined; (b) \(N^1\)- and \(N^4\)-acetylspermine levels were also similar in the control and treated distal colonic segments at 5 and 10 weeks; (c) at 15 weeks the level of \(N^1\)-acetylspermidine, but not \(N^4\)-acetylspermidine, however, was increased in the treated distal colonic segment secondary to increases in the activity of spermidine \(N^1\)-acetyltransferase; and (d) at 26 weeks, the level of \(N^1\)-acetylspermidine remained higher in treated distal “uninvolved” colonic tissue and were markedly elevated in colonic tumors in both segments. Based on these findings, it would appear that the reverse pathway for putrescine formation may be involved in the 1,2-dimethylhydrazine-induced malignant transformation process of the rat colon.

INTRODUCTION

Colorectal cancer is a major cause of death among patients with internal malignancies (1) and is, unfortunately, incurable in approximately one-half of these patients at the time of initial diagnosis (2). Previous investigators have utilized various chemical carcinogens to induce colonic tumors in experimental animals in order to elucidate the factors involved in the development of these malignancies as well as to determine better methods for the early detection of colonic cancer (3, 4). One such carcinogen, DMH,3 has been used extensively for these purposes (4). This colonic procarcinogen, which requires metabolic activation within the host, produces colonic tumors in virtually 100% of susceptible strains of rodents, with a latency period of approximately 6 months, when administered in weekly s.c. doses of 20 mg/kg of body weight (4). DMH-induced tumors closely parallel human large intestinal cancers in many respects (3, 4), including predilection for development in the distal segment of this organ (4). Moreover, using this model of experimental colon cancer, several laboratories (4–6), including our own (7, 8), have reported various biochemical alterations in rat colonic tissue prior to the development of overt tumors.

In this regard, during the past decade, the naturally occurring aliphatic polyamines, putrescine, spermidine, and spermine, have received considerable attention with respect to their possible role in the malignant transformation process in the colon and other organs (9). Alterations in intracellular polyamine levels in various neoplastic cells, including colonocytes (9) as well as cells undergoing neoplastic transformation by viruses (10) or chemical carcinogens (9, 11, 12), including DMH (12), have been reported. In particular, the activity of ODC, which enzymatically converts ornithine to putrescine (9, 11), and the content of putrescine have been found to be increased in these experiments. Based on these observations, it has been proposed that the increased biosynthesis of intracellular polyamines are intimately involved in the malignant transformation induced in the colon by the chemical carcinogens (9, 11). Additional support for this contention has recently been provided in studies using 2-difluoromethylornithine, an enzyme-activated irreversible inhibitor of ODC (11, 12). In these studies, concomitant administration of 2-difluoromethylornithine significantly reduced the incidence of DMH-induced colonic tumors in mice (12) and azoxymethane-induced colonic cancers in the rat (11).

Recently, a number of laboratories, however, have demonstrated that putrescine can be synthesized not only from ornithine via ODC but also by an alternative route involving the higher polyamines (spermidine and spermine). This “reverse” or “conversion” pathway requires the activities of two enzymes, spermidine/spermine \(N^1\)-acetyltransferase and polyamine oxidase (13, 14). The former enzyme uses acetyl-CoA to convert spermidine into \(N^1\)-acetylspermidine and spermine into \(N^1\)-acetyl spermine (13, 14). The second enzyme, polyamine oxidase, oxidizes the resulting \(N^1\)-acetylspermidine to putrescine and \(N\)-acetyl-3-aminopropionialdehyde (13, 14). The rate-limiting step in this conversion is the acetylase activity (13, 14). Several different lines of evidence in human colon cancer cells (15), as well as in cells undergoing neoplastic transformation by viruses (10, 16) or carcinogens (17), suggest that this reverse pathway may also play a role in neoplastic development. To date, however, studies addressing the possible involvement of this pathway in DMH-induced tumor production in the rat colon have not been performed. The present studies were, therefore, undertaken to determine whether alterations in spermidine/spermine \(N^1\)-acetyltransferase activity of the content of \(N^1\)-acetylspermidine exist in proximal or distal colonocytes of animals treated with DMH for 5, 10, and 15 weeks, i.e., before the development of colonic cancer, as well as in colonic tumors and “uninvolved” tissue after 26 weeks of DMH. The data from these present experiments demonstrate that alterations in \(N^1\)-acetylspermidine can be detected in distal colonocytes of animals treated with DMH for 15 weeks as well as in colonic tumors in both segments (26 weeks) and serve as the basis of the present report.

MATERIALS AND METHODS

Animals. Male albino rats of the Sherman strain weighing 75–100 g were given weekly s.c. injections of diluent or 1,2-dimethylhydrazine dihydrochloride (Sigma Chemical Co., St. Louis, MO) at a dose of 20 mg/kg body weight for 5, 10, 15, and/or 26 weeks. The stock solution for injections consisted of 400 mg of DMH dissolved in 100 ml of water containing 37 mg EDTA and was adjusted to pH 6.5 with sodium hydroxide (7, 8). The animals were maintained on a pelleted diet (Camm...
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Maintenance Rodent Diet, Camm Research Institute, Inc., Wayne, NJ) with water and food ad libitum. One week after the last injection at each time period studied, six to eight animals from each group were killed rapidly by cervical dislocation between 9 a.m. and 11 a.m. and their colons were excised. The cecum from each animal was discarded and the remaining large intestine was divided into two parts, proximal and distal (7). The mucosa from each of these segments was then rapidly scraped off and pooled separately for enzyme determinations and polyamine analyses (see below). Tumors from 26-week animals as well as tissue at least 1 cm from tumors (uninvolved) were also excised and homogenized for analysis of polyamines.

Enzyme Assays. Colonic mucosal scrapings from each segment of each group were homogenized in either 50 mM Tris/HCl, 5 mM DTT, pH 7.4 buffer (ornithine decarboxylase and spermidine-N\(^4\)-acytentransferase) or 100 mM glycine/NaOH, pH 9.5 buffer (polyamine oxidase), using a Brinkmann polytron at a setting of 4 for 60 s (4 × 15 s). Homogenates were then centrifuged at 105,000 × g for 60 min and the supernatants were used as the enzyme source. Protein was determined by the method of Lowry et al. (18), using bovine serum albumin as standard.

Ornithine decarboxylase was assayed by the method of Pegg and Williams-Ashman (19) as modified by Steeves and Lawson (20). The reaction mixture consisted of 50 mM Tris/HCl (pH 7.4), 5 mM DTT, 0.2 mM pyridoxal phosphate, 0.2 μM L-[\(^{14}\)C]ornithine and 0.5–1.0 mg of protein in a final volume of 0.3 ml. The reaction was incubated at 37°C in a 16 × 100-mm glass tube sealed with a rubber stopper supporting a center well (Kontes, Morton Grove, IL). The released \(^{14}\)CO\(_2\) was trapped on a disc of Whatman GFA glass fiber filter paper soaked with 200 μl of NCS (Amersham, Arlington Heights, IL) in the suspended center well. The reaction was stopped at 30 min by injecting 0.5 ml of 2 N HCl through the rubber stopper and it was allowed to sit at room temperature for 30 min to completely trap the released \(^{14}\)CO\(_2\).

The filter paper and center well were then removed and placed in a scintillation vial containing 2 ml of ethanol; 10 ml of Scintiverse E liquid scintillation fluid and radioactivity was measured.

Spermidine-N\(^4\)-acytentransferase activity was assayed by the method of Libby (21) as modified by Shinku et al. (22). The reaction mixture contained 300 nmo1 of spermidine, 10 μmol of Tris/HCl, pH 7.8, 0.9 nmo1 (0.05 μci) of [acetyl-L-\(^{14}\)C]CoA, and 50–100 μg of protein in a final volume of 0.1 ml. After incubation at 30°C for 3–6 min, the reaction was stopped by addition of 20 μl 1 M NH\(_2\)OH/HCl and heating in boiling water for 3 min. Aliquots (50 μl) of reaction mixture were then applied to a Whatman P-81 phosphocellulose filter disc and thoroughly washed with distilled water and then with ethanol on a sintered glass filter. Discs were then dried and placed in liquid scintillation fluid and radioactivity was measured.

Polyamine oxidase was measured as previously described by Holta (23). The reaction mixture contained 100 mM glycine/NaOH, pH 9.5, 0.4 mM [\(^{14}\)C]spermidine, 5 mM DTT, and 0.5–1.0 mg of protein in a final volume of 0.25 ml. Reactions were incubated at 37°C for 60 min and stopped by the addition of 50 μl of 50% trichloroacetic acid. After pelleting the protein with low speed centrifugation, a 25-μl aliquot of supernatant was applied to Whatman P-81 phosphocellulose paper. Polyamines were chromatographed by using 2 M sodium acetate, and bands were visualized by spraying with 0.2% ninhydrin in ethanol. Bands with similar R\(_f\) values to standard polyamines were then cut out and radioactivity was measured. All enzyme reactions were linear with respect to time and protein concentrations used in these assays.

Polyamine and Decarboxylated S-Adenosylmethionine Cellular Levels. Colonic mucosal samples were rapidly scraped into 0.2 M HClO\(_4\) (approximately 1 g tissue wet weight per 5–10 ml of acid). Samples were homogenized as described above, centrifuged at 10,000 × g for 30 min to remove precipitated proteins, and then filtered through 0.22-μm membrane (Millex-GV; Millipore, Bedford, MA). Aliquots of 25–100 μl of the samples were then analyzed for putrescine, spermidine, spermine, decarboxylated S-adenosyl-L-methionine, N\(^4\)-acetylspermidine, N\(^4\)-acetylspermine, and N\(^4\)-acetylcysteine by reversed-phase, HPLC according to the method of Wagner et al. (24). Sample components were found to be stable when kept at 4°C for weeks or –70°C for up to 6 months.

The HPLC system consisted of two pumps, Model 6000A and M45 controlled by a Model 680 automated programmer, WISP 710A autosampler, and a Model 481 spectrophotometer from Waters (Milford, MA). Post-UV detector derivatization of polyamines with o-phthalaldehyde was done with a P-3 peristaltic pump (Pharmacia, Sweden) and derivatives were analyzed in a Model 2070 fluorescence spectrophotometer from Varian (Sugarland, TX) with excitation and emission wavelengths of 340 nm and 455 nm, respectively. Detector outputs were integrated on Model 3393 Hewlett-Packard integrators (Naperville, IL).

Components were separated by using a gradient elution technique. Solvent A consisted of a mixture of 980 ml of 0.1 M NaH\(_2\)PO\(_4\) and 20 ml of acetonitrile adjusted to pH 2.65 with HPLC grade phosphoric acid. Solvent B contained 740 ml of 0.15 M NaH\(_2\)PO\(_4\) and 260 ml of acetonitrile at pH 3.25. Both solvents contained 8 × 10\(^{-4}\) M octane sulfonic acid used as the ion-pairing reagent. Optimal separation of compounds was obtained by using a linear gradient from 90% Solvent A to 100% Solvent B over 50 min, followed by 10 min of isocratic elution at a flow rate of 1.5 ml/min. An Ultrasphere ion-pair column (5 μm particle size, 250 × 4.6 mm inside diameter) from Beckman (Berkeley, CA) was used, fitted with a Guard-Pak precolumn module and C\(_4\) cartridge maintained at 25°C.

o-Phthalaldehyde reagent was made as previously published (24) and was delivered at a flow rate of 1.0 ml/min. The compounds were identified by their relative retention times and quantified by comparison of peak areas to that of known amounts of standard. Peak area responses were linear over the range tested. No internal standard was used as recovery has previously been shown to be quantitative (24).

The identity of N\(^4\)-acetylspermidine was further confirmed by acid hydrolysis and thin-layer chromatography as previously described by Matsu- zaki et al. (25).

Histological Studies. At 5, 10, and 15 weeks, 1-cm proximal and distal colonic segments from each animal of each group were immediately fixed in 4% paraformaldehyde. At 26 weeks, all macroscopic lesions as well as tissue at least 1 cm away from these lesions were also immediately fixed in paraformaldehyde. Fixed specimens were then embedded in paraffin for light microscopic examination and stained with hematoxylin and eosin (7, 8).

Statistical Methods. All results are expressed as mean values ± SE. Paired or unpaired t tests were used for all statistical analysis. A P value <0.05 was considered significant.

Materials. Spermidine, spermine, putrescine, N\(^4\)-acetylspermidine, N\(^4\)-acetylspermine, and N\(^4\)-acetylcysteine were obtained from Sigma Chemical Co. Decarboxylated S-adenosyl-L-methionine was generously provided by Dr. A. E. Pegg, Hershey, PA. Radiolabeled L-[\(^{14}\)C]-ornithine (54.3 μCi/mmole) and [acetyl-L-\(^{14}\)C]CoA (50.6 μCi/mmole) were purchased from New England Nuclear, Boston, MA. [tetramethy- lone-1,4,5]Spermidine trihydrochloride (101 μCi/mmole) was obtained from Amersham. HPLC-grade acetonitrile and octane sulfonic acid were purchased from Alltech Associates, Inc. (Arlington Heights, IL). All other chemicals were of reagent grade and were obtained from Sigma Chemical Co. unless stated otherwise.

RESULTS

Light Microscopic Studies. Earlier studies (3, 4) performed on the effects of DMH on colonic epithelial histology have revealed that carcinoma in situ did not appear until at least 14–16 weeks, microscopic adenocarcinomas developed between 12 and 18 weeks, and macroscopic adenocarcinomas were present in the majority of animals in the distal colon by 18–24 weeks. In agreement with earlier findings from our laboratory (7, 8), despite extensive sampling of the proximal and distal colons of each group, no evidence of severe atypia, carcinoma in situ, or microscopic carcinomas was seen at 5, 10, or 15 weeks by light microscopic examination (not shown). In the present experiments, minimal inflammation was also noted in the proximal and distal segments, was equally distributed in these segments, and did not appear to differ in intensity after 5, 10, or 15 weeks in each group. These results, therefore, indicate that inflam-
mation per se was not responsible for the biochemical alterations noted in the distal DMH-treated colonocytes (see below).

At the 26-week time period, control rats were not found to have any tumors in their entire colon. DMH-treated rats, however, were found to have both proximal (5) and distal (19) colonic adenocarcinomas at this time. One DMH-treated rat was also found to have a single distal colonic tubulovillous adenoma.

Cellular Polyamine Levels. As shown in Tables 1 and 2, after 5, 10, 15, and 26 weeks of administration of DMH, no significant differences were noted in the concentrations of spermidine, spermine, or decarboxylated S-adenosyl-L-methionine in control or treated animals in both the proximal and distal colonic mucosa. Similarly, at the 5- and 10-week periods, no differences were noted in putrescine levels in these segments. At 15 weeks, however, small but significant increases in putrescine were noted in both the proximal and distal segments of DMH-treated animals compared to their control counterparts (Table 1). Furthermore, at 26 weeks the level of putrescine was found to be significantly elevated in uninvolved and tumor tissue in both colonic segments compared to control values (Table 2).

After 5- and 10-week administration of DMH no significant differences were noted in N$^1$-acetylpermium levels in both segments of treated and control animals (not shown). As can be seen in Fig. 1, however, after 15 weeks of treatment with this carcinogen, the distal colonic segment of treated animals, but not the proximal segment, was found to possess significantly greater amounts of N$^1$-acetylpermium than its control counterpart.

Moreover, as shown in Fig. 1, at 26 weeks uninvolved distal colonic tissue continued to demonstrate increased levels of N$^1$-acetylpermium and distal tumors showed marked elevations in N$^1$-acetylpermium compared to control tissue. In contrast to these findings, proximal uninvolved tissue at 26 weeks again showed no increase in N$^1$-acetylpermium levels. At this time, however, proximal tumors were found to possess elevated N$^1$-acetylpermium levels compared to control tissue (Fig. 1). It should also be noted that at each of the time periods examined, N$^8$-acetylpermium levels were not found to differ significantly in either the proximal or distal segments of control and treated animals (not shown) and N$^1$-acetylpermium levels were undetectable.

Enzyme Analyses. After 5 and 10 weeks of DMH administration, no significant differences in ornithine decarboxylase-specific activity were found in either segment of treated and control animals (not shown). After 15 weeks, however, this enzymatic activity was found to be significantly increased (P < 0.05) in both the treated proximal (7.2 ± 1.2 pmol/30 min/mg protein; N = 6) and distal segments (3.5 ± 0.5; N = 6) compared to control proximal (3.4 ± 1.1; N = 6) and distal (1.0 ± 0.2; N = 6) values. Similarly, at 26 weeks, ornithine decarboxylase activity was also found to be significantly elevated (P < 0.05) in both the treated proximal uninvolved (4.8 ± 0.6 pmol/30 min/mg protein; N = 4) and distal uninvolved segments (11.4 ± 1.5; N = 4) compared to control proximal (2.1 ± 0.3; N = 4) and distal (1.1 ± 0.1; N = 4) values. Proximal and distal tumors also showed significant increases in this enzymatic activity compared to control tissue (not shown). These findings would, therefore, at least in part, explain the elevated putrescine levels found in these segments in treated animals at these time periods (Tables 1 and 2).

As shown in Fig. 2, at 5, 10, or 15 weeks after DMH administration, the levels of spermidine N$^1$-acyltransferase were not found to be significantly different in the proximal colonic segment of control and treated animals. In the distal

| Table 1 | Polyamine and decarboxylated S-adenosylmethionine levels of rat proximal and distal colonic mucosa after 5, 10, and 15 weeks of administration of DMH or diluent* | Duration of treatment (wk) | 5 | 10 | 15 |
| --- | --- | --- | --- | --- | --- | --- |
| P | C(D) | C(P) | DMHP | DMHD | C(D) | C(P) | DMHP | DMHD |
| Putrescine | 5.9 ± 0.5 | 5.3 ± 0.7 | 260.1 ± 4.0 | 239.2 ± 12.2 | 240.1 ± 12.1 | 239.2 ± 12.2 | 240.1 ± 12.1 | 239.2 ± 12.2 |
| Spermidine | 4.5 ± 0.3 | 5.0 ± 0.2 | 205.0 ± 3.0 | 205.0 ± 3.0 | 205.0 ± 3.0 | 205.0 ± 3.0 | 205.0 ± 3.0 | 205.0 ± 3.0 |
| Spermine | 6.7 ± 0.6 | 6.6 ± 0.6 | 230.1 ± 31.0 | 230.1 ± 31.0 | 230.1 ± 31.0 | 230.1 ± 31.0 | 230.1 ± 31.0 | 230.1 ± 31.0 |
| Decarboxylated S-adenosylmethionine | 9.1 ± 0.4 | 9.1 ± 0.4 | 308.2 ± 10.0 | 308.2 ± 10.0 | 308.2 ± 10.0 | 308.2 ± 10.0 | 308.2 ± 10.0 | 308.2 ± 10.0 |

* Values represent means ± SE of six separate preparations of each group. Values are expressed in fmol/mg protein.
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Table 2. Polyamine and decarboxylated S-adenosylmethionine levels in uninvolved mucosa and in tumors of rat proximal and distal colon after 26 weeks of administration of DMH or diluent.*

<table>
<thead>
<tr>
<th>Tissue preparations</th>
<th>Putrescine</th>
<th>Spermidine</th>
<th>Spermine</th>
<th>Decarboxylated S-adenosyl-L-methionine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proximal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.1 ± 1.7</td>
<td>343 ± 28</td>
<td>338 ± 25</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Uninvolved</td>
<td>10.2 ± 1.0*</td>
<td>338 ± 28</td>
<td>264 ± 19</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Tumors</td>
<td>27.1 ± 6.1*</td>
<td>338 ± 25</td>
<td>264 ± 19</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td><strong>Distal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.3 ± 0.5</td>
<td>345 ± 16</td>
<td>353 ± 15</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Uninvolved</td>
<td>8.8 ± 0.4*</td>
<td>373 ± 11</td>
<td>349 ± 11</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>Tumors</td>
<td>37.2 ± 2.5*</td>
<td>393 ± 22</td>
<td>313 ± 14</td>
<td>0.8 ± 0.1</td>
</tr>
</tbody>
</table>

* Values represent means ± SE of three separate preparations of each group. Values are expressed in nmol/mg protein. * P < 0.05 compared to control values of similar segment.

FIG. 1. Effect of 1,2-dimethylhydrazine administration for 15 and 26 weeks on the levels of N'G-acetylspermidine in rat proximal and distal colonic segments as well as in proximal and distal control mucosa. ■, values obtained in control mucosa; □, values obtained in DMH-treated, histologically normal (uninvolved) mucosa; ◇, at 26 weeks represent N'-acetylspermidine values in tumor tissue. *, P < 0.05 compared to control values in the same segment at the same time point.

From spermidine via sequential reactions catalyzed by spermidine N'-acytlytransferase and polyamine oxidase exists in a number of different cell types (13, 14, 26–32). Furthermore, acute changes in the activity of spermidine N'-acytlytransferase have been noted in various tissues after a variety of stimuli, including carbon tetrachloride (33), thioacetamide (34), spermidine (14), dialkylnitrosamines (17), folic acid (26), secretin (25), phytohemagglutinin (28), vitamin D3 (30), and trauma (31). Several lines of evidence have also suggested that this pathway may be involved in the malignant transformation process seen in the colon (15) and other tissues (17). Thus, Matsui and Pegg (17) have shown that spermidine-N'-acytlytransferase activity was induced in the liver of rats treated with the carcinogens dimethylnitrosamine and diethylnitrosamine. Levels of N'-acytlypermidine have also been reported to be elevated in human colorectal adenocarcinomas (15). Bachrach and Seiler (10) have demonstrated that spermidine-N'-acytlytransferase levels were increased in Rous sarcoma virus-transformed chick embryo fibroblasts compared to their normal counterparts. Polyamine oxidase activity has also been shown to be altered in kidney cells transformed by the avian sarcoma virus (16). Finally, the urinary excretion of various acetylated polyamines, including N'-acytlypermidine, has been reported to be elevated in patients with several different types of malignancies (35, 36).

DISCUSSION

Recent studies have clearly established that the so-called reverse or conversion pathway for the synthesis of putrescine segment no differences in this enzymatic activity were also noted in these animals after 5 and 10 weeks of carcinogen treatment. After 15 weeks, however, the specific activity of spermidine N'-acytlytransferase in this segment was found to be significantly increased compared to control values.

While no differences were noted in the specific activity of polyamine oxidase in either segment of treated and control animals at 5, 10, and 15 weeks, there appeared to be a tendency for this enzymatic activity to be lower in the distal segment of treated animals at each of these time points (Fig. 3). The latter differences, however, were not found to be statistically significant (P > 0.05).
generalized biphasic increase in intestinal ODC activity during increases in ODC activity, as being of critical importance in the noma model, have also shown that this carcinogen induces a ionic tumors in rats as well as in human colon cancers (40-42). Thus, ODC activity purposes but will obviously require further studies. A related polyamine may have potential in terms of screening week findings, therefore, suggest that elevations of this acety 15-week findings, therefore, suggest that elevations of this acetylated polyamine at this time were reflect the malignant transformation process than any effect of th agent per se. Moreover, the observation that these changes were also seen in the uninvolved distal mucosa away from tumors at 26 weeks further suggests the possibility that some form of “field change” induced in this segment occurred second ary to DMH. These latter data, taken together with the 15-week findings, therefore, suggest that elevations of this acetylated polyamine may have potential in terms of screening purposes but will obviously require further studies. Several aspects of these findings deserve further comment. Since the increases in N\textsuperscript{1}-acetylspermidine were first seen at 15 weeks after DMH administration, at a time when the colonic mucosa was histologically normal and before the development of colon cancer induced by this carcinogen, these alterations can be termed “premalignant.” Along these same lines, the changes noted in this acetylated polyamine at this time were restricted to the distal segment. This is especially interesting in view of the marked predilection for the development of cancers in this colonic segment in animals administered DMH previously noted (4) and confirmed in the present studies. While the exact reasons why there is an increased propensity for this carcinogen to induce tumors in this colonic segment are unclear, this finding lends support to the contention that the distal alterations in N\textsuperscript{1}-acetylspermidine noted may more likely reflect the malignant transformation process than any effect of the agent per se. Moreover, the observation that these changes were also seen in the uninvolved distal mucosa away from tumors at 26 weeks further suggests the possibility that some form of “field change” induced in this segment occurred second ary to DMH. These latter data, taken together with the 15-week findings, therefore, suggest that elevations of this acetylated polyamine may have potential in terms of screening purposes but will obviously require further studies. Previous studies have, in general, stressed the importance of increases in the biosynthesis of polyamines, particularly increases in ODC activity, as being of critical importance in the development of colon cancer (11, 37-42). Thus, ODC activity has been noted to be increased in azoxymethane-induced colonic tumors in rats as well as in human colon cancers (40-42). Luk et al. (11), using the azoxymethane-induced colonic carcinoma model, have also shown that this carcinogen induces a generalized biphasic increase in intestinal ODC activity during tumor development. Based on this biphasic response, these authors have suggested that the initial peak could be associated with initiation while the second peak could be associated with promotional effects of this carcinogen (11). Additionally, recent studies (11, 12), using various colonic chemical carcinogens, have shown that administration of difluoromethylornithine, a specific ODC inhibitor, decreases the incidence of colon cancer induced by these agents. Taken together, these studies would support a role for increases in ODC in colonic malignant transformation.

In the present studies, DMH administration for 15–26 weeks was found to significantly increase ODC activity in both colonic segments. This agent also produced increases in the levels of N\textsuperscript{1}-acetylspermidine at 15 weeks in the distal colon and at 26 weeks in the distal uninvolved mucosa as well as in proximal and distal tumors. Based on these findings, it is likely that at 26 weeks, N\textsuperscript{1}-acetylspermidine levels in colonic tumors were elevated secondary to the increased ODC activity present in these neoplastic tissues. The mechanism(s) responsible for the elevated levels of N\textsuperscript{1}-acetylspermidine at 15 weeks in distal-treated colonic mucosa and at 26 weeks in the distal-treated uninvolved mucosa are, however, unclear at this time. Since ODC was increased in both the proximal and distal colonic segments of treated animals at these time periods, it would appear that the increases in N\textsuperscript{1}-acetylspermidine in the distal segment at 15 and 26 weeks were not simply secondary to increase in the biosynthesis of the polyamines “driving” the reverse pathway. While speculative, the increased levels of this acetylated polyamine in the distal segment at these times may also be due to differences in compartmentalization or transport out of the proximal or distal colonocytes (13, 14). As noted previously (Fig. 3), there was also a tendency for polyamine oxidase activity to be lower in distal-treated tissue than their control counterparts. Additional studies will, therefore, be required to clarify this issue.

Regardless of the mechanism(s) involved, however, it does appear that alterations in the reverse pathway can be detected after 15–26 weeks of DMH administration in the colonic mucosa. In this regard, recent studies (43–46) have suggested that acetylated polyamines, including N\textsuperscript{1}-acetylspermidine, may affect the conformation of DNA, presumably by removal of polyamines from binding sites. For example, N\textsuperscript{1}-acetylspermidine has previously been shown to exert significant structural effects on the B-DNA to Z-DNA transitions of poly(dG-m\textsuperscript{3}dC)-poly(dG-m\textsuperscript{3}dC) and on the helix coil of calf thymus DNA (44). Acetylpolyamines have also recently been shown, using thermal denaturation and circular dichroism techniques, to stabilize and change the conformation of nucleosome core particles, thereby, facilitating replication and transcription in vivo (45). It is, therefore, possible that alterations in the reverse pathway in colonic mucosa of DMH-treated animals may be involved in the pathogenesis of the malignant transformation process induced by this carcinogen. In this regard, however, it should be noted that recent studies utilizing specific inhibitors of polyamine oxidase, which caused marked increases in N\textsuperscript{1}-acetylspermidine levels in various organs of the mouse, failed to induce significant changes in growth or toxic effects over a 6-week period (47). Furthermore, Claverie et al. (48) have recently shown that inhibitors of polyamine oxidase actually improved the antitumoral effect of ODC inhibitors on various cancers. These investigations suggested that polyamines produced by the reverse pathway were used by the tumors in order to cover their polyamine requirements (47).

Based on these latter observations, it would appear that the present alterations noted in the reverse pathway induced by
DMH-induced alterations in acetylated polyamines

DMH in the rat colon are less likely to be causally involved in the malignant transformation process but rather are secondary to this process. Further studies, however, using this experimental model of colon cancer together with specific inhibitors of polyamine oxidase, will be necessary to resolve this issue. Such experiments are now being conducted in our laboratory.

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1,2-Dimethylhydrazine-induced Alterations in $N^1$-Acetylspermidine Levels and Spermidine $N^1$-Acetyltransferase Activity in Rat Colonic Mucosa

Allan G. Halline, Pradeep K. Dudeja and Thomas A. Brasitus


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