Effects of Dietary **Trans** Fatty Acids on Mutagenesis of Known Carcinogens

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**ABSTRACT**

The metabolic activating potential of liver homogenates from animals fed a diet containing 46.6% **trans** fatty acids or a diet containing less than 1% **trans** fatty acids was compared in the Ames assay with 2-aminofluorene (AF), benzo(a)pyrene (BP), and dimethylbenz(a)anthracene (DMBA). The control fat had a similar fatty acid composition only consisting of **cis** fatty acid (**cis** fats). Since both the **cis** and **trans** fats contained moderate levels of satu­rated fatty acids, a comparison was made between these two fats and corn oil. All three fats were incorporated into high fat, 20%, and low fat, 5%, diets and fed to male Sprague-Dawley rats for three weeks. Although the muta­gentic potentials of AF and BP increased with increasing mutagen concentration and with increasing level of dietary fat, there was no consistent difference in mutagenic potential between the **cis** and **trans** fats. DMBA was mutagenic only at the two highest concentrations with livers from corn oil-fed rats. The mutagenic activating potential of S-9 from animals fed **trans** fat diets generally was similar to that of animals fed **cis** fat diets, but did not follow the trend of animals fed corn oil diets. Thus the amount and/or type of polyunsaturated fatty acids (essential fatty acids) present in the diet may be key factors in evaluating the in­crease in mutagenic activity of DMBA by dietary fat.

A large proportion of vegetable fats consumed by Americans is modified chemically by partial hydrogenation to increase stability and to achieve desirable physical properties (6,10,24). Partial hydrogenation results in conversion of variable amounts of the naturally occurring **cis** unsaturated fatty acids into a variety of isomers including **trans** fatty acids (5,7,11,12).

Dietary **trans** fatty acids are readily absorbed, incorpo­rated into tissue lipids, and utilized for energy (8,9). The deposition of **trans** fatty acids is influenced by their level in the diet and may be selective. Generally, adipose, liver and heart tissues contain higher levels per gram of tissue than brain or lung tissue (14,25).

It has been established that dietary **trans** fatty acids are incorporated into membrane phospholipids and can alter enzyme activities in tissues of animals (17,22). However, recently Nishiyama et al. (19) concluded that the difference in the geometry of dietary fatty acids had little effect on modulating the hepatic mixed function oxidase system.

To date the effect of dietary **trans** fatty acids on the metabolism of mutagenic and carcinogenic compounds has not been adequately addressed. Erickson et al. (13) found that promotion of growth of transplanted mammary tumors was similar for animals fed **trans** or **cis** fatty acids, but **trans** fatty acids were less effective than **cis** fatty acids in promoting bloodborne implantation and distant survival of the tumor cells. Selenskas et al. (23) concluded that diets containing saturated fat, **cis** fatty acid or **trans** fatty acids were significantly less effective than were corn oil diets in promoting development of mammary neoplasia in rats at either 5 or 20% fat. Ponder and Green (21) found that **trans** fatty acids potentiate and the mutagenic activating potential of rat liver homogenates from butylated hydroxytoluene-induced ani­mals with 2-acetylaminofluorene in the Ames Salmonella assay.

This research explored the role of dietary **trans** fat on the in vitro metabolism of the mutagens 2-aminofluorene (AF), benzo(A)pyrene (BP), and 7,12-dimethylbenz(a)anthracene (DMBA). The specific aim was to investigate the effect of feeding rats a fat high in **trans** fatty ac­ids (**trans** fat) on the metabolic activating potential of the liver homogenate from these animals in the Ames/Salmonella microsome assay. The corresponding control fat (**cis** fat) had a similar fatty acid composition, consisting only of **cis** isomers. Since both the **cis** and **trans** fats were moderately satu­rated, a comparison was made between these two types of fat and corn oil. All three fats were incorporated into a high fat (20%) and a low fat (5%) diet. The activation potentials of the liver homogen­ates were compared for animals fed a high or low fat diet containing **trans** fatty acids, **cis** fatty acids, or corn oil to determine the effect of **trans** fatty acids on mutagenic potential.

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MATERIALS AND METHODS

Reagents
Nicotinamide adenine dinucleotide phosphate, BP, AF, DMBA, and dimethylsulfoxide (DMSO) were from Sigma Chemical Company (St. Louis). Oxoid nutrient broth No. 2 was from Oxoid USA, Inc. (Columbia, MD). Bacto-agar was from Difco Laboratories (Detroit). Aroclor 1254 was from Analabs, Inc. (New Haven).

Animals and diet composition
Six to eight-week-old male Sprague Dawley rats (Charles River Breeding Laboratories, Inc.) were housed in separate wire mesh cages and maintained under controlled temperature, light, and humidity conditions. They were allowed access to food and water ad libitum. After a 1-week period of adjustment, the standard laboratory diet was removed and six groups of six animals were randomly chosen. Each group was fed a different fat-modified diet for a 3-week period. The six different diets examined were 5 or 20% lipids derived from 1% corn oil and 19% test fat. The rats were housed with members of the Institute of Shortenings and Edible Oils. The corn oil (Mazola) was from Best Foods (Union, NJ). The diets were formulated in such a manner that all nutrients except fat and dextrose were equivalent on a caloric basis (Table 1). To provide the minimum requirement of essential fatty acids (EFA), 1.0% corn oil was added to 4.0% and 19.0% fat diets.

The fatty acid compositions of the dietary fats were analyzed by capillary column gas chromatography (Table 2). Total trans fatty acids were determined by infrared spectroscopy (Table 2). Salmonella typhimurium mutagenicity assay
Aroclor 1254 was used to induce hepatic microsomal enzymes in all six groups of animals. Aroclor, dissolved in corn oil, was administered to each animal as a single intramuscular injection of 500 mg/kg of body weight 5 d before sacrifice. S-9 fractions from the livers of Aroclor-induced rats and S-9 activation mix were prepared according to the method of Maron and Ames (18). For each S-9 fraction, the livers of three animals were pooled to minimize animal-to-animal variation and portions frozen at -70°C. The two S-9 fractions per treatment group were compared for any significant differences in protein content by the Lowry method (16). No differences were found in the S-9 fractions per treatment group in protein content.

The mutagenicity tests were done as described by Maron and Ames (18) with Salmonella typhimurium strain TA98 obtained from B. N. Ames, University of California, Berkeley. For general screening of compounds for mutagenicity Maron and Ames recommend S. typhimurium strains TA97, TA98, TA100, and TA102. This experiment used only TA98 which detects frameshift point mutations because the mutagens tested were known to revert TA98. Both BP and AF give a dose response curve with TA98 with revertant colonies in a range that can be counted with reasonable accuracy.

The mutagens were dissolved in DMSO and stored in sterile, capped tubes in the dark with selected concentrations prepared on the day of the experiment. Four concentrations of each mutagen were selected from the lower end of the linear portion of the dose response curve for that mutagen. Each data point represents the mean ± standard deviation of two different experiments with three plates/concentration. Solvent control plates, culture control plates, and spontaneous revertant plates were included with each experiment. For statistical analysis of data, the mean numbers of revertant colonies were converted using the power transformation model $Y=(\text{revertants/plate})^2$ (26) and then compared using the Student's $t$ test.

RESULTS

Table 3 shows the numbers of mean revertant colonies per plate at different concentrations of AF, BP, and DMBA in the Ames assay from animals fed 5 or 20% levels of trans fat, cis fat, or corn oil. All concentrations tested of both AF and BP were mutagenic toward S. typhimurium (TA98) in the presence of the rat liver homogenate from animals fed 5 or 20% levels of the three fat diets. The liver homogenates from animals fed the 20% level of fat whether cis, trans, or corn oil resulted in greater mutagenic activity with AF compared to the homogenate from animals fed the 5% level. With BP, liver homogenates from animals fed the 20% cis or trans diet resulted in greater mutagenic activity; corn oil fed animals did not show a fat level response. DMBA gave a positive response only in the assays with the liver fractions from rats fed corn oil and only at the two highest levels of DMBA. No...
TABLE 3. Effect of dietary fat on the mutagenic potential of 2-aminofluorene, benzo(a)pyrene, and dimethylbenz(a)anthracene in the Ames/Salmonella microsome assay.

<table>
<thead>
<tr>
<th>Mutagen (tug/plate)</th>
<th>Trans fat 5%</th>
<th>20%</th>
<th>Cis fat 5%</th>
<th>20%</th>
<th>Corn oil 5%</th>
<th>20%</th>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1</td>
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<td>479±121b</td>
<td>119±41</td>
<td>498±138b</td>
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<td>2</td>
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<td>1114±308b</td>
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<td>1242±512b</td>
<td>266±57</td>
<td>559±102b</td>
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<td>5</td>
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<td>1693±338b</td>
<td>549±133</td>
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<tr>
<td>Dimethylbenz(a)anthracene</td>
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<td>26±7</td>
<td>34±11</td>
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</table>

aValues represent mean ± std. dev. after spontaneous revertant colonies have been subtracted; n=6.

bMean differences for 5% vs 20% of the respective fat significantly different p 0.05 with t test.

SRc revertant colonies (SR) consist of the mean ± S.D. of the number of revertant colonies resulting from the incorporation of 100 ul bacteria culture, 20 ul DMSO, and 500 ul liver homogenate mix.

No positive mutagenic response, revertant colonies per plate show less than a two fold increase over spontaneous revertants.

significant difference was found between the mutagenicity of DMBA at the 5 or 20% level of corn oil.

When comparisons were made at the 5% dietary fat level, the liver homogenates from the animals fed the 5% trans diet resulted in the highest mutagenic response for AF. Homogenates from animals fed the cis diet or corn oil diet had progressively less activating potential. On the other hand, the homogenate from the animals fed the 5% corn oil diet resulted in the highest mutagenic response for BP at the lower levels of mutagen. Only with the two higher concentrations of BP did the homogenate from the animals fed the trans fat diet produce a higher mutagenic response than the homogenate from the animals fed the cis fat diet.

At the 20% level, with AF and BP there was no significant difference in the activating potential between liver homogenates from the cis and trans fat groups. However, the activating potentials from both the cis- and trans-fed animals at the 20% level were significantly greater (p<0.05) than the activating potential for the corn oil group with AF.

DISCUSSION

The effect of dietary fat on mutagenicity is inconsistent, since both the quantity and type of fat altered the mutagenic response in the Ames assay. The mutagenic response also varied with the specific mutagen tested.

Increasing the level of dietary fat from 5 to 20% significantly enhanced the mutagenic potential of AF in liver homogenates from rats fed trans fat, cis fat and corn oil. This finding supports studies (2-4) which suggest that a high level of dietary fat increases mutagenesis. The mutagenic potential of BP was significantly (p<0.05) enhanced when the level of dietary fat was increased from 5 to 20% in the cis fat groups and in the trans fat group with the two lower concentrations of BP, but not with the corn oil group. The mutagenic potential of BP in the 5% corn oil assay was as great as in the assays with 20% cis and trans fat greater than with 5% cis fat.

It is difficult to generalize about the different types of fat fed because the effect varied with the mutagens tested. With AF, liver homogenates from animals fed the corn oil had lower mutagenic activity than either cis- or trans-fed animals. With BP, homogenates from animals fed corn oil tended to have greater mutagenic activity than the cis- or trans-fed animals.

Only with DMBA did type of fat affect the positive and negative outcomes of the Ames assay. It should also be noted that the mutagenic response with DMBA is low in comparison to the other mutagens tested. DMBA required activation with liver homogenates from animals fed the corn oil diet to produce a positive mutagenic response demonstrating enhanced metabolic activation. Ip et al. (15) found an apparent requirement of 4.4% linoleic acid (EFA) in the diet to promote the maximum DMBA mammary tumor response. While cancer is a multi-stage process and enhanced tumor yields could involve enhancement at any stage, it is of interest that the 20% corn oil diet is the only diet used which provided more than 4.4% EFA.

The formation of trans isomers is at the expense of linoleate in partially hydrogenated fats, and the potentiating effect of oils high in linoleate content has been demonstrated (1,3,15). The S-9 derived from animals fed the cis or trans
fats, which contained a relatively low level of linoleic acid, tended to behave differently from the S-9 derived from animals fed corn oil, which contains a high level of linoleic acid. This response may reflect inadequate essential fatty acids (EFA) in both the cis and trans fats.

In contrast to the current study, Ostlund-Lindqvist et al. (20) reported the mutagenesis of 2-AF is decreased with the liver S-9 from rats fed a diet containing 36.6% trans fatty acids as compared to the control fat containing no trans fatty acids. However, there are several important differences in the two studies. Ostlund-Lindqvist et al. (20) fed a dietary fat level of 10% (we fed 5 and 20% fat) and investigated a smaller concentration range of 2-AF, 1.4 µg compared to 1-10 µg in this study. They did not report a dose response curve in the assay with S-9 from animals fed the diet containing trans fatty acids. In our study, there were no significant differences in the mutagenesis of 2-AF due to diet at the 20% level while at the 5% level the trans fed animals had the higher activating potential (p<0.05).

In summary, this study found that (a) the effects of quantity and type of fat on mutagenicity vary with the specific mutagen under investigation; (b) the metabolic activating potential of livers from animals fed diets containing trans fatty acids tend to behave similarly to livers from animals fed diets containing cis fatty acids with respect to enhancement of the mutagenic potential of specific carcinogens, and (c) the mutagenic potential of livers from animals fed a high level of fat tended to be greater than that from animals fed a low level of fat. In conclusion, the amount and type of polyunsaturated fatty acids present in the diet (EFA content), rather than the type and quantity of isomerized fatty acids, appear to be key factors in the enhancement of DMBA-induced, but not AF- or BP- induced mutagenic activity by dietary fat.

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