A Rat Model for Studying Colonic Cancer:

Effect of Cholestyramine on Induced Tumors*

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IN RECENT YEARS great strides have been made toward the development of an animal model for studying colonic cancer. Intestinal tumors, both adenomas and carcinomas, can be induced in some animals by a variety of chemical compounds. Among the most effective are the aminobiphenyls and a newer group of related compounds which include 1, 2-dimethylhydrazine, azoxymethane, and methylazoxymethanol.5,7

Several studies have shown that rats, which rarely develop bowel cancer spontaneously, are good animals to use for the induction of intestinal tumors by these chemical carcinogens.1 The agents are best given subcutaneously at weekly intervals. The tumors appear after 5 or 6 months, depending upon the amount of carcinogen given. Tumors of various degrees of malignancy are reported to develop in both the small and the large intestine. Histologically, they are similar to human intestinal neoplasms.5

The objectives of our studies were two-fold: to compare the effectiveness of the newer intestinal carcinogens, and to improve the yield of tumors in the large intestine.

Method

Two studies were done, using young, non-inbred, male, Sprague-Dawley rats, weighing 100 to 150 g.

The aim of the first study was to compare the effectiveness of dimethylhydrazine and azoxymethane. Twenty-four rats were given weekly subcutaneous injections of dimethylhydrazine, 15 mg/kg. Another 24 rats were given azoxymethane, 8 mg/kg, in the same manner. There were 24 control rats. All rats were fed the regular Purina rat chow diet. The injections were continued until the rats died or were sacrificed because death appeared imminent. All animals alive at the end of nine months were sacrificed. Necropsies of all animals, including the controls, were done. Intestinal tumors were counted, photographs taken, and histologic sections of representative tumors made.

The objective of the second experiment was to study the effects of a moderate amount of cholestyramine§ given orally on the yields and distributions of intestinal tumors induced in rats by three carcinogens. Eighty rats were divided into two equal groups. In the first group, ten rats were given dimethylhydrazine, 18 mg/kg; azoxymethane, 8 mg/kg, was administered to another ten rats; methylazoxymethanol, 6 mg/kg, was given to a third set of ten rats; ten rats served as controls. All these 40 rats were fed the regular Purina rat chow diet. The second group of 40 rats was treated in the same manner in all respects except that cholestyramine was added to the diet in the amount of 2 per cent of the granulated Purina chow. The cholestyramine was given

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§ Supplied by Merck, Sharp and Dohme.
in this manner throughout the experiment. The carcinogens, given as outlined, were injected subcutaneously at weekly intervals. All animals were examined after death or when sacrificed, as was done in the first experiment. The duration of the study was nine months.

**Results**

Table 1 compares the number of rats developing intestinal tumors in the dimethylhydrazine and aoxymethane groups within nine months in our first study. Twenty of the rats on dimethylhydrazine developed intestinal tumors. The total number of tumors in the group was 91. All of the 24 rats on aoxymethane developed tumors, which totalled 131. The average numbers of tumors per rat were 4.5 for dimethylhydrazine and 5.5 for aoxymethane.

There were a surprising number of tumors in the small bowel. With dimethylhydrazine, there were nearly as many lesions in the small intestine as in the large intestine, a fact best seen in Figure 1; whereas there were more tumors in the small intestine than in the large intestine in the rats receiving aoxymethane (Fig. 2).

Figures 1 and 2 also show that there was a striking preponderance of tumors in the proximal half of each segment of the intestinal tract with either carcinogen.

At least 75 per cent of all lesions were polypoid, with the remainder being sessile; some of the latter were ulcerated. They ranged in size from 2 mm to 5 cm. Abdominal carcinomatosis with ascites occurred in about 15 per cent of all rats with tumors. Histologically, the tumors ranged from well-differentiated lesions to highly anaplastic carcinoma. Only one rat in this study had metastatic implants in the lung. Several primary tumors occurred in sites other than the intestine: two in the liver, one in the kidney, four in the ear, and two at the site of injection. No tumor occurred in any control animal.

In the second study, the number and distribution of tumors in the bowel of the rats on a normal diet without cholestyramine were much the same as in the first experiment (Table 2). In addition, a third carcinogen, methylazoxymethanol, not given before, was administered at a dosage level of 6 mg/kg; only 60 per cent of the rats developed tumors.

There was a striking change in the number and distribution of intestinal tumors in the rats given cholestyramine with each of the three carcinogens used. A comparison of these data between the rats on a normal diet and those on the same diet to which cholestyramine was added is shown in Figures 3, 4, and 5.