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Intestinal Tumors in Mice Treated with a Single Injection of *N*-Nitroso-*N*-butylurea[1]

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SUMMARY

N-Nitroso-*N*-butylurea was injected once at dosage levels of 150 or 75 mg/kg into 3- or 6-week-old male C57BL/6 mice. Intestinal tumors occurred in 100% of the mice that survived more than 15 weeks after injection with a high dose of *N*-nitroso-*N*-butylurea at 6 weeks of age, and in 35 to 70% of the mice in other treatment groups. These intestinal tumors were seen primarily at the junction of the pylorus and duodenum and in the anterior portion of the small intestine, with a few in the cecum, colon, and rectum. The tumors at the junction were not very invasive tumors and frequently appeared as polypoid growths. Tumors not at this location were adenocarcinomas that invaded all the layers of the gut wall but which did not metastasize. Colorectal tumors were adenomas and adenocarcinomas. *N*-Nitroso-*N*-butylurea also induced tumors of the stomach, hematopoietic system, lung, and liver.

INTRODUCTION

Nitrosoureas are generally potent carcinogens, some of which have induced tumors of the brain, leukemia and other organs with only 1 exposure in rats and mice (7, 14). After BNU[2] induced s.c. sarcomas in mice (2), it was found to cause leukemias and other types of tumors in rats and mice by p.o. administration (4, 6, 9, 10, 18, 22-24), but all after repeated exposure. In this study we wished to determine whether there would be any effect from 1 injection of BNU in weanling and adolescent mice.

MATERIALS AND METHODS

BNU was synthesized by nitrosation of *n*-butylurea (21). Weanling male C57BL/6 mice were obtained from Laboratory Animal Supply Co., Indianapolis, Ind. One hundred fifty or 75 mg of BNU were dissolved in 10 ml of Mazola liquid corn oil (Best Foods, Englewood Cliff, N. J.). Weanling (3-weeks-old) or adolescent (6-week-old) mice were given i.p. injections of the carcinogen at dosage levels of 150 or 75 mg/kg. Groups of untreated control and vehicle-treated mice were also maintained. Mice were housed 10/cage in polycarbonate cages with filter tops, on hardwood bedding, and were fed Wayne Lab Blox (Allied Mills, Chicago, Ill.) and weighed weekly. Surviving mice were killed 67 weeks after the injection. All animals were necropsied. The entire gastrointestinal tract was examined carefully, and individual tumors were placed in Bouin's fixative. Other tissues were placed in buffered neutral formalin; routine techniques were used to prepare histological slides.

RESULTS

There was a dose response to the injection of BNU in mice in both age groups. Animals given BNU at a level of 150 mg/kg lost weight for the 1st 1 to 2 weeks, then gained weight at a normal rate. However, they weighed 15 to 20% less than control mice. Mice given BNU at a level of 75 mg/kg gained weight at a rate 10% less than that of the control mice.

A few mice in each treated group died 1 to 2 weeks after injection with lesions of bronchial and bronchiolar hyperplasia and inflammation. The 1st tumors, lymphomas, appeared after 23 weeks in the mice given injections as weanlings, and after 15 weeks in the older mice. Thymic lymphomas generally produced signs of interference in respiration. The 1st intestinal tumor was detected at 31 weeks when a mouse died with an intussusception caused by a mass in the small intestine. Generally, however, intestinal tumors did not cause death. The number of mice of each group surviving to 67 weeks is presented in Table 1.

Pathology. The tumors found in mice of each group are given in Table 1. There was a dose response to the carcinogen as evidenced by the number of intestinal tumors. Mice given injections at 6 weeks of age were more susceptible to intestinal tumor induction, although a few other types of tumors were also noted.

The distribution of tumors in the intestines is shown in Chart 1. There was no effect of dosage on tumor distribution. Most of the tumors were in the doudenum and jejunum. Mice given injections at 6 weeks of age had more tumors in the duodenum at the junction of the pylorus. Frequently, there was more than 1 tumor in the same mouse. Cecal, colon, and rectal tumors were seen in a few

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[2] The abbreviation used is: BNU, *N*-nitroso-*N*-butylurea.

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Table 1

Tumors in mice treated with a single injection of BNU

Age at injection (wk)	Dosage rate (mg/kg)	Initial no. of mice	After 15 wk: Mice surviving	After 15 wk: No. with intestinal tumors	After 15 wk: No. of intestinal tumors	At wk 67: Mice still alive	At wk 67: No. with intestinal tumors	At wk 67: No. of intestinal tumors	At wk 67: Intestinal tumors/mouse with intestinal tumors	Mice with leukemia and lymphoma	At wk 15–67, mice with tumors of the: Lung	Liver	Stomach	Miscellaneous
6	75	20	20	14	17	18	13	13	1.0	2	2	1	1	1
	150	20	17	17	34	5	5	14	2.8	4	4	2	3	0
3	75	20	17	6	8	15	7	9	1.2	1	10	3	2	0
	150	20	16	10	18	9	6	12	2.0	5	10	1	1	1
Untreated controls		20	20	1	2	19	0	0	0	0	0	0	0	0
Vehicle controls		20	20	4	5	19	4	5	1.2	0	0	1	0	0

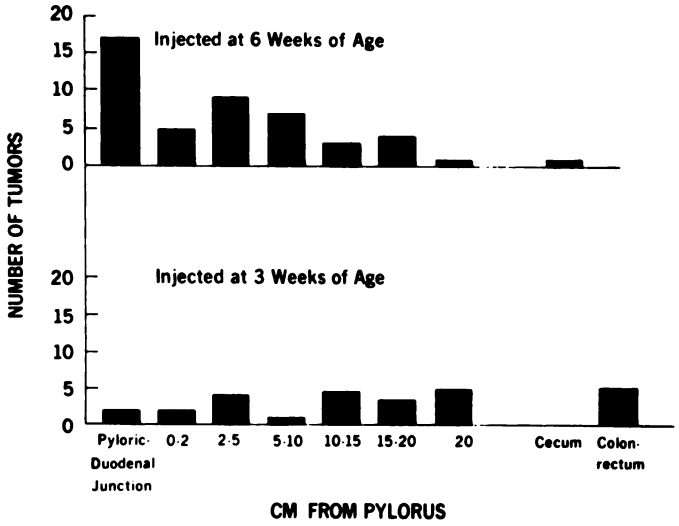

Chart 1. Shown is the distribution of tumors in the intestines of male C57BL/6 mice given 1 injection of BNU at dosage levels of 150 or 75 mg/kg. There was no effect of dosage on distribution. Includes animals dying prior to or sacrificed at 67 weeks after injection. Six of 7 intestinal tumors in control mice were at the junction of the pylorous and duodenum.

Table 2

Classification of tumors of the small intestine induced by BNU, according to depth of invasion

Includes mice of all treatment groups.

Depth of invasion	No. of tumors induced in: Duodenum, jejunum, and ileum	No. of tumors induced in: Pyloric-duodenal junction
Mucosa	19	16
Submucosa	9	2
Tunica muscularis	15	1
Serosa	12	0

mice. All tumors of the small intestines were histologically similar. They appeared to develop from surface foci of atypical and basophilic epithelial cells (Fig. 1). As these foci enlarged, the tumor grew deeper into the intestinal wall, until the serosa was invaded (Table 2; Figs. 2 to 4). Cystic areas, fibrosis, and lymphocytic foci were noted in larger tumors. However, tumors did not metastasize. Tumors of the doudenum at the junction of the pylorus tended to be less malignant than those of the remaining portion of the small intestine (Table 2). Early lesions were associated with cystic dilations of Brunner's glands (Fig. 5). Most tumors formed as polypoid growths (Fig. 6), and a few invaded the intestinal wall. Although these junctional tumors appeared less malignant than tumors in other portions of the small intestine, the cytology of the surface epithelium of these tumors and of the more malignant ones appeared similar. The cells were basophilic, with hyperchromatic nuclei. There was loss of polarity, and the degree of differentiation varied, although tall columnar cells with much cytoplasm were more common in junctional tumors.

Tumors in the cecum, colon, and rectum were usually polypoid adenomas that did not invade the intestinal wall (Fig. 7). One rectal adenocarcinoma was accompanied by diffuse hyperplastic lesions (Fig. 8).

Pulmonary adenomas were primarily induced by BNU in mice given injections at 3 weeks of age, and these tended to be multiple, with the maximum number in 1 lung being 6. Stomach tumors were papillomas of the forestomach, except for 1 small adenocarcinoma of the glandular portion. Hematopoietic tumors included thymic lymphomas, granulocytic leukemias, and reticulum cell sarcomas of Peyer's patches in the small intestine. Liver tumors were well-differentiated hepatocellular neoplasms that did not metastasize.

Untreated and vehicle control mice developed some intestinal tumors, almost all located in the duodenum at the

junction of the pylorus. Only 1 tumor was in the more distal portion of the duodenum.

DISCUSSION

There have been several previous reports on the induction of leukemias and lymphomas in rats and mice by BNU, as well as reports of several other types of tumors (4–6, 10–13, 18, 23, 24) such as those of the liver and mammary gland. However, this is the 1st report on the induction of a high incidence of intestinal tumors by a single injection of a nitrosourea. In 1 previous study with BNU (24), a single s.c. injection in newborn rats led to many neurogenic tumors. An analog of BNU, namely *N*-methyl-*N'*-nitro-*A*-nitrosoguanidine, in which the carbonyl (=O) group of the urea is replaced by an imino (=NH) group, has induced intestinal tumors in rats (8, 16). However, in order to obtain these neoplasms, repeated intrarectal instillation of the carcinogen was necessary. Additionally, since the tumors were found only in the region exposed to the carcinogen, direct contact with the *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine may be implicated as the cause of tumor formation.

In this study, there was a difference in response to BNU in the 3-week and 6-week-old mice. The older mice were more susceptible to intestinal tumor induction, and doudenal tumors at the pyloric junction occurred primarily in this group. The younger mice developed more lymphomas and lung tumors. A similar tendency of younger mice had been observed previously with urethan and other carcinogens (19).

The duodenal tumors in the older mice appeared benign and were similar to spontaneous tumors reported in C57BL/6 mice (15). The small intestinal tumors were similar to those induced by other chemicals in mice (17), although no mucinous carcinomas or metastases were found in our study. The colorectal tumors were also identical to some spontaneous tumors in this mouse strain (3).

With p.o. exposure, BNU generally leads to leukemia and lymphoma and a few intestinal tumors (20), while injection into mice yields many intestinal tumors and few leukemias. An analogous situation holds with dibutylnitrosamine where administration p.o. leads mostly to liver and esophageal tumors and a few bladder lesions. Conversely, s.c. injection of this latter carcinogen gives practically all bladder tumors and a few liver tumors (1). Thus the route of administration has a profound effect on the main target sites not only with dibutylnitrosamine but also with BNU.

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Fig. 1. Focal areas of early neoplastic changes on the luminal surface of the small intestine (*arrows*). H & E, × 140.

Fig. 2. Umbilicated 6-mm adenocarcinoma in the jejunum. There is no invasion into the tunica muscularis, although inflammatory lesions are noted. H & E, × 25.

Fig. 3. Mass (11 mm) in ileum near cecum. Cystic dilation of adenocarcinoma invading to the serosa. H & E, × 15.

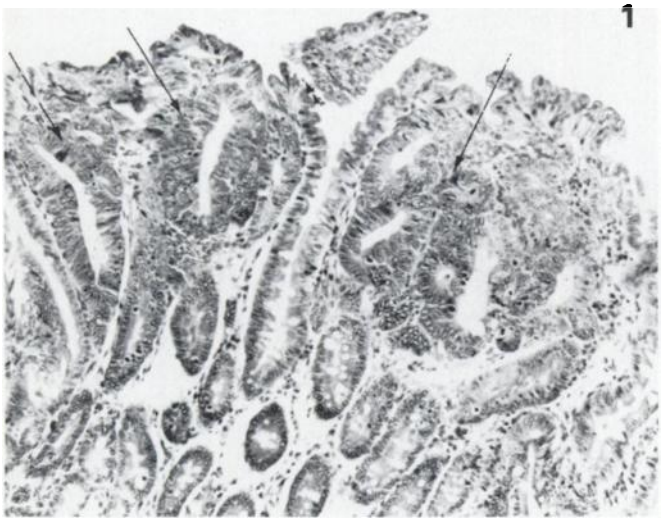
Fig. 4. High magnification of tumor in Fig. 3. Cystic structures are lined by neoplastic epithelial cells and stroma with inflammatory cells is seen. H & E, × 200.

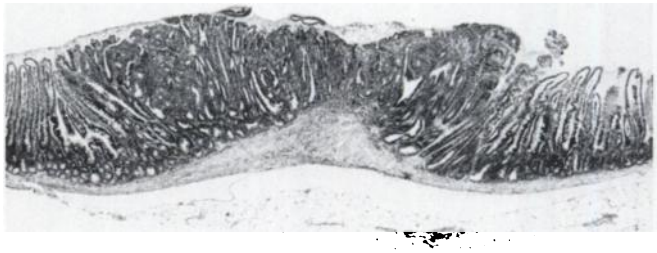
Fig. 5. Lesion (4 mm) in duodenum at junction with pylorus in untreated mouse. Neoplastic changes in surface epithelium have caused cystic dilatation of Brunner's glands. H & E, × 20.

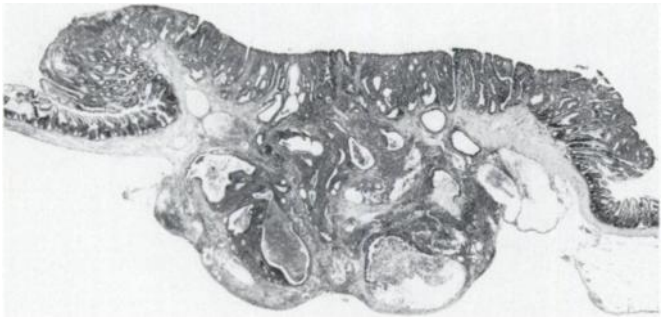
Fig. 6. Polypoid tumor (7 mm) in duodenum at junction with pylorus. A well-developed stalk is not observed. H & E, × 20.

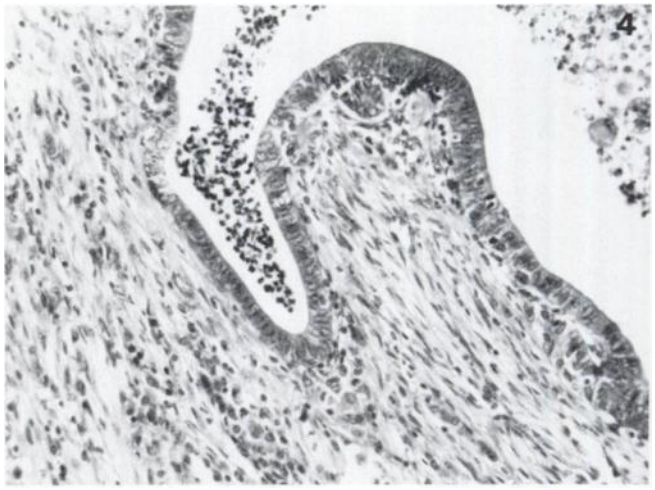
Fig. 7. Polypoid tumor (12 mm) in descending colon. H & E, × 20.

Fig. 8. Rectal adenocarcinoma and diffuse hyperplasia of epithelium. H & E, × 60.









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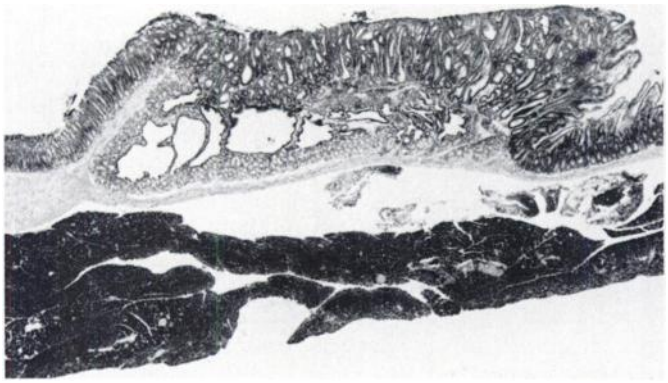

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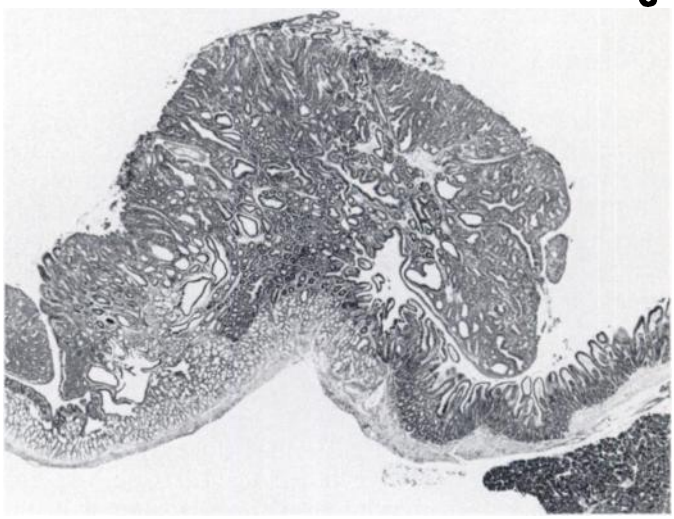

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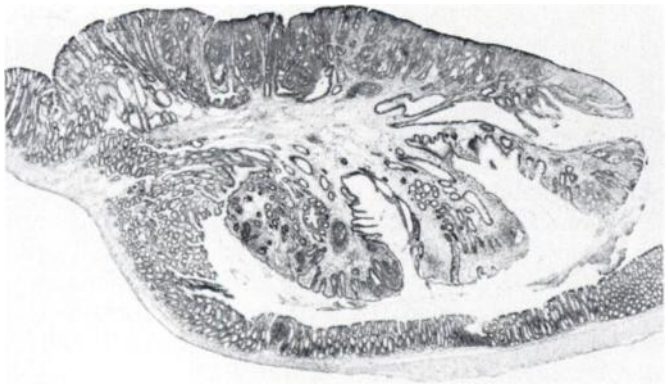

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