

Effects of 1,2-Symmetrical Dimethylhydrazine on Jejuno-colic Transposition in Sprague-Dawley Rats

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ABSTRACT

In this experiment, a segment of the left colon including the upper part of the rectum was transposed to the upper jejunum, and a segment of upper jejunum was transposed to the left colon of the same animal. In another group, the same colon and jejunum segments were transected and reanastomosed in place. A third group served as a normal control. After a recovery period, weekly s.c. 1,2-symmetrical dimethylhydrazine injections were begun. Each animal received a total of 20 injections at a dose of 20 mg/kg. Five weeks after the last 1,2-symmetrical dimethylhydrazine injection, 15 of 19 (79%) of the animals had one or more tumor(s) in the transposed colon segment, while none had tumor in the transposed jejunal segment. Transected and reanastomosed animals showed the same distribution of tumors as did the normal control animals. All three groups had tumors at other sites in the colon and rectum. In addition, about 20% had tumors of the duodenojejunal area. These data indicate that the colonic mucosa is the primary target for the carcinogenic effect of 1,2-symmetrical dimethylhydrazine, independent of other variables such as the fecal stream.

INTRODUCTION

DMH² is widely used in rodents as a colon carcinogen (1-8). A dosage of 20 mg/kg weekly for a total of 20 injections s.c. in SD rats produces colon cancer in the majority of animals. Most of the tumors occur in the left colon and rectum. Occasionally, tumors also appear around the duodenojejunal junction (4-7). These organ specificities are not well understood. Occurrence of carcinoma primarily in the left colon has been attributed to several factors such as the activation of the carcinogen by colonic flora (9-11), the slower turnover rate of the left colon mucosa (9), and less effective repair of damaged DNA (12).

The purpose of this study is to evaluate the significance of luminal *versus* mucosal factors in the process of DMH colon carcinogenesis.

MATERIALS AND METHODS

Equal numbers of 6- to 8-week-old male and female SD rats, 150 to 200 g in weight, were used. Animals were housed 5/ cage, fed with standard mouse and rat diet (Teklad Mills, Winfield, Iowa) containing 4% fat, and provided tap water *ad libitum*. DMH (Aldrich Chemical Co., Milwaukee, Wis.) was

dissolved in normal 0.9% NaCl solution containing 1.5% EDTA, and the solution was adjusted to a final pH of 6.5 with N NaOH. All DMH-treated animals were weighed prior to each injection and received DMH (20 mg/kg) s.c. each week for 20 weeks. Five weeks after the last injection, all surviving animals were killed.

For autoradiography, 100 μ Ci [³H]thymidine (specific activity, 17.9 Ci/mmol) were injected i.p. 1 hr before animals were killed, and tissue specimens were fixed in 10% formalin. Slides were coated in thick emulsion (Ilford T-4; Polysciences, Inc., Warrington, Pa.) for 3 weeks, developed, and stained with hematoxylin and eosin. Fresh specimens were obtained and fixed in 4% glutaraldehyde solution for electron microscopy studies.

Equal numbers of male and female SD rats were randomly assigned to the following groups: Group 1, normal control plus normal 0.9% NaCl solution-EDTA (16 animals); Group 2, normal animal plus DMH (28 animals); Group 3, jejuno-colic transposition plus normal 0.9% NaCl solution-EDTA (14 animals); Group 4, jejuno-colic transposition plus DMH (30 animals); Group 5, transection control plus normal 0.9% NaCl solution-EDTA (12 animals); and Group 6, transection animal plus DMH (21 animals).

Surgical Technique. Midline laparotomy was done under light ether anesthesia. A 4- to 6-cm segment of left colon, including the upper rectum, was transected with its blood supply intact. An equal-length segment of upper jejunum (25 cm distal to ligament of Treitz) was likewise prepared. The colon segment was then anastomosed in place of the jejunal segment, and the jejunal segment was anastomosed in the colon (Fig. 1). Anastomoses were done with continuous one-layer 7-0 silk suture.

In the transection groups, similar segments were prepared and anastomosed back in their original positions. Food was removed 12 hr before surgery and was returned 36 hr after surgery. DMH injections were begun 12 to 15 days after surgery. Control animals received similar treatment of normal 0.9% NaCl solution-EDTA, s.c. (0.5 ml).

RESULTS

Surgery and DMH treatment had no effect upon weight gain ($p > 0.005$). After a recovery period of 7 to 10 days from surgery, steady weight gain was observed in all surgical groups. After the 21st week, all DMH-treated animals exhibited diarrhea. In addition, by the 25th week, bowel obstruction and occasional bloody stools were observed.

Groups 1, 3, and 5 showed no evidence of tumor in either the gastrointestinal tract or at the injection site. In Group 2 (normal animals plus DMH), 20 animals survived through the 25th week. No tumors were seen at the site of injection. However, all animals had one or more colon tumors, the ma-

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² The abbreviations used are: 1,2-symmetrical dimethylhydrazine; SD, Sprague-Dawley.

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majority of which were localized in the left colon and rectum. Four animals also showed tumors of the duodenojejunal junction (Fig. 2; Table 1).

In the jejuno-colic transposition group, 19 animals survived to the 25th week. Mortality in this group was 36.7%, the most common causes being obstruction and anastomotic leaks. Again, there were no tumors at the injection site. However, 15 of 19 (79%) had one or more tumors in the transposed colon segment, but none had tumors in the transposed jejunal segment. The majority of animals had a dilatation above the colo-jejunal anastomosis. Tumors as large as 2 cm were seen in this area (Fig. 3; Table 2). This dilatation most likely was due to partial obstruction of the colojejunal anastomosis. Additional tumors were seen in other areas of the colon and rectum.

Group 4 (transsection) showed a tumor distribution similar to that of Group 2 (Table 3). There was one tumor at the proximal colonic anastomosis and one tumor at the proximal jejunal anastomosis.

Histological Findings. The main characteristics of the colon and jejunum were constant after transposition. Slight mucosal hyperplasia was seen in the transposed colon segment, whereas slight mucosal atrophy was noted in the transposed jejunum. Transsection and reanastomosis had no significant effect on the histological features of the bowel. In the dilated segment above the colojejunal anastomosis mucosal hypertrophy, submucosal inflammatory infiltrate was a common finding.

Autoradiographic studies of control animals showed no changes in the proliferation zone of the mucosa after transposition (20% of cells labeled in normal colon crypt *versus* 23% in transposed colon; 40% of cells labeled in normal jejunum crypt *versus* 36% in transposed jejunum). These differences were not statistically significant. After DMH treatment, significant elongation and widening of the crypts and labeling of the surface epithelial cells were observed in both normal and transposed segments.

Electron microscopic studies confirmed the slight hyperplasia of transposed colonic mucosa as increased numbers of microvilli, mitochondria, and goblet cell activity. Slight atrophy

Table 3
Tumor distribution in transection group

Localization of tumor	No. of animals	No. of tumors	Benign	Malignant
Duodenojejunal junction	4	4		4
Cecum	1	1		1
Ascending colon	5	6	2	4
Transverse colon	3	3	2	1
Left colon and rectum	14	18	6	12

of transposed jejunal mucosa was similarly confirmed. Following DMH treatment, the primary electron microscopic finding was loosened cellular interdigitation, clubbing of microvilli, and increased vacuolization of the epithelium.

The majority of the tumors in the cecum and ascending colon were poorly differentiated and signet ring cell carcinomas. However, tumors located in the left colon and rectum were well-differentiated papillary carcinomas. Multiple small, invasive carcinomas were common along with adenomas. No malignant degeneration of benign lesions was seen in this experiment. Similar findings were reported by Chang (3) and Pzharisski *et al.* (25).

Three lung metastases (5.4%) and 11 lymph node metastases (12.7%) were seen in the entire DMH-treated groups, but no liver metastases were observed. No sex difference was seen in colon tumor incidence.

DISCUSSION

Epidemiological studies have shown that environmental and dietary factors have an important role in the etiology of colon cancer (13–16). High-fat and animal protein diets appear to increase risk for colon cancer predominantly of the left side (17, 18, 20). Experimental animal studies have shown that high-fat and protein diets tend to promote DMH colon carcinogenesis (19, 21, 22). However, no study using high-fat and protein diets or increased bile salt administration has produced colon tumors without the addition of a carcinogen (23).

In the present study, in order to evaluate the importance of luminal factors, the most susceptible target segment (left colon) was transposed into the upper jejunum, where the luminal factors are significantly different from those of normal location. Similarly, a more resistant segment of jejunum was transposed into the left colon to be exposed to a more likely carcinogenic environment. If the luminal factors were the dominant ones for carcinogenesis, tumors would be expected to occur in the transposed jejunum rather than the transposed colon segment. Contrary to this expectation, tumors occurred exclusively in the transposed colon segment (Fig. 4) in the colon proximal and rectum distal to the transposed jejunum (Fig. 5). No tumors occurred in the transposed jejunum. There were tumors very close to the anastomosis line on the rectum (Fig. 6). These results support those of Gennaro *et al.* (7). Diversion of the fecal stream by colostomy decreases but does not prevent occurrence of tumors in the distal colon (26). These findings and our data indicate that colon mucosa is susceptible to DMH carcinogenesis independent of luminal factors.

Increased incidence of tumors in the dilated splenic flexure was noted. We have no explanation of this finding. However, chronic irritation and inflammation which was noted may play

Table 1
Tumor distribution in Group 2 (normal animal + DMH)

Localization of tumor	No. of animals	No. of tumors	Benign	Malignant
Duodenojejunal junction	4	4		4
Cecum				
Ascending colon	8	9	3	6
Transverse colon	5	6	2	4
Left colon and rectum	16	22	6	16

Table 2
Tumor distribution in jejuno-colic transposition Group 4

Localization of tumor	No. of animals	No. of tumors	Benign	Malignant
Transposed colon segment	15	24	5	19
Cecum	2	2		2
Ascending colon	3	4	1	2
Transverse colon	4	4	2	2
Dilated splenic flexure	16	19	3	13
Transposed jejunal segment				
Rectum	10	14	4	10

a role. Uncomplicated healing of anastomosis did not increase tumor incidence.

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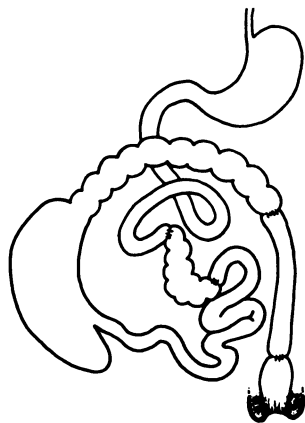


Fig. 1. Jejunocolic transposition.

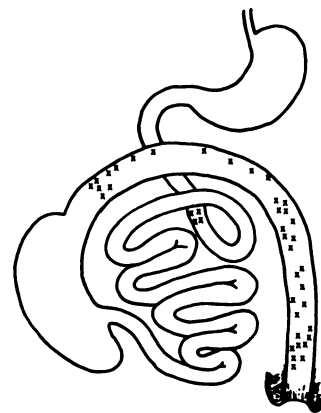


Fig. 2. Distribution of tumors in normal control animals.

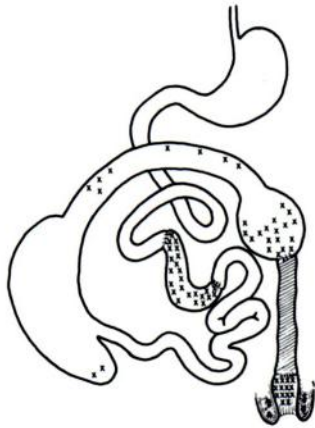


Fig. 3. Distribution of tumors in jejuno-colic transposition.

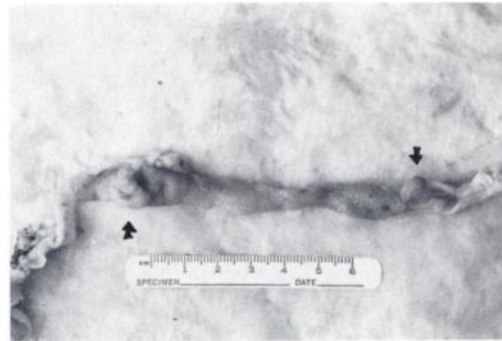


Fig. 5. Ulcerated tumor in the colon proximal to the anastomosis and polyploid tumor distal to the jejuno-rectal anastomosis.

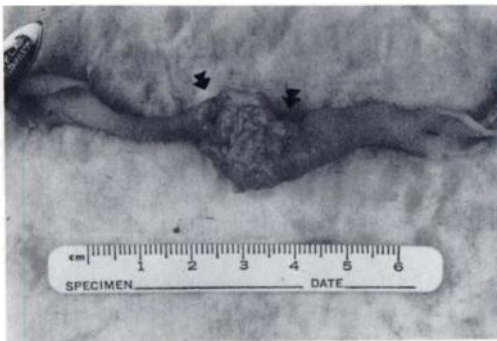


Fig. 4. Ulcerated carcinomas in the transposed colon segment close to both anastomoses.



Fig. 6. Polyploid tumor of the rectum very close to the anastomosis line (arrow).