Effects of Dietary Fat on Hepatic Mixed-Function Oxidases and Hepatocellular Carcinoma Induced by Aflatoxin B1 in Rats

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ABSTRACT

Some groups of male Sprague-Dawley rats were fed a diet containing either 28% beef fat and 2% corn oil or 30% corn oil (high fat), and others were fed either 13% beef fat and 2% corn oil or 15% corn oil (moderate fat), for varying periods of time, with or without exposure to aflatoxin B1 (AFB1). Assays for hepatic microsomal oxidases [p-nitroanisole demethylase and benzo(a)pyrene hydroxylase] were performed on liver samples from animals exposed to AFB1, corn oil permitted a significantly higher induction of enzyme activity by AFB1. In a 14-month study of rats fed the high corn oil diet, those exposed to the diet during and after AFB1 treatment developed significantly more liver tumors than those consuming the diet only after administration of a carcinogenic dose of AFB1. In animals fed beef fat, tumor incidence was depressed compared to those fed corn oil, but no differences were observed between groups fed beef fat throughout exposure to AFB1 and afterward and those fed beef fat only after AFB1 exposure. These observations suggest that corn oil may act as a promoter through microsomal enzyme induction and AFB1 activation.

INTRODUCTION

The nutritional state of an animal may appreciably influence its response to toxic substances and the way it metabolizes exogenous or endogenous chemicals in the liver (4, 5, 15, 23, 31). In a number of studies, we have observed a marked effect of diet on tumor induction by various chemical carcinogens (27). The diets used in many of our investigations have been deficient or only marginally adequate in the lipotropes (choline, methionine, folic acid, and vitamin B12) but high in beef fat. The control diet traditionally has used an unsaturated fat, either cottonseed or corn oil (20, 27, 30, 32, 33, 35). The results reported here were derived from investigations designed to examine the effects of beef fat or corn oil on the induction of hepatocarcinomas in rats exposed to AFB1. Selected hepatic microsomal enzyme activities were also examined. The diets used in these studies were adequate in all known respects, and only the fat quality and quantity were varied.

MATERIALS AND METHODS

Male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, Mass.), about 4 weeks old, 45 to 50 g body weight, were fed Diets 1 to 4 ad libitum (Table 1) from weaning for various periods of time. The numbers of animals used in each experiment are indicated in Tables 2 to 5. The rats were housed individually in screen-bottomed stainless steel cages in climate-controlled animal facilities; they were weighed when placed on the experimental diets and weekly thereafter. AFB1 (Makor Chemicals, Inc., Jerusalem, Israel) was administered, by gastric intubation where indicated, either as an LD50 dose of 7 mg/kg body weight (Table 2) or as 5 or 15 daily doses (Tables 4 and 5) of 25 µg/dose. At the end of the trial period, the rats were decapitated for autopsy, collection of samples for analysis of enzyme activity, and histopathology. Microsomal oxidases (PNA and BPOH) were measured in 6 to 10 rats per group per time period (15, 31). Fat extracted from the liver was determined gravimetrically.

At autopsy, after liver samples were taken for enzyme assay, the major organs were fixed in 10% neutral buffered formalin, processed by routine histological methods, and examined in sections stained by hematoxylin and eosin. Tumor incidences were compared statistically by the χ2 test.

RESULTS

An acute experiment was conducted to determine the short-term effect of an LD50 dose of AFB1 on rats fed either a high or a moderate level of each of the dietary fats. The male weanling rats were fed the respective diets for 2 weeks and then given a single dose of AFB1 (7 mg/kg body weight) dissolved in 0.2 ml dimethyl sulfoxide, by gastric intubation. Table 2 lists the mortality rate associated with each of the diets and total liver fat. The rats fed beef fat had higher total liver fat and a higher mortality rate than those fed corn oil. The group fed the lower level of corn oil (Group 4) tended to have a slightly higher mortality rate also, compared to the high-corn oil group (Group 2).
weanling rats for 1 week and then gave them doses of AFB, in corn oil was added to prevent essential fatty acid deficiency.

In this study, we fed the respective diets to male rats. It thus appears that at 3 weeks there was a depression of liver microsomal enzyme activity, both before and after exposure to AFB, (Table 2) differed in gross and in microscopic appearance. Lower incidences were observed with those fed beef fat, whether or not the diets were fed during AFB1 exposure (Group 3); Group 4, fed the high-corn oil diet after the rats had been exposed to AFB1, had the next highest incidence. Lower incidences were observed with those fed beef fat, whether or not the diets were fed during AFB1 exposure (differences of 66 versus 51 to 53%; p = 0.02). It appeared to make a difference in tumor incidence if the high-corn oil diet was fed during the time the rats were exposed to AFB1, which might imply an effect of corn oil on the metabolism of AFB1, presumably by increasing the proportion of the AFB1, caused increased activity in all groups.

Another study was conducted to assess the sequential effects of the 4 dietary treatments on liver enzyme activity over a 3-week period. Groups of male weanling rats were fed the respective diets, and at weekly intervals enzyme assays were performed on 6 rats per dietary group at each time point. Four groups of 6 rats each were selected at random on arrival from the supplier and assayed for base-line levels of enzyme activities to serve as initial, pretreatment reference points. Results are listed in Table 3. The activity of PNA increased with time in all groups; however, those groups fed corn oil at both the moderate and high levels had the most rapid increase and reached the highest activity of any of the groups after 3 weeks. Those groups fed beef fat had increases in PNA activity, but these were significantly less than those observed in the corn oil groups. It thus appears that at 3 weeks there was a depression (or decreased rate of induction) in PNA activity in the beef fat groups (Groups 1 and 3) relative to that in the corn oil groups (Groups 2 and 4).

In an additional experiment, we examined the effects of the 2 types of fat and the 2 different concentrations of each on liver microsomal enzyme activity, both before and after exposure to AFB1. In this study, we fed the respective diets to male weanling rats for 1 week and then gave them doses of AFB1, dissolved in dimethyl sulfoxide as noted in Table 4. Enzyme assays were done on the seventh day following the first of 5 doses (2 days after the fifth and last dose). Table 4 lists the results of this phase of the series of studies. The important observations in the case of both enzymes (PNA and BPOH) were that corn oil enhanced the induction of both enzymes (or, conversely, beef fat depressed induction) and that it was much more effective in this regard, although AFB1 caused increased activity in all groups.

A long-term study using 4 groups of 60 male weanling rats each was initiated to investigate the effects of dietary fat on the induction of liver tumors during, and during and after, administration of a carcinoogenic dose of AFB1. Starting weight was roughly the same for all groups (45 to 50 g). Table 5 describes the diet fed prior to and during AFB1 treatment. Beef fat resulted in better weight gains, with about 50 g difference after 24 weeks. This was attributable in part to less food intake by those fed corn oil. Two spot checks of food intake for 1-week periods revealed that the beef fat groups consumed about 10% more diet than those fed corn oil.

The most notable difference in tumor incidence (Table 5) was in the group fed the high-corn oil diet during and after AFB1 exposure (Group 3); Group 4, fed the high-corn oil diet after the rats had been exposed to AFB1, had the next highest incidence. Lower incidences were observed with those fed beef fat, whether or not the diets were fed during AFB1 exposure (differences of 66 versus 51 to 53%; p = 0.02). It appeared to make a difference in tumor incidence if the high-corn oil diet was fed during the time the rats were exposed to AFB1, which might imply an effect of corn oil on the metabolism of AFB1, presumably by increasing the proportion of the AFB1, that became activated to the ultimate carcinogen (16).

Table 3: Quality and quantity of fat and liver microsomal oxidase activity (effect of diet only)

<table>
<thead>
<tr>
<th>Dietary fat (%)</th>
<th>Enzyme activity*</th>
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<tbody>
<tr>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td>Diet</td>
<td>Beef</td>
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<tr>
<td>1</td>
<td>28</td>
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<tr>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
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<td>4</td>
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* PNA (g p-nitrophenol per g liver per hr); BPOH [quinine units (see Refs. 3 and 17)]. Values are from dry, fat-free liver tissue.

Mean ± S.D.

a Significantly different from beef fat groups (Groups 1 and 3); p < 0.02.

b Significantly different from beef fat groups (Groups 1 and 3); p ≤ 0.05; 6 to 10 rats per group per time period.
The fifth dose), the rats were sacrificed and enzyme activities were assayed (6 to 10 rats per group per period).

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was not a result of lipid accumulation, determined by either... DEXTRIN, 14; Rogers-Harper salts mix, 5; vitamin mix, 2; lard, 4; corn oil, 2; and beef fat, 4. The vitamin mix was the same as described in Table 1. All rats received crystalline AFB, dissolved in dimethyl sulfoxide, 15 daily doses of 25 μg each.

Dietary fat, microsomal enzymes, and aflatoxin B1.

Aflatoxin B1 was administered daily in 5 doses by gastric intubation, 25 μg/dose, dissolved in 0.2 ml dimethyl sulfoxide. On Day 7 after the first dose (2 days after the fifth dose), the rats were sacrificed and enzyme activities were assayed (6 to 10 rats per group per period).

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<th>BPOH</th>
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<tr>
<td>Diet 1</td>
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<td>6.2 ± 0.3</td>
</tr>
<tr>
<td>Diet 2</td>
<td>30</td>
<td>6.8 ± 0.7</td>
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<tr>
<td>Diet 3</td>
<td>32</td>
<td>6.5 ± 0.7</td>
</tr>
<tr>
<td>Diet 4</td>
<td>30</td>
<td>6.2 ± 0.3</td>
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* Mean ± S.D.

Effects of dietary fat during and after exposure to a carcinogenic dose of AFB1, on tumor incidence.

Prior to feeding the diets described in Table 1, rats were fed a diet as follows (in percentage): casein, 20; sucrose, 15; dextrose, 14; dextrin, 14; Rogers-Harper salts mix, 5; vitamin mix, 2; lard, 4; corn oil, 2; and beef fat, 4. The vitamin mix was the same as described in Table 1. All rats received crystalline AFB1, dissolved in dimethyl sulfoxide, 15 daily doses of 25 μg each.

Table 5

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* Mean ± S.D.

grossly visible as splotches that were slightly depressed below the surface of the lobe. Microscopically, there was severe damage (Fig. 1). In less damaged areas, there was lipid infiltration (Fig. 2); this was not observed in livers of rats fed corn oil. Fig. 3 illustrates the typical lesion observed in the corn oil group, in which focal necrosis was present but the liver was much less extensively damaged. The increase in liver weight as a percentage of body weight (Table 2) in the corn oil group was not a result of lipid accumulation, determined by either gravimetric assessment or histological evidence. The weight increase presumably was a result of increased protein synthesis associated with microsomal enzyme induction.

The gross (Figs. 4 and 5) and microscopic (Fig. 6) appearances of the hepatocellular carcinomas were similar, but those tumors in rats fed corn oil were more widespread, had often infiltrated the abdominal cavity, and had a higher incidence of lung metastases. Tumors in rats fed beef fat tended to be less widespread, with no infiltration into the abdominal cavity and with significantly fewer metastases to the lung (Table 5).

**DISCUSSION**

There is little doubt that dietary factors do in some obscure manner influence the development of cancer in humans (29) and animals (16). The complexities of dietary interactions with chemical carcinogens in animal models are evident from many studies (19, 25, 28, 29, 43, 44), and the situation very probably is no less complicated in the case of human cancer. Epidemiological evidence for diet and neoplasia is convincing in the case of human colon (11, 41, 45) and breast cancer (2, 8, 10). However, very little data are available to help explain mechanisms. In the case of colon cancer, a number of suggestions may be proposed: (a) diet may influence intestinal microflora and through this mechanism produce, or convert chemicals in the gastrointestinal tract to carcinogenic molecules; (b) nutritive factors (e.g., vitamin A deficiency) may change the sensitivity of the colon mucosa; (c) promoters or accelerators may be synthesized de novo or simply increased, to act on the colon mucosa [Rats fed a diet high in fat excreted more bile acids and steroid metabolites and were more susceptible to dimethylhydrazine-induced colon cancer than rats fed a diet low in fat]; and (d) dietary fiber may increase transit time of ingested material that may be potential or proximate carcinogens, providing less contact time with intestinal epithelia. It is likely that all of these factors and more impinge on nutrient-carcinogen interactions.

Breast cancer is not common in women in developing societies or in Japanese women in their native habitat (2). However, the incidence increases in these population groups when they migrate to the United States. Further, breast cancer may be influenced by height and weight (possibly obesity) but would appear to be more closely related to total body mass (8, 10, 26). In experimental animals, diets high in corn oil enhanced mammary tumor induction by 7,12-dimethylbenz(a)anthracene in one study (42). However, studies in our laboratory (18) have shown that diets low in lipotropes but high in fat decreased the incidence of mammary tumors induced by acetylamino- fluorourea or 7,12-dimethylbenz(a)anthracene in 2 strains of rats. Thus, conflicting observations on breast cancer induced in rats fed high-fat diets require resolution.

In some population groups of Africa, South China, Hawaii, Thailand, Mozambique, and other areas, primary liver cancer is a major problem, particularly in males (6, 12, 22, 36, 39, 40). In most populations, the livers of hepatic cancer patients generally do not consume excessive levels of fat. It should be noted, however, that the fat they do consume is most often unsaturated (fish or vegetable) and that their diets frequently are contaminated with mycotoxins, nitro- amines, or other hepatic carcinogens; they also suffer from a high incidence of viral hepatitis, in some cases, and liver parasitism, in others. Any or all of these factors may interact with dietary nutrients to enhance liver cancer.
Whatever the mechanisms involved, nutrition and liver cancer appear to be interrelated in human populations; in experimental animal models, nutrients have a profound influence on tumor induction. AFB\textsubscript{1}, nitrosamines, and acetylaminofluorene all induced tumors earlier or with a higher incidence in rats fed diets high in fat (27, 34).

Two metabolic changes for AFB\textsubscript{1} are O-demethylation and ring hydroxylation (9, 24); these changes may be carried out by PNA and BPHO systems, respectively. These reactions, clearly affected by dietary corn oil and beef fat, suggest that such activity may be related to acute and chronic responses, although we have no basis for assuming that this was in fact the case.

PNA activity was consistently enhanced by corn oil, whether the oil was included in the diet at moderate or at high levels (Table 4). Both beef fat and corn oil actively enhanced enzyme induction, but corn oil was most effective, whether considered on a per g liver or whole liver basis.

The diets low in lipotrope but high in beef fat have been shown in other studies (28) to result in depressed PNA and BPHO activities, although liver tumors induced by AFB\textsubscript{1} were increased. In the present study, enzyme activities were increased by corn oil, parallel to an increase in liver tumor incidence. Thus, decreased MFO enzyme activity (low lipotrope-high beef fat) and increased enzyme activity in rats fed high corn oil both can be associated with enhanced tumor induction by AFB\textsubscript{1}, creating an apparent paradox. These observations would suggest that activation of AFB\textsubscript{1} by MFO enzyme systems may not be the primary effect leading to enhanced carcinogenesis. Other considerations would include: (a) alterations in glutathione levels, which in turn might have modified AFB\textsubscript{1}-nucleic acid adduct formation through removal of the activated carcinogen as a mercapturic acid (1, 21) [Degen and Neumann (7) have documented and characterized the formation of a glutathione adduct of AFB\textsubscript{1}]; (b) the fact that measuring enzyme activities at one or only a few time points may not reflect long-term consequences of AFB\textsubscript{1}; (c) a suggestion that the growth environment of the initiated neoplastic tissue may be altered by the type of fat; and (d) a difference in rate of DNA repair related to dietary fat.

It seems clear in this study that, contrary to results reported by some investigators (13, 38), cholesterol was not involved in the increased tumor incidence in rats fed corn oil. A recent report by Kitagawa (14) provides additional convincing evidence that a promoting agent can markedly enhance hepatocarcinomas induced by a weak carcinogen. Perhaps corn oil is a promoting agent for AFB\textsubscript{1}.

The observations reported here imply that quality of dietary fat had an effect on liver tumor induction and that tumor induction was associated with altered microsomal enzyme activities. However, whether AFB\textsubscript{1} activation was modified is not answered by these data.

REFERENCES


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**Fig. 1.** Liver section from rat fed beef fat and given an LD₅₀ of AFB₁ (7 mg/kg body weight) as a single dose. Note widespread hemorrhagic necrosis. H & E, × 60.

**Fig. 2.** Area from liver of rat treated as in Fig. 1 but with less damage. Lipid accumulation was present in areas where hemorrhagic necrosis did not occur. H & E, × 90.

**Fig. 3.** Liver from rat fed corn oil and given an LD₅₀ of AFB₁ (7 mg/kg body weight) as a single dose. Note relatively intact liver except for single area of focal necrosis. H & E, × 90.

**Fig. 4.** Gross appearance of liver from rat fed beef fat and exposed to AFB₁, 15 daily doses of 25 μg each. Tumor, while multicentric, is restricted to the liver, and in none of the rats fed beef fat were abdominal extensions observed. However, lung metastases were present in 20 to 25% of the animals (Table 5).

**Fig. 5.** Gross appearance of liver from rat fed corn oil and exposed to AFB₁, 15 daily doses of 25 μg each. The tumor is multicentric but also had widely infiltrated the abdominal cavity, surrounding and invading the stomach wall (arrows). Such extensions were common in corn oil-fed rats, as were lung metastases (Table 5).

**Fig. 6.** Histological appearance of hepatocellular carcinoma from rats fed corn oil and given doses of AFB₁. Many of the tumors of both groups were trabecular, but in corn oil groups they were often anaplastic, had frequent mitotic figures (arrows), and generally were more necrotic. H & E, × 370.
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