Variable Effects of a Lipotrope-deficient, High-Fat Diet on Chemical Carcinogenesis in Rats¹

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SUMMARY

Earlier studies demonstrated enhanced chemical carcinogenesis in the liver, colon, and probably esophagus of male rats that were fed a lipotrope-deficient, high-fat diet. In further experiments, designed to examine the range of the dietary effect on chemical carcinogenesis, rats were fed either the marginally lipotrope-deficient, high-fat diet or an adequate control diet, and treated with N-2-fluorenylacetamide, 3,3 diphenyl-3-dimethylcarbamoyl-1-propyne, N-methyl-N-nitroso-N'-nitroguanidine, N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide, aflatoxin G₁, or ethionine. N-2-Fluorenylacetamide induced hepatocarcinomas more rapidly and in higher incidence in deficient rats than in control rats. 3,3-Diphenyl-3-dimethylcarbamoyl-1-propyne induced a higher incidence of hepatocarcinomas but not gastric tumors in deficient rats. Aflatoxin B₁, included as a positive control, was significantly more hepatocarcinogenic in deficient rats. Gastric tumor induction by N-methyl-N-nitroso-N'-nitroguanidine and induction of tumors of the urinary bladder by N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide were not influenced by diet. Aflatoxin G₁ and ethionine were toxic to deficient rats, and carcinogenic doses could not be administered.

INTRODUCTION

The nutritional condition of experimental animals, and probably of people, is a major factor in determining response to chemical carcinogens. Rats are rendered more susceptible to chemical hepatocarcinogenesis by nutritional deficiencies of choline, methionine, and folate (lipotrope deficiency) and to N-nitrosodimethylamine renal carcinogenesis by nutritional deficiency of protein (2, 10, 13, 16, 19, 22). Deficiencies of these and other nutrients may be responsible for the enhanced susceptibility of cirrhotic patients to hepatic and esophageal carcinogenesis (7, 21). Nutritional deficiencies may alter carcinogenesis by altering metabolism of chemical carcinogens by the drug-metabolizing enzymes of the smooth endoplasmic reticulum. In deficiency of either lipotropes or protein, the enzymes are significantly reduced (1, 12).

A diet marginally deficient in lipotropes, which supported normal weight gain and life-span, enhanced AFB₁, N-nitrosodimethylamine, DBN, and 1,2-dimethylhydrazine carcinogenesis and induced depression of hepatic drug-metabolizing enzymes in rats (12–14, 16). The studies reported here were designed to examine further the range of the dietary effect on chemical carcinogenesis.

The carcinogens studied were AAF, DDCP, MNNG, FANFT, AFG₁, and ethionine. AFB₁ was included as a positive control for the dietary effect. AAF, DDCP, MNNG, and FANFT are carcinogens of chemical classes not studied previously in lipotrope-deficient rats (3, 5, 20). MNNG and DDCP, administered intragastrically, induce gastric tumors; the effect of lipotrope deficiency on gastric carcinogenesis was not examined in earlier studies. MNNG, unlike the other compounds studied, does not require activation by the tissues (20). FANFT is a bladder carcinogen and was studied for comparison with DBN, which was a more effective carcinogen for the liver but not for the bladder of lipotrope-deficient rats (16). AFG₁ was included to assess the dietary effect on renal carcinogenesis by a class of carcinogen known to have enhanced activity in lipotrope-deficient rats. Ethionine hepatocarcinogenesis was reported to be inhibited by addition of lipotropes to an adequate diet (4).

MATERIALS AND METHODS

Male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, Mass.) were used in all experiments, except in the studies of AFB₁ and AFG₁, in which male Fischer rats (A. R. Schmidt Co., Madison, Wis.) were used. Rats weighing 40 to 50 g were fed either Diet 1 (adequate) or Diet 2 (high-fat, marginally lipotrope-deficient) for 3 weeks. Diet 1 was composed of vitamin-free casein, 22%; dextrose, sucrose, and dextrin, 56%; Wesson oil, 15%; vitamins, 2%; and Rogers-Harper's salts, 5% (11). The content of choline was 0.3%. Diet 2 was composed of vitamin-free casein, 3%; alcohol-extracted peanut meal, 12%; gelatin, 6%; fibrin, 1%; L-cystine, 0.5%; cellulose fiber, 2%; sucrose, 36.3%; beef fat, 30%; Mazola oil, 2%; vitamins, 2%; Rogers-Harper's salts, 5%; and choline, 0.2%. No folate was added to the diet (15). There were 25 to 35 rats per diet
and treatment group, unless otherwise noted, and 5 untreated rats per diet group served as controls for each treatment group.

Rats were treated with carcinogen by one of the regimens given in Table 1. AFB1, AFG1, and DDCP were dissolved and administered in dimethyl sulfoxide; MNNG and ethionine were given dissolved in distilled water. Rats were weighed weekly and maintained on Diet 1 or 2 until they developed hematuria or persistent weight loss or were moribund, at which time they were killed and autopsied. Food intake was measured 1 week of every 4 when carcinogens were fed. The major organs were fixed in 10% neutral buffered formalin, processed by routine histological methods, and examined in sections stained with hematoxylin and eosin. In rats fed FANFT, the urinary bladder was distended with formalin before being opened. Cumulative probability of death with tumor was calculated as described by Saffiotti et al. (18). The calculation is based on the number of animals at risk and the number dead with tumor each week and is made by the equation:

\[
\rho(n) = 1 - \left\{ \frac{(N_1 - t_1)}{N_1} \times \frac{(N_2 - t_2)}{N_2} \times \ldots \times \frac{(N_n - t_n)}{N_n} \right\}
\]

\(N\) is the number of animals alive at the beginning of the week; \(t\) is the number that died with a tumor during the week; \(p\) is the probability of death with tumors. Tumor incidence was compared statistically by the \(\chi^2\) test.

**RESULTS**

Untreated rats, that were fed either diet grew well (Chart 1). Rats fed Diet 2 had scattered, fat-containing hepatocytes at autopsy but were otherwise normal. Rats fed the diet for periods of 3 weeks to 18 months have 1.5 to 2 times the normal hepatic lipid content (12).

The compounds examined can be divided into 3 groups on the basis of the effect of the experimental diet on their action: (a) compounds that were more effective carcinogens in rats fed Diet 2 than in rats fed Diet 1; (b) compounds that were equally effective in the 2 dietary groups; (c) compounds that were so toxic to rats fed Diet 2 that the tolerated dose was too low to induce a significant number of tumors in either dietary group.

**Group 1 Compounds**

The 1st group of compounds included AFB1, AAF, and probably DDCP.

**AFB1.** As in earlier studies, the carcinogenicity and toxicity of AFB1 were greatly enhanced in rats fed Diet 2 (Chart 2; Table 2). Rats fed Diet 2 were unable to tolerate 15 \(\mu\)g/day 5 days a week, the dose used previously, and all rats were given 15 \(\mu\)g, 3 times a week after the 1st 2 weeks. During AFB1 treatment weight gain was 12% less than in diet controls in rats fed Diet 1 and 15% less in rats fed Diet 2; the total AFB1 dose on a body weight basis was 2.4 mg/kg in rats fed Diet 1 and 2.6 mg/kg in rats fed Diet 2.

Rats fed Diet 2 had an incidence of hepatocarcinoma 8 times that of rats fed Diet 1 \((p < 0.001)\). Twenty-seven \% of tumors in rats fed Diet 2 metastasized to other abdominal organs or the lung; there were no detectable metastases in rats fed Diet 1.

**AAF.** AAF, comprising 0.02\% of the diet, reduced weight gain in rats fed Diet 2 within 1 week and was removed from both diets after 2 weeks because of decreased weight gain in rats fed Diet 2 (Chart 1). AAF was then fed at 0.0125\% for 5 weeks, raised to 0.02\% for 1 week (which led to weight loss in both dietary groups), removed from the diets for 1 week, and then fed at 0.0125\% for the remainder of the feeding period. In rats fed Diet 1, daily intake of AAF averaged 12.7, 5.0, 7.4, 5.7, and 4.3 mg/kg body weight in the 5 periods of measurement during the 16 weeks in which it was fed; in rats fed Diet 2 the intake at the same periods was 12.4, 5.4, 7.8, 5.3, and 4.1 mg/kg. Average total intake of AAF per rat was 279 mg (Diet 1) and 239 mg (Diet 2).

Both the cumulative probability of death with hepatocarcinoma and the final tumor incidence were greater in rats.
fed Diet 2 than in rats fed Diet 1 (Chart 3; Table 2). The incidence of squamous carcinoma of the ear canal was 5% in rats fed Diet 1 and 11% in rats fed Diet 2.

**DDCP.** In the 1st week of administration, rats were given 3 doses of DDCP, 10 mg each, that killed 3 of 10 of the rats fed Diet 1 and 3 of 11 of those fed Diet 2. In all cases there was extensive, hemorrhagic, hepatic necrosis. The remaining rats tolerated administration of 5 mg once or twice a week and began to die with tumor 29 weeks after the 1st dose of DDCP. The incidence of hepatocarcinoma in rats fed Diet 2 was twice that of rats fed Diet 1; squamous carcinoma of the forestomach, accompanied in 1 rat by mucinous adenocarcinoma of the duodenum, occurred in higher incidence in rats fed Diet 1, but the incidence of gastric carcinoma and polyps combined was the same in the 2 groups (Table 2).

**Group 2 Compounds**

The 2nd group of compounds included MNNG and FANFT.

**MNNG.** There was little evidence of MNNG toxicity in rats fed either diet. The weekly dose of 10 mg/kg was raised to 15 or 20 mg/kg after 3 weeks to give a total dose of 155 mg/kg. Rats began to die with squamous gastric polyps at the end of MNNG administration, and the 1st carcinoma was found at 14 weeks (Chart 4). Gastric tumors were induced in virtually every treated rat; 97% of rats fed Diet 1 and 100% of rats fed Diet 2. Sixty-nine % of rats fed Diet 1 and 87% fed Diet 2 had squamous carcinoma; the remaining tumors were squamous polyps. There were marked abnormalities of the nontumorous forestomach epithelium, but there were no tumors or other significant abnormalities of the glandular stomach. There was no effect of diet on cumulative probability of death with gastric carcinoma (Chart 4). Two rats fed Diet 2 bore mucinous adenocarcinomas of the duodenum in addition to gastric tumors.

**FANFT.** FANFT was the only carcinogen tested in this or earlier studies that was more toxic to rats fed Diet 1 than to rats fed Diet 2. In both diet groups weight gain was depressed by FANFT, but the depression was greater in rats fed Diet 1 during the latter half of the feeding period. Facial and ventral abdominal alopecia and cutaneous erythema were marked in rats fed Diet 1 during FANFT administration, but they cleared rapidly after rats were returned to carcinogen-free diet. FANFT was removed from the diet for 2 weeks after 9 weeks of feeding and for 1 week after a

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**Table 2**  
**Incidence of hepatocarcinoma in rats given AFB, AAF, or DDCP**

<table>
<thead>
<tr>
<th>Carcinogen</th>
<th>Total dose</th>
<th>No. of rats*</th>
<th>Hepatocarcinoma (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diet 1</td>
<td>Diet 2</td>
<td>Diet 1</td>
</tr>
<tr>
<td>AFB,</td>
<td>375 μg</td>
<td>375 μg</td>
<td>27</td>
</tr>
<tr>
<td>AAF</td>
<td>279 mg</td>
<td>239 mg</td>
<td>21</td>
</tr>
<tr>
<td>DDCP*</td>
<td>195 mg</td>
<td>195 mg</td>
<td>7</td>
</tr>
</tbody>
</table>

* Number of rats that completed carcinogen treatment.
  * Significantly greater than rats fed Diet 1, p < 0.001.
  * In addition to hepatocarcinoma, 57% of rats fed Diet 1 had squamous carcinoma of the forestomach, and an additional 14% had squamous polyps; in rats fed Diet 2 the incidences were 25% and 38%, respectively; total gastric tumor incidence was 71% in rats fed Diet 1 and 63% in rats fed Diet 2.
Chart 4. Cumulative probability of death with gastric carcinoma in rats fed Diet 1 or 2 and treated with MNNG.

Further 3-week feeding period. In rats fed Diet 1, FANFT intake averaged 87, 97, 68, 65, and 51 mg/kg body weight/day during the periods of measurement; in rats fed Diet 2 the daily intake during the same periods was 78, 82, 67, 56, and 40 mg. Total intake was 2.8 g in rats fed Diet 1 and 2.6 g in rats fed Diet 2.

Tumors were induced only in the urinary bladder, except for a transitional cell carcinoma of the renal pelvis in 1 rat fed Diet 2. Ninety-one % of benign polyps or papillomas and 56% of the malignant tumors were entirely or predominantly transitional cell; the remainder were squamous tumors. Cumulative probability of death with tumor, tumor incidence, and number of tumors per tumor-bearing animal were not significantly different in the 2 diet groups (Chart 5; Table 3).

Group 3 Compounds

The 3rd group of compounds included AFG1 and ethionine.

AFG1. Administration of 50 µg/day, 3 or 5 days a week for 9 doses, resulted in death of 82% of rats fed Diet 2; no rats fed Diet 1 died. AFG1 was given to a 2nd group of rats at a lower dose, 25 µg, 1 to 3 times a week for 22 weeks to give a total dose of 1.025 mg/rat, and then discontinued because of toxicity to rats fed Diet 2; their average body weight was 324 g compared to 346 g in rats fed Diet 1. Tumors were found only in the small number of rats that survived to 18 months. Of 5 rats fed Diet 1, 1 had hepatocarcinoma and renal adenoma, 1 had renal adenocarcinoma, 2 had renal adenoma and hyperplastic hepatic nodules, and 1 had only hyperplastic hepatic nodules. Of 7 rats fed Diet 2, 2 had hepatocarcinoma and the other 5 had hyperplastic hepatic nodules; there were no renal tumors.

Ethionine. In a preliminary experiment 0.1% DL-methionine and DL-ethionine were added to the experimental diets, but rats fed Diet 2 lost an average of 7 g body weight in the 1st week, while rats fed Diet 1, containing the same amount of ethionine and methionine, gained an average of 50 g.

Chart 5. Cumulative probability of death with bladder tumor in rats fed Diet 1 or 2 containing FANFT.

Table 3

<table>
<thead>
<tr>
<th>Diet</th>
<th>No. of rats*</th>
<th>Polyp, papilloma*</th>
<th>Carcinoma</th>
<th>No. of tumors/tumor-bearing rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26</td>
<td>38</td>
<td>15</td>
<td>2.4</td>
</tr>
<tr>
<td>2</td>
<td>31</td>
<td>26</td>
<td>35</td>
<td>2.3</td>
</tr>
</tbody>
</table>

* Number of rats that completed carcinogen treatment.
* Rats with polyp or papilloma only; rats with bladder carcinoma had polyps or papillomas as well.

Alternate feedings of ethionine-free diet and diet containing ethionine also were tolerated poorly by rats fed Diet 2. A 2nd group of rats was given ethionine and methionine by gastric intubation, 50 mg/kg of each compound once or twice a week for 29 weeks to give a total dose of 2.125 g/kg; average body weights at that time were 686 g (Diet 1) and 610 g (Diet 2). All surviving rats were killed at 18 months; there were no hepatic tumors in either diet group.

DISCUSSION

Enhancement of chemical carcinogenesis in the liver of male rats by the marginally lipotrope-deficient, high-fat diet has been demonstrated with several different carcinogens. Neither the dietary component(s) nor the nature of the alteration(s) in the liver responsible for the dietary effect is known. The experiments described were designed to examine the range of the dietary effect by studying carcinogens of different chemical structures, or active in other target organs than chemicals previously studied. Enhancement of hepatocarcinoma induction in rats fed Diet 2 demonstrated earlier with AFB1, N-nitrosodimethylamine, and DBN, has
been found with AAF and probably with DDCP, although a firm conclusion cannot be drawn from the small number of animals given DDCP. The dietary effect, therefore, occurred with 4 groups of hepatic carcinogens: aflatoxins, nitrosamines, aromatic amines, and benzylc compounds. The only exception found was N-nitrosodimethylamine (16).

Effects of Diet 2 on carcinogenesis in other organs are more variable. It enhanced 1,2-dimethylhydrazine induction of tumors in the colon and N-nitrosodiethylyamine induction of esophageal tumors but did not affect DBN induction of esophageal, urinary bladder, or pulmonary tumors (14, 16). In a subsequent study in which N-nitrosodiethylyamine was fed for a shorter time, there was more marked enhancement of hepatic carcinogenesis by Diet 2, but the incidence of esophageal tumors was low and unaffected by diet (17). In this study no dietary effect was found on bladder tumor induction by FANFT or on induction of squamous tumors in the forestomach by MNNG or DDCP.

Differences in tumor incidence were not caused by differences in carcinogen dosage or in body weight gain. The small difference in total AFB, dose, calculated on the basis of body weight, is well below that expected to influence tumor incidence or the latent period for tumor development (24). The daily intake of AAF per kg body weight varied, but the average over the 5 measurement periods was the same in the 2 diet groups. Weight gain was slower in rats fed Diet 2 than in rats fed Diet 1 in both cases, a factor that would be expected to retard rather than enhance tumor development.

The hepatocarcinogens tested all are thought to require activation in the liver; probably the lipotrope-deficient, high-fat diet affects carcinogenesis by increasing activation or decreasing deactivation of the carcinogens (1, 9, 23). The diet-induced differences in effective carcinogenesis offer models in which metabolism and its relation to carcinogenesis can be studied. Reduced hepatic microsomal oxidases, failure of AFB, induction of the enzymes, and retarded clearance of N-nitrosodiethylyamine from the blood have been measured in rats fed Diet 2 (12, 17). No dietary effect on hepatic DMN metabolism was found (16). There is, therefore, evidence of a correlation between dietary effects on metabolism and carcinogenicity, but more detailed studies are needed.

Carcinogenicity of chemicals for the urinary tract apparently is not enhanced by Diet 2, despite the metabolic effects of severe lipotrope deficiency on the kidney (6). The marginal deficiency in rats fed Diet 2 induced no histological renal changes. The greater toxicity of FANFT to rats fed Diet 1 may have been the result of their slightly greater intake of the compound, but this was not reflected in tumor incidence.

The induction of gastric tumors by MNNG, which requires no activation, or by DDCP, which presumably does require activation, was unaffected by diet, but DDCP was the more effective carcinogen in the livers of rats fed Diet 2.

Diet 1 and 2 differ not only in lipid and lipotrope content but also in their sources of protein and carbohydrate. Studies are in progress to examine the effects of these components, as well as of lipid and the lipotropes, on carcinogenesis by the compounds discussed above.

In earlier studies of lipotrope deficiency in which aflatoxin-contaminated peanut meal or purified AFB, were used, enhancement of hepatocarcinogenesis was blocked by addition of choline and methionine to the diet (10, 19). Male rats fed 0.006% AAF in an adequate diet to which a large amount of methionine (2%) or cystine or casein was added had a decreased incidence of hepatic tumors (9). Addition of methionine, choline, or betaine to an adequate diet inhibited to varying degrees the induction of liver tumors by ethionine (4). It is likely, therefore, that deficient dietary levels of the lipotropes and the metabolic load imposed by the high dietary level of fat are responsible for the enhancement of hepatocarcinogenesis in rats fed Diet 2.

REFERENCES

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