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Effect of Quality and Quantity of Dietary Fat and Dimethylhydrazine in Colon Carcinogenesis in Rats[1] (39181)

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Based on epidemiological studies of the geographic distribution of colon cancer and data dealing with migrant populations, Wynder *et al.* (1) and Berg *et al.* (2) proposed that dietary factors, particularly high dietary fat and beef protein, play an important role in large bowel carcinogenesis. The suspected effect of diet on colon carcinogenesis may be mediated through changes in intestinal microflora and also in the composition of compounds such as acid and neutral sterols secreted into the gut (3, 4).

Large bowel cancer can be induced in experimental animals by 1,2-dimethylhydrazine (DMH) and azoxymethane (AOM) among other chemicals (5). The intestinal microflora appeared to play a modifying role in the carcinogenicity of DMH in rats (6, 7). We have now extended our studies to elucidate the effect of quality and quantity of dietary fat on colon tumor induction by DMH in rats.

Inasmuch as men in various population groups usually eat comparable regimens over generations, we have designed our dietary experiments in a manner so that animals are exposed to a given regimen for two generations. A low dose of DMH was selected to avoid the production of large numbers of colon tumors since our aim was to determine the promoting or accelerating effect of varying levels of dietary fat.

Materials and methods. Weanling female Fischer rats were randomly divided into five groups and fed *ad libitum* Purina lab chow (PLC) or semipurified diets with varying amounts of corn oil, a typical fat with a high degree of unsaturation, or lard, an example of a highly saturated fat: 5% (normal) corn oil; 20% (high) corn oil; 5% (normal) lard; 20% (high) lard. The normal corn oil or lard contained these ingredients: casein, 25%; sucrose, 59%; corn oil or lard, 5%, alphacel, 7%, salt mixture, 4%, vitamin diet fortification mixture at recommended levels. The fat content in high-fat diets was adjusted at the expense of sucrose. Purina lab chow contained about 4.5% fat.

At puberty, female rats were mated with males and reared on the respective experimental diets. The litter size from each mother was reduced to eight and weaned to the same experimental diets consumed by their mothers. At 7 weeks of age, all animals from the second generation in each dietary group, except controls, were given weekly sc injections of 10 mg/kg body wt/week DMH, for 20 weeks. Before injection, DMH dihydrochloride was brought to pH 6.8. All animals were autopsied 10 weeks after the last injection, and organs, including the intestine, were examined grossly and microscopically for the number and type of tumors. Tissues were fixed in 10% formalin and embedded in paraffin, and the sections were stained with hematoxylin and eosin. The standards for histologic diagnosis of intestinal neoplasma (8), kidney tumors (9), and ear duct tumors (10) were as described by various investigators.

Results. Table I summarizes the tumor incidence in rats fed semipurified diets containing lard or corn oil at 5 or 20% level or in rats fed Purina lab chow and injected with DMH. There was no difference in body weights among those animals fed different diets. The number of rats with ear duct tumors and small intestinal tumors were increased in rats fed diets containing 20% corn oil or 20% lard as compared to the number in rats fed 5% corn oil, 5% lard or PLC. Tumors of the small intestine predom-

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TABLE I. TUMOR INCIDENCE IN RATS TREATED WITH DMH AND FED TWO LEVELS OF DIETARY CORN OIL OR LARD[a]

Diets	Animals with tumors (%)				Animals with multiple colonic tumors (%)	Total colon tumors (per rat)
	Ear canal	Kidney	Small intestine	Colon		
Corn oil, 5%	32	4	27	36	14	0.77
Corn oil, 20%	59	14	36	64	32	1.55
Lard, 5%	13	0	4	17	4	0.22
Lard, 20%	67	0	50	67	29	1.50
Purina Lab Chow	15	0	20	25	0	0.25

[a] Number of animals per group ranged from 20 to 24. Animals received weekly sc injections of 10 mg/kg of body weight for 20 weeks and were autopsied 10 weeks after the last injection.

inated in the duodenum approximately 2 cm from the pylorus.

Animals fed a 20% corn oil or 20% lard diet and treated with DMH had a higher incidence of colon tumors than did rats fed a 5% corn oil, 5% lard or PLC diets. The number of colon tumors per animal was higher in rats fed high fat diets than in rats fed 5% fat or PLC diets. The quality of fat (corn oil vs lard) had no major influence on the incidence of colon tumors in animals fed 20% fat. But the animals fed a 5% corn oil diet had a slightly higher incidence of colon tumors than did rats fed a 5% lard diet. The tumor sizes ranged from 0.1 to 1.0 cm in diameter in all groups. Aside from those noted, none of the other organs showed any tumors. There were no tumors in untreated animals.

The macroscopic and microscopic findings of neoplasms in the large intestine, small intestine, kidney, and ear duct of groups of animals fed various types of diets were similar. Duodenal and other small intestinal carcinomas were polypoid type with or without central ulceration. They were well-differentiated adenocarcinomas and, in a few cases, poorly differentiated adenocarcinomas and signet-ring cell carcinomas. Most of them were invading extensively into the muscularis propria or into the serosa, and a few invaded in the peritoneal cavity.

Adenomatous polyps of the large intestine were pedunculated and sessile types. The majority of large intestinal carcinomas were polypoid and well-differentiated adenocarcinomas and signet-ring cell carcinomas. Most of signet-ring cell carcinomas were located in the proximal colon.

Tumors of the ear duct were squamous with sebaceous cell differentiation. Kidney tumors were of mesenchymal type.

Discussion. The dose of DMH used in the present study was rather low, accounting for a low incidence of intestinal tumors in all experimental groups. Thus, factors increasing tumors could be visualized. Our results indicate that the animals fed a high-fat diet containing either corn oil or lard were more susceptible to colon tumor induction by DMH compared to rats fed normal fat diets or PLC, by as yet unclear mechanisms. These observations are in line with epidemiologic findings in man that populations in a high-risk area for colon cancer consume diets high in fat whereas people in a low-risk area eat food low in such components (1, 4).

We have hypothesized that the high-fat diet modifies the composition of intraluminal compounds of endogenous origin such as bile acids and neutral sterols and also the composition of the intestinal microflora, which, in turn, may produce cocarcinogenic compounds from these intraluminal compounds (3, 4). Our preliminary studies also indicate that the rats fed a high-fat diet excreted higher levels of biliary bile acids than those fed normal diets. Fecal excretion of microbially modified bile acids were higher in animals fed diets containing high fat than in those fed a normal diet (11). Narisawa *et al.* (12) observed that lithocholic acid and taurodeoxycholic acid acted as colon tumor promotors in rats initiated with a single dose of MNNG. The results of Chomchai *et al.* (13) add more support to the concept that bile salts in the colon have some role in colon carcinogenesis.

Another factor requiring consideration is the amount of enzyme inducers or inhibitors

present in the diet, which alters the capacity of the animal to metabolize the carcinogen. Available evidence indicates that many of these enzymes are diet-dependent (14). Therefore, this difference in susceptibility between rats fed different levels of fats may be explained in terms of diet-mediated enzyme changes in the gut.

Our results also indicate that the small intestinal tumors were increased in rats fed high-fat diets. These tumors were mainly localized in the proximal duodenum where the bile enters into duodenum. On the basis of evidence, including the carcinogenicity of DMH in germfree rats (6, 7, 15) and preliminary observations on the metabolism of DMH,[3] it would appear that this kind of carcinogen may be delivered into the intestine via both bile and circulation, and may be converted to the ultimate carcinogen by enzymes in the large bowel. Tumors in the small intestine may be due, likewise, to metabolism to an active form of the carcinogen by the tissue affected. It could be also that an active form is delivered to site by the bile, since most small intestinal tumors are localized in an area of high biliary concentration. The high incidence of duodenal tumors in rats fed high-fat diets may also be a result of excretion of elevated amounts of biliary bile acids in these rats.[4]

Although the mechanism needs elucidation, our data show that dietary intake of high fat exerts a promoting effect in colon carcinogenesis.

Summary. The effect, quality, and quantity of dietary fat on colon tumor induction by DMH were studied in rats exposed to a given regimen for two generations prior to treatment with DMH. Animals fed a 20% corn oil or 20% lard and treated with DMH had a higher incidence of colonic tumors than did rats fed a 5% corn oil, 5% lard or Purina lab chow and treated similarly. The quality of fat had no major difference on the incidence of colonic tumors.

[3] Fiala, E. Unpublished observations, 1975.

[4] Reddy, B. S. Unpublished observations, 1975.

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