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Promotion of Azoxymethane-induced Intestinal Cancer by High-Fat Diet in Rats[1]

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ABSTRACT

Promotional properties of a high-fat diet in intestinal cancer were studied by feeding a 30% beef fat diet to 8 groups of rats (25 rats/group) for time periods varying from 1 to 21 weeks after 8 weekly s.c. injections of azoxymethane (AOM) (8 mg/kg). Two other groups were fed the high-fat diet, one for 8 weeks prior to and the other during AOM injections. A 5% fat diet was fed to rats when not on the 30% fat diet and to a control group of 25 animals.

High-fat diet increased intestinal tumor frequency up to 2-fold when given for at least 4 weeks after but not during or prior to AOM injections; this increase occurred even after a prolonged interval (10 weeks) between the last AOM injection and the high-fat diet. In general, tumor frequency increased according to the length of time animals were fed the high-fat diet after AOM. Therefore, the high-fat diet in this model exhibited most of the properties of promoters developed from murine skin cancer, thus adding support to the concept that excess dietary fat acts at the promotional phase of carcinogenesis.

INTRODUCTION

Environmental factors appear to have an important role in the etiology of many human cancers. However, it is not clear how they influence the carcinogenic process. The initiation-promotion concept, developed from studies of cancer formation in the skin of mice (2), is a useful model for speculation and research on the role of various causative factors in cancer of other organs. Most investigators believe that initiation is an action by which a carcinogen damages the genetic material in cells of the target tissue while promotion selectively accelerates the growth of such altered cells.

The properties of promoting agents that were formulated from studies of cancer of the skin of mice include the following: (*a*) promoters are not carcinogenic in themselves; (*b*) promoters increase the yield of tumors when given after, but not before, an initiating agent; (*c*) promoters increase the tumor yield even when given long after the last exposure to the carcinogen. Several detailed reviews of tumor promotion are available (4, 15).

Animal studies indicate that other cancers also develop by a multistep process (6). That promotion may be involved in intestinal cancer is suggested by the observation that the carcinogenic response to a variety of intestinal carcinogens is enhanced by substances which in themselves are not carcinogenic. These include high levels of dietary fat (12, 13), ingestion of hypocholesteremic agents such as cholestyramine and candicidin (1, 11), and other methods which increase the concentration of biliary steroids in the lumen of the intestinal tract (5, 8). The objective of the present study is to determine to what extent excessive dietary fat exhibits the properties of promoting agents in azoxymethane-induced intestinal cancer in rats.

MATERIALS AND METHODS

Materials. AOM[2] was obtained from Ash-Stevens Co., Detroit, Mich., and was prepared as an aqueous solution for injection. Dextrose was purchased from a bakery supply house. Beef fat was donated by the Belmont Packing Co. and was rendered in the laboratory. Other dietary components were supplied by Bio-Serv, Inc., Frenchtown, N. J. Male Sprague-Dawley rats weighing 150 to 175 g (8 weeks old) were purchased from Spartan Research Animals Inc., Haslett, Mich.

Methods. Animals were caged individually, diets and water were given *ad libitum*, body weights were recorded every 4 weeks, and food consumption was determined every 8 weeks. Animals were fed 1 of 2 diets prepared weekly in the laboratory. The dietary composition in weight percentage is presented in Table 1. The low-fat diet [5% (w/w) beef fat and 3.69 cal/g food] contained as percentage of total calories approximately 35.8% protein, 12.2% beef fat, and 52.0% dextrose. The high-fat diet [30% (w/w) beef fat and 4.94 cal/g food] contained 26.7% protein, 54.7% beef fat, and 18.6% dextrose as percentage of total calories. Both diets were adequate for the animals and yielded body weight curves similar to those obtained from rats fed commercial rat diets (11).

The experimental design is presented in Chart 1. There were 25 animals in each group with the exception of a noncarcinogen control group (Group I) which contained 10 animals. For the first week of the experiment, all rats were fed the low-fat diet. Then AOM (8 mg/kg body weight) was given s.c. once a week for 8 weeks beginning the second week of the experiment to all groups except Groups I, K, and L. Animals in Groups K and L were given the carcinogen in the same manner beginning the 12th week of the experiment. All rats received the low-fat diet during the administration of the carcinogen except those in Group G which were fed the high-fat diet during this period. Group K animals received the high-fat diet for 8 weeks followed by 2 weeks of the low-fat diet prior to AOM administration. Group L served as low-fat control for Group K. After AOM injections, all rats were fed the low-fat diet for 2 weeks. The 2-week delay before feeding the high-fat diet was to ensure complete metabolism and excretion of the carcinogen. Then, the high-fat diet was fed to the following groups for varying time periods: A, 16 weeks; B, 8 weeks; C, 4 weeks; D, 2 weeks; E, 1 week; F, 0 weeks; and H, 21 weeks. Group J

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[2] The abbreviation used is: AOM, azoxymethane.

animals received the low-fat diet for 10 weeks after the last AOM injection and then were fed the high-fat diet for 8 weeks. After receiving the high-fat diet, the animals were fed the low-fat diet for the remainder of the experiment. The noncarcinogen group (Group I) was fed the high-fat diet for 21 weeks.

Animals in Groups A through G and J were sacrificed at the end of 27 weeks; those in Groups H and I were sacrificed at the end of 32 weeks; while those in Groups K and L were sacrificed at the end of 36 weeks. The animals were killed at different times to allow at least 18 weeks to elapse from the last AOM injection to the time of sacrifice, permitting adequate time intervals for the development of tumors. One or 2 rats in each group died early in the experiment and were not included in the data.

Necropsies were performed on all animals. Abdominal and thoracic tissues were examined grossly for evidence of tumors. The number, size, and location of all intestinal tumors were recorded. Eight representative tumors were removed from each group. The specimens were fixed in 10% formalin, embedded in paraffin, and stained with hematoxylin and eosin. All data were analyzed with Student's *t* test (3).

Table 1

Dietary composition in weight percentage

Component	5% fat diet	30% fat diet
Protein[a]	33	33
Salt mix[b]	7	7
Vitamin mix[c]	2	2
Beef fat	5	30
Dextrose	48	23
Cellulose	5	5

[a] Vitamin-free casein, Bio-Serv No. 11319.

[b] Salt mix USP XIV, Bio-Serv No. 20232 containing (per kg salt mix): 7.8 mg cupric sulfate; 1.53 g ferric ammonium citrate; 20 mg manganese sulfate; 9.2 mg ammonium aluminum sulfate; 4.1 mg potassium iodide; 51 mg sodium fluoride; 68.8 g calcium carbonate; 308.3 g calcium citrate; 112.8 g calcium biphosphate; 35.2 g magnesium carbonate; 38.3 g magnesium sulfate; 124.7 g potassium chloride; 218.8 g dibasic potassium phosphate; 77.1 g sodium chloride.

[c] Vitamin mix, Bio-Serv No. 20315 containing (per kg of mixture): 4.5 g vitamin A (200,000 units/g); 0.25 g vitamin D (400,000 units/g); 5.0 g α-tocopherol acetate; 5.0 g inositol; 45.0 g ascorbic acid; 158.25 g choline dihydrogen citrate; 2.25 g menadione; 5.0 g *p*-aminobenzoic acid; 4.5 g niacin; 1.0 g riboflavin; 1.0 g pyridoxine hydrochloride; 1.0 g thiamine hydrochloride; 3.0 g calcium pantothenate; 20 mg biotin; 90 mg folic acid; 1.35 mg vitamin B_{12}; 765.24 g sucrose to make 1 kg.

RESULTS

Body Weight and Food Consumption. The average body weights of animals are presented in Chart 2. Although not directly proportional, the body weights tended to increase according to the length of time the rats were on the high-fat diet. There was no significant difference in food consumption (g consumed per 100 g body weight) between groups fed the high-fat diet and those fed the low-fat diet. Since the high-fat diet contained more cal/g than did the low-fat diet, rats on the high-fat diet had a higher caloric intake than did those consuming the low-fat diet. This difference in caloric intake is responsible for the increased body weight of rats fed the high-fat diet for longer periods of time.

Tumor Frequency. All animals given injections of AOM developed intestinal tumors while there were none in the noncarcinogen controls. As shown in Chart 3, rats given the high-fat diet for 4 or more weeks after the last injection of the carcinogen (Groups A, B, C, H, and J) had a significantly higher ($p < 0.005$ to < 0.0005) tumor frequency (average number of tumors/rat) than did Group F, animals fed the low-fat diet for the entire period following administration of AOM. Rats fed the high-fat diet for 8 weeks either before (Group K) or during (Group G) the administration of the carcinogen developed significantly fewer ($p < 0.005$ to < 0.0005) tumors compared to the animals in Group B, those fed the high-fat diet for the same time period after the carcinogen was administered.

The distribution of tumors in the small and large intestines is presented in Table 2. Although there were differences in tumor frequencies in the small intestine in some groups, they were not all significant and do not correlate well with the length of time the rats were fed the high-fat diet. The tumor frequency in

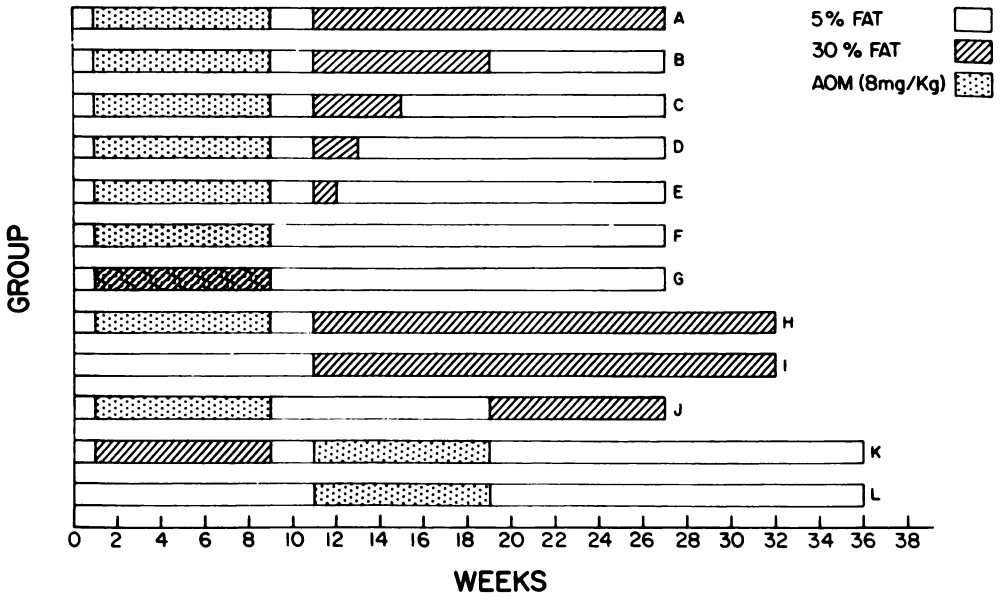

Chart 1. Design of the experimental animal groups which contained 25 animals/group except the noncarcinogen control group (Group I), which contained 10 animals.

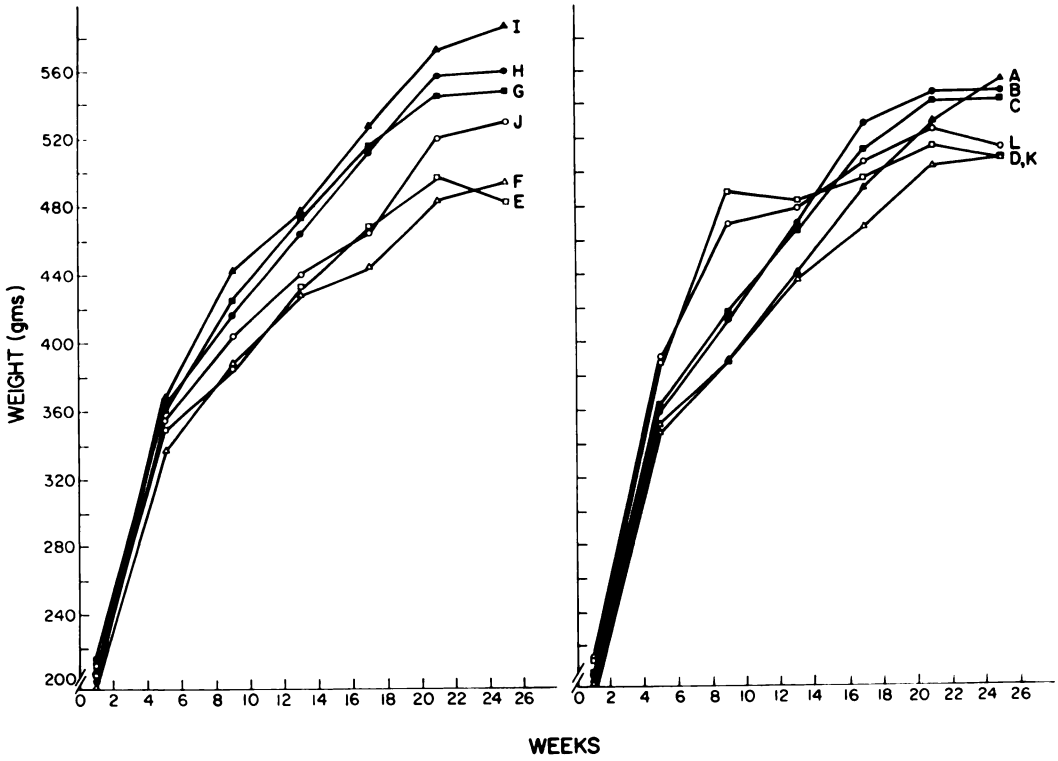

Chart 2. Average body weights of animal groups as a function of time. There is an increase in average body weight the longer the high-fat diet is given, but it is not statistically significant.

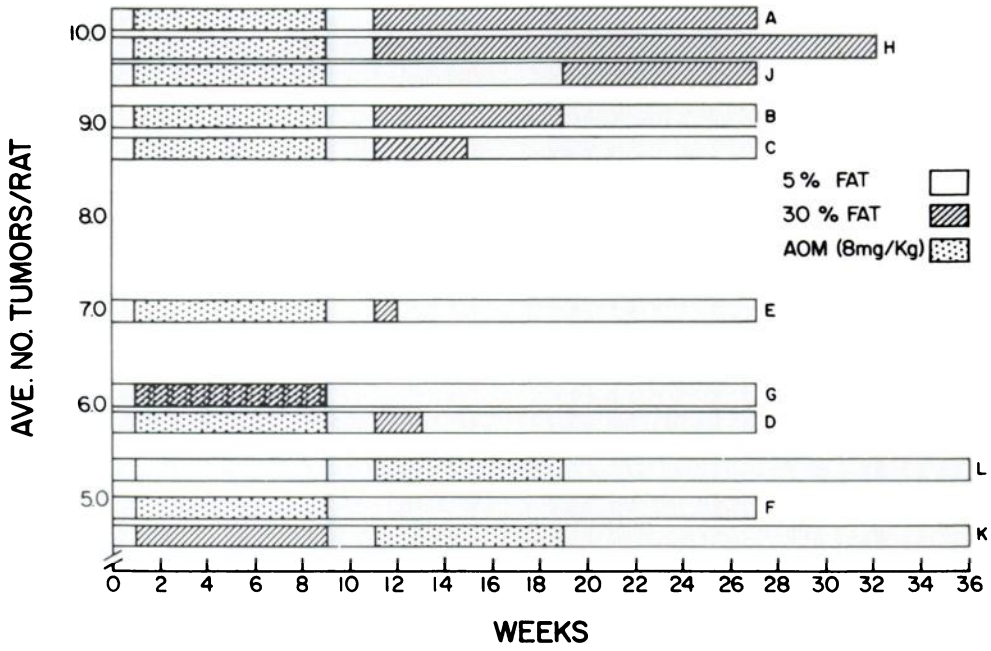

Chart 3. Tumor frequency (average number of tumors/animal) of the entire intestinal tract for each AOM-treated group. There was a significant increase ($p < 0.0005$ to < 0.005) in groups receiving high-fat diet after AOM treatment, except for Group D. Groups receiving high-fat diet before or during AOM treatment did not have a significant increase in tumor frequency.

the large intestine was significantly increased ($p < 0.0005$) in those groups receiving the high-fat diet for 4 weeks or more after the AOM (Groups A, B, C, H, and J).

Metastases occurred i.p. in all animal groups receiving the carcinogen. The number of animals with these metastases in the various groups did not correlate well with the alteration in diet. Some animals developed metastatic disease of the liver and lungs. Here there was some correlation in that animals on the high-fat diet longest developed more implants in the liver and lung, although these differences were not statistically

Table 2
Tumor distribution

Group	Small intestine (Av. no. of tumors/rat)	Large intestine (Av. no. of tumors/rat)
A	4.2	5.8
B	2.5	6.4
C	3.2	5.6
D	1.6	4.2
E	2.7	4.2
F	1.6	3.3
G	1.6	4.5
H	3.3	6.3
J	3.7	5.9
K	1.4	3.4
L	1.6	3.8

significant. Tumors of the external ear canal occurred in 1 or 2 animals in all groups except Groups K and L.

Eight representative intestinal tumors from each group, matched for size and location, were examined histologically. All lesions regardless of size were adenocarcinomas with varying degrees of differentiation and invasion of the intestinal wall. However, these characteristics were not significantly different among the various groups.

DISCUSSION

This experiment shows that a high-fat diet exhibits most of the characteristics of promotion. (*a*) The rats fed the high-fat diet for 21 weeks without AOM did not develop any tumors (Group I). (*b*) The ingestion of a high-fat diet increased tumor frequency in the intestinal tract when given after the administration of AOM, but not during or before, as demonstrated by the following results. When the high-fat diet was given for 8 weeks prior to the injection of AOM (Group K), there was no increase in tumor frequency compared to rats on a low-fat diet during the same time period (Group L). When the high-fat diet was fed during the 8 weeks of AOM injections (Group G), tumor frequency did not increase significantly over the group receiving only the low-fat diet (Group F). On the other hand, the animals fed the high-fat diet for 8 weeks after AOM injections (Group B) developed almost twice as many tumors compared to animals fed the 5% fat diet during the same time period (Group F). A 21-week exposure to the high-fat diet after AOM injections (Group H) did not increase the number of tumors over that in rats with the 16-week exposure (Group A). This suggests the possibility of an upper limit to the enhancement of carcinogenesis by high levels of dietary fat. (*c*) Finally, a delay of 10 weeks between the last AOM injection and the administration of a high-fat diet (Group J) yielded virtually the same increase in tumor frequency as the group with only a 2-week delay (Group B).

While there is no generalized theory of the mechanism of promotion, the experimental results in chemically induced intestinal cancer suggest that promotion results from some aspect of the metabolism of fat (5, 8, 11). It seems to act through a mechanism involving increased fecal steroid content, which results from the high levels of dietary fat.

Thus, it appears that experimental intestinal cancer is at least a 2-step process similar to that of the skin. The carcinogenic process in the human may have similar characteristics since there is a good correlation between the findings in a variety of animal studies and those done in humans (7). Due to the wide variety of initiating agents and the possible difficulties in removing them from the environment, the promotional phase of carcinogenesis may be a more promising area for development of preventive measures. It is tempting to speculate that the incidence of cancer of the large bowel may be markedly reduced by a combination of several dietary alterations including a reduction of dietary fat, an increase in fiber consumption (10), and the addition of inhibiting agents (9, 14). Studies are currently under way to gain more information in an attempt to accomplish this objective.

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