ABSTRACT

Potential enhancement of intestinal neoplasia by compensatory mucosal hyperplasia was tested in rats subjected to 50% proximal small bowel resection (PSBR) 10 days after the last of 16 weekly injections of azoxymethane. Azoxymethane alone increased jejunal contents of RNA and DNA each by 26% at 17 to 18 weeks (p < 0.01) before there was macroscopic evidence of neoplasia. Three months after PSBR alone, ileal hyperplasia was characterized by increased amounts of RNA (42 to 76%) and DNA (68 to 95%), taller villi, deeper crypts, and luminal dilation (p < 0.05 to 0.001); however, the colon showed only patchy hyperplasia. When the combined effects of azoxymethane and PSBR were observed 26 to 30 weeks after the first injection, rats with PSBR had an increased number of colonic tumors per animal (2.9 versus 1.6 for controls; p < 0.02). Despite the intense ileal hyperplasia produced by PSBR, ileal neoplasia did not occur. Enhanced colonic carcinogenesis followed sequential exposure of the mucosa to the carcinogen (azoxymethane) and to the promoting factor (PSBR).

INTRODUCTION

Abnormal patterns of cell proliferation in bowel mucosa may predispose to intestinal cancer (7, 15, 16). Since hyperplasia precedes neoplasia in the gut (15, 39), compensatory growth of the ileum and colon after PSBR (23, 24, 41, 43) might enhance intestinal carcinogenesis. Adaptive hyperplasia could explain the enhanced incidence of enteric and colorectal cancer in patients with Crohn's disease (12, 20, 38) and the promotion of chemical carcinogenesis in rodents by chronic mucosal injury (27) or bacterial inflammation (2).

This paper reports the effect of proximal enterectomy on the number and distribution of intestinal tumors produced by azoxymethane in the rat. Azoxymethane has a particular affinity for the colonic mucosa, but at higher dosage it also produces tumors in the upper small bowel; the ileum is seldom affected (35, 36).

MATERIALS AND METHODS

Male Fischer rats (Charles River Breeding Laboratories, Wilmington, Mass.) were received 1 to 2 weeks before the start of the experiment and were housed in cages with open wire-mesh bottoms. The quarters were lighted in alternate 12-hr cycles. Rats received Purina rat chow and water ad libitum.

Rats weighing 130 to 160 g (n = 76) were allocated to 1 of 4 groups. Two groups received azoxymethane (Ash Stevens, Inc., Detroit, Mich.), and 2 groups received the vehicle (water) alone. One group receiving azoxymethane and 1 receiving water alone each underwent PSBR later (Chart 1). Control rats for the resection had no operation.

Weekly s.c. injections of azoxymethane (10 mg/kg/week) or water were given for 16 weeks. Rats from each group were operated upon 8 to 12 days after the last injection. Under light ether anesthesia the small bowel was delivered and was measured from Treitz's ligament to the ileocecal valve. The proximal 50% (about 45 cm) of the small bowel was resected, and intestinal continuity was restored by end-to-end anastomosis with a single layer of 6-0 silk sutures. Two contiguous 5-cm segments of bowel were cut from the center of the resected specimen of proximal small bowel, and mucosal scrapings were obtained as previously described (24, 41, 43). Reliable and reproducible yields of mucosa were obtained by this technique, leaving few residual crypts.

Rats were weighed weekly and were observed for the development of tumors. The number and distribution of tumors were recorded in rats dying only after 26 weeks. Animals were killed when moribund or at the end of 30 weeks. A thorough autopsy was performed with particular inspection of the entire intestinal tract. Tumors and metastases were sent for histological confirmation.

Mucosal scrapings for biochemical analysis were obtained from 5-cm segments of small and large intestine in healthy animals from all 4 groups at the time of sacrifice. Small bowel segments were situated proximal from the ileocecal valve by 39 to 44 cm (upper ileum) and 11 to 16 cm (lower ileum). Large bowel segments were situated distal to the cecum by 3 to 8 cm (right colon) and 14 to 19 cm (left colon). Specimens adjacent to these were stored in formalin for subsequent histological analysis. The internal circumference of the opened small bowel segments was measured to the nearest 0.5 mm.

Mucosal scrapings were frozen in liquid nitrogen within 10 min of death and were stored at -70° for estimation of RNA and DNA contents (11, 30). Histological specimens of small bowel were coded and were used for measurements of villous height and crypt depth. Five villi and crypts sectioned from top to bottom without interruption were...
Promotion of Colonie Neoplasia

Azoxymethane Hlimg/Ky/wk (or vehicle) Control & Vehicle Control & Carcinogen PSBR & Vehicle PSBR & Carcinogen

IO 15 20 25 30 Weeks after first injection

Chart 1. Mean body weight.

measured per section by ocular micrometry. In the colon crypt depth was measured. Student's t test was used for statistical analysis unless otherwise stated. The 1-tailed $\chi^2$ test and Fisher's exact probability test were used for other data.

RESULTS

Weight Gain (Chart 1). Azoxymethane did not affect weight gain until the last few weeks of the experiment when carcinogen-treated rats began to develop cancers in sufficient quantity to cause debility. PSBR caused an initial 10 to 15% loss in body weight, followed by a tendency to return toward control weights.

Mortality. Five rats died of pneumonia during the early weeks of the experiment. Six rats died as a result of PSBR, giving a postoperative mortality rate of 15%. Three unoperated animals receiving azoxymethane died before the 24th week with small bowel obstruction from intussuscepted jejunal tumors. No colonic tumors were found in these rats, and they were not included in the statistical analysis because death occurred before any enhancement of neoplasia by hyperplasia secondary to PSBR was likely to have developed. Rats surviving for 26 to 30 weeks were as follows: 14 of 15 (control plus vehicle); 18 of 22 (control plus azoxymethane); 14 of 17 (PSBR plus vehicle); 16 of 22 (PSBR plus azoxymethane).

Jejunal Adaptation after Azoxymethane (Chart 2). In jejunal mucosa obtained at operation from rats that had received a full course of azoxymethane, RNA content increased 27%, and DNA content increased 26% over values in rats receiving vehicle alone. RNA/DNA was unaltered (1.07 versus 1.10; $p = 0.6$).

Ileal Adaptation after PSBR (Chart 3). Irrespective of preoperative treatment the amounts of both RNA and DNA remained elevated for 3 months after PSBR as compared with values from unoperated controls ($p < 0.001$). Increases were seen throughout except in the lower ileum of rats receiving vehicle. No consistent differences between rats given carcinogen or vehicle were seen in the amounts of RNA or DNA at this time. PSBR increased the mean circumference of upper and lower ileum by 19 to 22% ($p < 0.001$). Villous height was increased by 34% in both upper and lower ileum after operation, and crypt depth was increased by 19% in upper ileum only (Chart 4).

Colonic Adaptation after PSBR (Chart 5). Few differ-

Chart 2. RNA and DNA contents of jejunal mucosa obtained 8 to 12 days after the last of 16 weekly injections of carcinogen or vehicle (mean ± S.E.). The individual values used for these calculations were each derived from the mean of 2 values obtained from contiguous 5-cm segments of bowel. Significance, carcinogen versus vehicle: *, $p < 0.01$; **, $p < 0.05$.

Chart 3. RNA and DNA contents of ileal mucosa in vehicle and carcinogen groups at 30 weeks (3 months postoperative) (mean ± S.E.). Eight to 10 rats were used for each determination. Significance versus unoperated control: *, $p < 0.005$; **, $p < 0.001$. 
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Significant increases were observed in nucleic acid contents in colonic mucosa. PSBR increased right colonic RNA by 23% in rats receiving vehicle and increased DNA throughout the colon by 23 to 48% in rats receiving carcinogen. As in the ileum there was no consistent difference between animals receiving vehicle or carcinogen. Unlike the taller villi and deeper crypts in the ileum, PSBR did not increase the depth of colonic crypts after 3 months (Chart 4).

Small Bowel Tumors. PSBR affected neither the number nor the site of duodenal tumors (Chart 6), most of which arose within 1 cm of the pylorus. In control rats tumors occurred throughout the duodenal loop and within the proximal 12 cm of jejunum. Beyond this point only 2 enteric tumors were found; these were adjacent to one another in the same animal, about halfway between Treitz’s ligament and the ileocecal valve. After PSBR small bowel tumors were restricted to the duodenum, apart from 4 that arose at the site of anastomosis just distal to Treitz’s ligament; 1 of these 4 was associated with a stitch abscess. Three other rats had anastomotic granulomata, but these were discounted. No tumors were seen in the ileum distal to the anastomosis (Chart 6).

Tumors were classified into 3 histological types. Early neoplastic lesions included adenomatous and hyperplastic polyps and foci of severe mucosal atypia. Adenocarcinomas showed papillary, tubular, or mixed histological patterns with varying degrees of differentiation and invasion. The third group of mucinous (colloid) adenocarcinomas was characterized by the presence of signet ring cells. Most duodenal tumors were adenocarcinomas (Table 1), but mucinous cancers predominated in the jejunum and ileum. Nearly all small bowel tumors were larger than 5 mm in diameter.

Large Bowel Tumors. PSBR raised the incidence of colonic tumors from 1.6 to 2.9/rat (p < 0.02) (Chart 6), the increase being mainly in early neoplastic lesions (p = 0.06).
and mucinous adenocarcinomas ($p < 0.05$) (Table 2). PSBR did not affect the size of the tumors; 60% were less than 5 mm in diameter in both the operated and unoperated groups. Frequently, they were multiple, and occasionally they reached 20 to 30 mm in diameter.

In both control and operated groups, tumors most frequently arose in the descending colon (Table 1). As in the small bowel, adenocarcinoma was the commonest histological type overall, varying from carcinoma in situ to invasive cancer (Table 1). Mucinous cancers, usually found adjacent to the large lymphoid follicle in the lower ascending colon, tended to be the most aggressive with extensive local invasion and metastasis. No cecal tumors were seen, and there was relative sparing of the rectum. Colonic obstruction was more commonly caused by intussusception of a polypoid tumor than by annular stricture.

**Ear Canal Tumors.** These occurred with equal frequency in control and PSBR groups and were often bilateral. Larger tumors invaded deeply into the soft tissues of the face and occasionally caused hemorrhage or difficulty in feeding. Histological patterns included sebaceous adenoma, squamous cell carcinoma, and malignant fibrohistiocytoma. One squamous cancer contained areas of metaplastic ossification.

**Metastases.** Duodenal, jejunal, and colonic cancers all frequently metastasized, either to the regional lymph nodes or occasionally to the lung, liver, and peritoneal cavity (Table 3). Lymphatic metastases were commonest (73%). No other sites of primary tumor were found.

### Table 1
**Histological types of tumor in the small and large bowel**

<table>
<thead>
<tr>
<th>Location</th>
<th>Early neoplastic lesions</th>
<th>Adenocarcinoma</th>
<th>Mucinous adenocarcinoma</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small bowel</td>
<td>3</td>
<td>26</td>
<td>9</td>
<td>38</td>
</tr>
<tr>
<td>Duodenum</td>
<td>2</td>
<td>24a, b</td>
<td>2</td>
<td>28</td>
</tr>
<tr>
<td>Jejunum and ileum</td>
<td>1</td>
<td>2</td>
<td>7a, c</td>
<td>10</td>
</tr>
<tr>
<td>Large bowel</td>
<td>13</td>
<td>49</td>
<td>14</td>
<td>76</td>
</tr>
<tr>
<td>Ascending colon</td>
<td>1</td>
<td>7</td>
<td>8a</td>
<td>16</td>
</tr>
<tr>
<td>Transverse colon</td>
<td>5</td>
<td>17d</td>
<td>1</td>
<td>23</td>
</tr>
<tr>
<td>Descending colon</td>
<td>7</td>
<td>25e</td>
<td>5</td>
<td>37</td>
</tr>
</tbody>
</table>

a Commonest for this histological type: $p < 0.001$ (Fisher's test).
b Commonest histological type at this site: $x^2_{18} = 34.58, p < 0.001$.
c Commonest histological type at this site: $x^2_{18} = 29.82, p < 0.001$.
d Commonest histological type at this site: $x^2_{18} = 18.08, p < 0.001$.
e Commonest histological type at this site: $x^2_{18} = 19.68, p < 0.001$.

### Table 2
**Types of colonic cancer found in control and operated rats**

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 18)</th>
<th>PSBR (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early neoplastic lesions</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>23</td>
<td>28</td>
</tr>
<tr>
<td>Mucinous adenocarcinoma</td>
<td>3</td>
<td>11a</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>47b</td>
</tr>
</tbody>
</table>

a $p < 0.05$ versus control.
b $p < 0.02$ versus control.

discussion

Proximal enterectomy virtually doubled the yield of colonic cancers induced by azoxymethane without affecting the incidence of cancers in the small bowel or the ear canal. Raised levels of RNA and DNA in jejunal mucosa at the time of operation show that azoxymethane causes hyperplasia of the intestinal epithelium before the appearance of macroscopic tumors. Preliminary data have shown that dimethylhydrazine causes similar hyperplasia of colonic mucosa (25) in agreement with cytokinetic studies showing increased cell proliferation in rat and mouse colon within a few weeks of the administration of dimethylhydrazine (31, 33, 34). Some of these abnormalities in epithelial cell renewal after the administration of dimethylhydrazine have been seen in patients with familial polyposis either in "normal" colonic mucosa obtained from disease-free relatives or from nonpolypoid areas in affected patients (7, 15, 16).

The enhancement of colonic carcinogenesis by proximal enterectomy presumably depends upon the promoting effect of adaptive hyperplasia on the intestinal epithelium. Although limited hyperplasia of colonic mucosa within 48 hr of jejunal resection is still detectable 1 month after operation (41), this study shows that by 3 months there is little residual hyperplasia in the colon, perhaps because ileal adaptation is complete at that time and has compensated for the loss of proximal small bowel. Ileal resection, which causes more intense colonic hyperplasia (22), may also enhance the development of colonic cancer induced by chemical carcinogens (25).

Alternatively, enhanced carcinogenesis might result from greater concentrations of bile in the fecal stream. Partial enterectomy brings the colon closer to the bile duct, and bile acids and their degradation products may act as carcinogens or cocarcinogens in the colon and rectum (10). Increasing the fecal excretion of bile acids in rats by distal transposition of the bile duct (5, 40) or administration of cholestyramine (21) promotes colonic neoplasia by azoxymethane. On the other hand, biliary transposition also causes adaptive hyperplasia in distal bowel (42). Moreover, our unpublished data suggest that subtotal jejunoileal resection increases the number of neoplasms in proximal small bowel; this phenomenon can more readily be attributed to a consequence of adaptive hyperplasia than to altered luminal concentrations of bile.

Had intestinal resection preceded the course of carcinogen, it might have been argued that any increased tumor yield had resulted from a relatively greater dose of azoxymethane acting upon the remaining shorter bowel. For this reason operation was delayed until 8 to 12 days after the last injection. By that time azoxymethane, which is rapidly
metabolized, should have been cleared from the body. Enhancement of colonic neoplasia thus followed sequential exposure of the bowel to the carcinogen and the cocarcinogenic factors. Our data partly corroborate the concept of Richards (28) of a 2-stage process of cancer induction by dimethylhydrazine. In the first stage (initiation) the carcinogen increases the number of dividing cells, and in the second stage (promotion) it induces malignant transformation of some of the cells from this enlarged population (28).

Hyperplasia therefore both precedes the development of mucosal cancer and predisposes to it, perhaps by further increasing the number of cells at risk of malignant transformation. Consistent with this finding the hyperplastic stimulus imparted to colonic mucosa by Citrobacter freundii reduces the latent period for appearance of tumors after administration of dimethylhydrazine (2). As a corollary mucosal hyperplasia following fecal diversion or the use of liquid elemental diets is associated with a lower incidence of colonic tumors (3, 4, 13).

Despite causing ileal hyperplasia far more intense than the response in the colon, jejunectomy did not make the ileum susceptible to cancer. Intense ileal hyperplasia persisted for 3 months, whether animals received vehicle or carcinogen. Possibly because of its protective immune mechanisms, the ileum is a rare site of adenocarcinoma in humans (6, 17, 26) except in patients with regional enteritis (12, 19). The data suggest that the greater incidence of ileal cancer in Crohn’s disease does not simply reflect mucosal hyperplasia resulting from loss of functioning enteric mucosa. There must be some additional factor specific to this condition, for example, chronic mucosal injury (27), that might be absent in patients with other types of diffuse enterocolic disease or resection in whom no increased incidence of cancer has yet been reported.

The latent period between the administration of dimethylhydrazine or azoxymethane and the appearance of the first tumor is inversely proportional to the dose administered (39). Histological foci of atypia have been reported in murine colonic mucosa as early as 38 days after dimethylhydrazine (33), and tumors have been found in rats from the 15th to 18th week onward (1, 19). Jejunal tumors are often the first to manifest themselves (39).

Mucinous adenocarcinoma was largely confined to the jejunum and ascending colon. In the colon it was usually associated with a large lymphoid follicle, as reported elsewhere (36, 37). As in humans (32) colloid cancers were highly malignant with rapid invasion and metastasis (37). For some reason this type of cancer was commoner after small bowel resection.

Although the production of cancer in rats by azoxymethane cannot necessarily be extrapolated to humans, this study confirms the close resemblance of this experimental model to human colorectal cancer (18, 21, 37). As in familial polyposis a continuing spectrum of histological patterns was observed from mucosal atypia to invasive carcinoma. In both humans (8, 14) and the rat (35), colonic cancers appear to develop entirely from existing adenoma rather than to arise de novo from normal mucosa since small foci of carcinoma in situ are virtually never observed in nonadenomatous mucosa. At a low dose dimethylhydrazine and azoxymethane cause a predominance of tumors in the right colon (25, 29), but at higher doses (as in this study) the left colon is more frequently involved (2, 33, 37). Correspondingly, in humans, communities with a low incidence of colorectal cancer have a relative excess of right-sided lesions, but in high-risk areas the left colon predominates, especially the rectosigmoid region (9).

ACKNOWLEDGMENTS

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REFERENCES


Promotion of Azoxymethane-induced Colonic Neoplasia by Resection of the Proximal Small Bowel


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