RELATIONSHIP OF EXPERIMENTALLY INDUCED INTESTINAL TUMORS TO LAXATIVE INGESTION

JOSEPH C. CLEVELAND, MD, AND JACK W. COLE, MD

Colonic neoplasms are produced in the rat by repeated injection of 3:2′-dimethyl-4-aminodiphenyl. The mechanism presumably is by contact of carcinogen with the mucosal cell. This animal model was used to determine whether change in transit time and stool consistency might alter the induction of colonic neoplasia. Five percent magnesium sulfate solution substituted for drinking water was used to cause decreased transit time and watery diarrhea in a group of animals receiving the carcinogen. No difference was noted in incidence of neoplasia. It is concluded that length of contact time and concentration of carcinogen as induced by laxative ingestion is not a limiting factor in the induction of colonic tumors in the animal model used.

INTESTINAL NEOPLASMS ARE INDUCED IN THE rat by repeated subcutaneous administration of 3:2′-dimethyl-4-aminodiphenyl.[1-3, 6, 7] These tumors occur predominently in the colon. Adenomas are more common but adenocarcinomas also develop. Prior work strongly suggests that this carcinogen acts by contact with the intestinal mucosa.[1, 2, 4] In an earlier experiment, we showed that lack of mucosal contact with the fecal stream prevented neoplasm formation.[1, 2] This work was confirmed by others.[4] The present experiment was designed to determine if diminution of contact time of feces (presumably containing the carcinogen) with the intestinal mucosa and decreased concentration of the carcinogen in the stool would significantly modify the incidence of intestinal neoplasia. Oral administration of magnesium sulfate, a laxative, was used to study this question in this biologic model system in which a high rate of intestinal neoplasia develops.

METHOD

Thirty Wistar rats (half male and half female) were obtained from a relatively homogeneous but open colony and were randomly divided into 2 groups. Animals weighed about 175 gm at the start of the experiment; they were weighed weekly and observed for signs of illness. If they had gained weight and appeared healthy, they received a weekly subcutaneous injection of 3:2′-dimethyl-4-aminodiphenyl dissolved in peanut oil (4 cc chemical diluted to 100 cc with peanut oil). Usual amount injected was 1/2 cc. One group of animals (CONTROLS) received tap water for drinking purposes throughout the experiment; the other group (EXPERIMENTALS) received 5% magnesium sulfate (5 gm $MgSO_4$ made up to 100 ml in tap water) for drinking purposes from 9 am Monday morning till 5 pm Friday. Tap water was allowed for both groups from 5 pm Friday to 9 am Monday. Animals were sacrificed 6 months after the study began, or if they appeared seriously ill, or when passage of bloody stools or abdominal distention signaled the presence of an intestinal neoplasm. Autopsy was performed if the animal was found dead in the cage. Animals who died before 3 months of injections were discarded from the experiment.

Representative stools were obtained (immediately after passage) from both control and experimental animals. Wet weight and dry weight (after 24 hr in a vacuum-drying oven) of these stool specimens was determined. The percentage water concentration (by weight) was calculated.

Animals from both experimental and control groups were force-fed carmine red dye through a stomach tube. The stomach-to-anus transit time was then noted.

From the Department of Surgery, Yale University School of Medicine, 333 Cedar Street, New Haven, Conn. 06510.

Supported by American Cancer Society, Grant #T-169E. Dr. Cleveland is an Advanced Clinical Fellow, American Cancer Society.

Received for publication November 22, 1968.

RESULTS

Control animals passed firm, dessicated, pellet-like stools, normal for rats. After 12–24 hr of ingestion of 5% magnesium sulfate solution, the experimental group passed watery, formless stools and continued to do so until tap water was substituted. The water concentration (% of wet wt) of normal rat pellets (control animals) ranged from 50–58%. The average was 54%. The water content of diarrheal stool from erperimental animals ranged from 63–86%. The average water content was 76%.

The stomach to anus transit time for control animals after being force-fed carmine red ranged from 16–20 hr. The average was 18 hr. For experimentals, the range was 2–11 hr, with an average of 9 hr.

The experimental group of 13 animals showed 5 (38%) that developed intestinal neoplasms. Two rats had multiple tumors. One of these developed 17 separate polypoid colonic tumors (Fig. 1). The second rat had 3 separate lesions (colonic, ileal, jejunal). These 2 instances represent the greatest tendency toward multiplicity of neoplasm formation that we have seen in approximately 200 animals. The animal with 17 separate, gross, polypoid tumors is quite unique. Although synchronous (double) neoplasia was seen before, the usual finding was a single neoplasm.

FIG. 1. Rat colon (gross specimen) showing 17 separate tumors.

The control group showed that 5 of 11

TABLE 1.

Animal number	Sex	Time between 1st injection and death	Total wt. gained	Total cc's of carcinogen soln	Gross findings
1E	F	235 days	+128	9 1/2	Tumor—colon Tumor—ileum Tumor—jejunum
2E	M	206 days	+87	8	No tumor
3E	F	206 days	+54	7 1/2	Tumor—distal colon
4E	M	206 days	+40	8 1/2	No tumor
5E	M	235 days	+228	12	No tumor
6E	F	235 days	+84	11 1/4	Tumor—ileum
7E	F	238 days	+152	9 1/2	Large acoustic sebaceous tumor—multiple tumors entire colon.
8E	M	209 days	+151	10	No tumor
9E	M	209 days	+202	9 1/4	No tumor
10E	F	209 days	+184	11 1/2	No tumor
11E	F	210 days	+148	9 3/4	No tumor
12E	M	210 days	+96	9	Tumor—mid colon
13E	F	210 days	+107	10	No tumor
1C	F	234 days	+9 GMs	8 3/4	Tumor—ileum
2C	M	234 days	+77	11	No tumor
3C	M	224 days	+13	7 1/4	No tumor
4C	F	225 days	−26 GMs	8 1/4	Tumor—right colon
5C	F	234 days	+70	9 1/4	Acoustic sebaceous gland tumor; no other tumor
6C	M	204 days	+77	7 1/4	Tumor—distal colon Tumor—rectum Tumor—terminal ileum
7C	M	188 days	+64	7 3/4	No tumor
8C	M	202 days	−30 GMs	7 3/4	Tumor—distal colon
9C	F	199 days	+50	7 1/2	Two breast tumors
10C	M	181 days	+30	6 1/4	No tumor
11C	F	205 days	+87	8	Tumor—rectum

TABLE 2.

Animal number (experimentals)	Site of lesion	Histopathology
1E	Jejunum	Adenocarcinoma
1E	Ileum	Adenocarcinoma
1E	Colon	Adenoma
3E	Colon	Adenocarcinoma
6E	Ileum	Adenoma
7E	Colon and rectum	Multiple adenomas and adenocarcinomas
12E	Colon	Adenocarcinoma
Animal number (controls)	**Site of lesion**	**Histopathology**
1C	Ileum	Andeocarcinoma
4C	Colon	Adenoma
6C	Ileum	Adenoma
6C	Colon	Adenoma
6C	Rectum	Adenoma
8C	Colon	Adenocarcinoma
11C	Colon	Adenoma

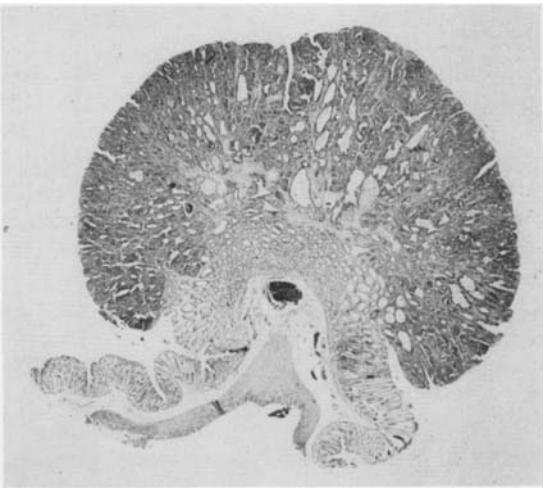

FIG. 2. Typical adenoma of colon induced by 3:2'-dimethyl-4-aminodiphenyl (low power).

animals (45%) developed intestinal neoplasms. One of these animals had multiple (3) tumors.

A tabulation of results is given in Tables 1 and 2. Table 1 shows the animals' sex, the length of time of drug administration, the total weight gained or lost, the amount of carcinogen received, and the gross findings at autopsy. Table 2 shows the site of tumefaction and the histopathology of the neoplasms.

Fig. 2 represents a characteristic adenomatous lesion viewed with low magnification. Fig. 3 shows a typical adenocarcinoma.

The difference in incidence of tumefaction for the experimental and control animals is not significant (p 0.5).

DISCUSSION

In experiments with other carcinogenic aromatic amines, a definite threshold dosage is necessary before tumor induction will occur.[8]

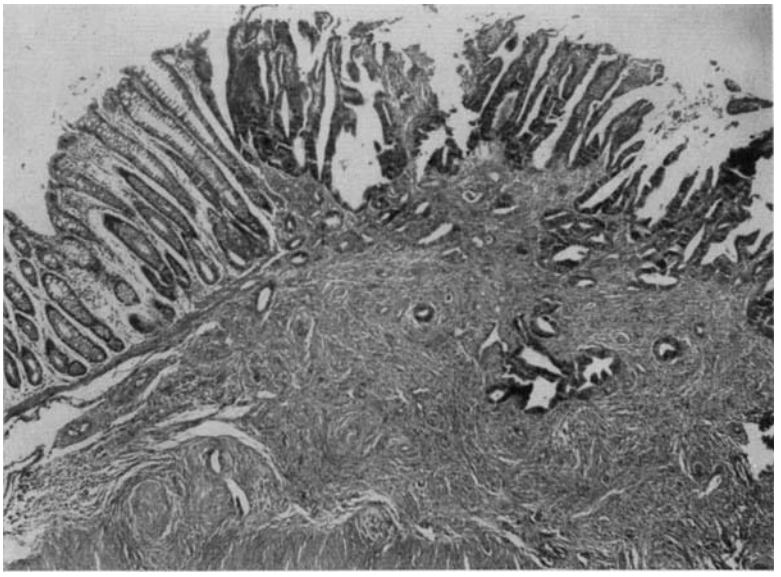

FIG. 3. Adenocarcinoma of colon induced by 3:2'-dimethyl-4-aminodiphenyl (high power).

The ingestion of 5% magnesium sulfate solution by the rat results in prompt induction of a diarrheal state. Thus, we were interested in whether or not the change in transit time and water concentration of stool might alter the threshold in the fecal stream of this aromatic amine, 3:2′-dimethyl-4-aminodiphenyl. There was no significant difference in the percentage of animals developing neoplasms of the GI tract between the experimental and control groups. Thus, it seems that the increased water content of diarrheal stool achieved by laxative ingestion in our experiments was not effective in lowering the carcinogen's concentration to less than a threshold level.

Animal 8E presents a striking situation. This animal's colon harbored 17 polypoid lesions, most of them adenomas. However, there were also carcinomas. The tumors were limited to the colon. This is the only animal in a total of about 200 (in several experiments) that has shown this degree of multiple involvement.

The role of chronic laxative ingestion as an etiologic factor in human colonic cancer has remained controversial. The use of laxatives is certainly widespread. Mineral oil was highly suspect for a time when it contained many organic impurities (some proven carcinogenic hydrocarbons). It is less suspect now that purification procedures and standardization is practiced in its manufacture. In a study of normal mice, feeding of 6 different cathartics until the animal reached an advanced age of 24 months gave no indication of any carcinogenic effect.[5] Our study shows no reduction in incidence of neoplasia related to laxative ingestion.

REFERENCES

1. Cleveland, J. C., and Cole, J. W.: Identification of the route of action of the colonic carcinogen 3:2′-dimethyl-4-aminodiphenyl. *Surg. Forum* 17:314, 1966. 52nd Clinical Congress, Amer. Coll. Surg.

2. ———, Litvak, S. F., and Cole, J. W.: Identification of the route of action of the carcinogen 3:2′-dimethyl-4-aminobiphenyl in the induction of intestinal neoplasia. *Cancer Research* 27:708, 1967.

3. King, E. S. J., and Varasdi, G.: Experimentally induced tumors of the intestine. *New Zeal. J. Surg.* 29:38, 1959.

4. Navarrete-Reyna, A., and Spjut, H. J.: The Effect of colostomy on experimentally produced neoplasms of the colon of the rat. *Fed. Proc.* 25:2, 1966.

5. O'Gara, R. W.: Personal Communication.

6. Spjut, H. J., and Spratt, J. S.: Endemic and morphologic similarities existing between spontaneous colon neoplasma in man and 3:2′-dimethyl-4-aminobiphenyl induced colonic neoplasms in rats. *Ann. Surg.* 161:309, 1965.

7. Walpole, H. L., Williams, H. M. C., and Roberts, D. C.: The carcinogenic action of 4-aminodiphenyl and 3:2′-dimethyl-4-aminodiphenyl. *Brit. J. Indust. Med.* 9:255, 1952.

8. Weisberger, J. H.: Carcinoma of the Alimentary Tract. Salt Lake City, University of Utah Press, 1965, p. 114.