Modulation of Carcinogenesis by Dietary Factors

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The purpose of this report is to present recent data on two modulating factors of carcinogenesis that are found in Western-type diets: a beef-derived mutagenesis modulator that has been shown to inhibit the initiation of epidermal carcinogenesis in mice, and the possible role of net energy rather than dietary fat per se in the enhancement of rat mammary carcinogenesis.

Introduction

It is well established that foods contain substances that can initiate and/or enhance carcinogenesis as well as substances that can inhibit carcinogenesis (1–3). In fact, it would be virtually impossible to consume a diet devoid of carcinogens and enhancers or inhibitors of carcinogenesis. The full implication of this observation in terms of human health is not yet known, but it is possible that the balance between opposing factors in the diet is of greater importance than the presence of any single factor per se (4).

The purpose of this report is to present our recent findings with regard to two modulating factors found in Western-type diets: first, a beef-derived mutagenesis modulator that has been shown to inhibit the initiation of epidermal carcinogenesis in mice, and second, the role of net energy rather than dietary fat per se in the enhancement of mammary carcinogenesis in rats.

Results

Beef-Derived Mutagenesis Modulator

Previously we reported that methylene chloride extracts of fried ground beef contained a mutagenesis modulator as well as bacterial mutagens (4,5). Similar, possibly identical activity was also detected in extracts of uncooked ground beef. The mutagenesis modulator was not toxic for the bacteria under the conditions of test, and appeared to act on the rat liver S-9 preparation (9000g supernatant) added to the system to activate the mutagens for mutagenesis. Depending upon the type of rat liver S-9 (uninduced, or induced with phenobarbital or Aroclor 1254) and the mutagen under test, inhibition, enhancement, or no effect on mutagenesis was observed (5).

Three mutagenesis modulator preparations were tested, and they are identified as preparations A, B, and C. Preparation A was extracted using a modification (6) of the method of Bjeldanes et al. (7) designed to extract the heterocyclic amine mutagens from fried ground beef. However, partially purified modulator preparations are not soluble in water (6), and for this reason the Bjeldanes et al. method (7) is not suitable for efficient extraction of the modulator activity. Therefore, a more efficient method based on extraction with acetonitrile was developed (6). Preparation B was extracted with acetonitrile using the method described above (6). Preparation C was obtained by further purification of preparation B by using column chromatography (6).

Figure 1 demonstrates that the modulator activity extracted from fried ground beef inhibits 7,12-dimethylbenz[a]anthracene (DMBA) mutagenesis in Salmonella typhimurium TA 98 mediated by liver S-9 from phenobarbital-treated rats. The inhibitory effect is due to a decrease in mutagenesis rather than to bacterial toxicity. The data also indicate that the extraction method developed specifically for the modulator (preparation B, Fig. 1B) extracts the modulator more efficiently than the method developed for mutagen extraction (Preparation A, Fig. 1A). For example, the amount of mod-
The properties exhibit or water, 1.2 N stable and were conducted in triplicate except for the control where 6 replicates were performed. Because of limited material, toxicity was assessed by examining the bacterial lawn, which appeared normal on all plates. The amount of modulator tested in this experiment was not precisely quantified and is therefore given as gram-equivalents of original ground beef starting material. (B) preparation B, data presented as means of duplicate determinations except for the control for the mutagenesis assay, where three determinations were performed and the result plotted as the mean ± SEM. The specific activity of this preparation (based on that amount necessary to inhibit mutagenesis by 50%, which is defined as one unit) (6) was calculated to be 9.5 units/mg. (C) preparation C, data presented as means of duplicate assays. The specific activity of this preparation was calculated to be 31 units/mg (6).

We have also found that the modulator is insoluble in water, 1.2 N HCl, and 2.5 N NaOH, but soluble and stable in concentrated H2SO4. These solubility properties appear to eliminate several possible molecular structures, for example, proteins, peptides, charged or polar lipids, carbohydrates, molecules with five carbons or less, phenols or polyhydroxy phenols, hydroxy acids, amino acids, amides, amines, acids or anhydrides (6,8). The solubility in organic solvents, notably hexane, indicates that it is a very nonpolar molecule, a conclusion supported by its behavior on column chromatography (6). It remains possible that the modulator activity is effected by a class of closely related compounds.

Figure 2 shows that all three modulator preparations inhibited the initiation of mouse epidermal tumors by DMBA. The modulator-treated mice consistently exhibited fewer papillomas and lower papilloma incidences. Moreover, some modulator-treated mice were completely protected from the carcinogenic effects of DNBA during the experimental period and beyond.

Each experiment began with two groups of 20 mice, except for the modulator-treated group in Figure 2A, where 17 mice were used. The mouse tumor assay system employed was based on a report of Slaga and Boutwell (9). Female SENCAR (10) or CD-1 mice were treated with modulator dissolved in 0.2 mL acetone or acetone alone (controls). Five minutes later (except where otherwise specified) an initiating dose of DMBA (10 nmole/mouse for SENCAR mice; 50 nmole/mouse for CD-1 mice) in 0.2 mL acetone was applied. One to two weeks later, twice weekly applications of TPA (2 g/mouse for SENCAR mice; 6 g/mouse for CD-1 mice) in 0.2 mL acetone was commenced and continued for the remainder of the experiments. The three experiments reported were conducted independently at different times. For positive control versus modulator-treated mice, the means of papillomas per mouse preparation for A at 20 weeks (30.2 ± 3.4 vs. 13.4 ± 2.4, mean ± SEM), for preparation B at 19 weeks (9.6 ± 0.8 vs. ± 4.5 ± 0.9, mean ± SEM) and for preparation C at 12 weeks (10 ± 1.7 vs. 3.2 ± 1.1, mean ± SEM), were all significantly different (p < 0.01) by the t-test. There was no evidence that treatment with the modulator produced toxicity. SENCAR mice were used for preparation A. The amount of modulator preparation tested in this experiment was not precisely quantified, but each mouse was treated with an amount of modulator equivalent to that derived from 500 g ground beef (using the Preparation A extraction system). Papillomas were evident at 6–7 weeks but were not systematically quantified until week 10 as indicated. One group of five mice treated with modulator alone (no DMBA) followed by TPA had no tumors at 20 weeks. Although the number of papillomas per mouse appears not to have plateaued by week 20, the respective means at 18 and 20 weeks for the control mice were not significantly different (p > 0.2) by the t-test. This was also true for the modulator-treated mice. In B, SENCAR mice were used. Modulator preparation B was applied at 20 mg/mouse representing an amount derived from 20 g ground beef using this extraction system. Four modulator-treated mice that had not developed tumors at week 19 (termination of experiment) were retained; as of this writing (week 42) two of these mice were still tumor-free. In C, CD-1 mice were used, and it was therefore necessary to increase the DMBA dose. For this reason the modulator dose was also increased to 45 mg/mouse. Of the 20 modulator-treated mice, 10 were treated with modulator 5 min. prior to DMBA and 10 were treated 30 min prior to DMBA (controls received

![Figure 1](image1.png)

**FIGURE 1.** Inhibition of rat liver S-9 mediated DMBA mutagenesis by three different preparations of modulator. The control (DMBA without modulator) for each experiment is indicated on the abscissa by the letter C. *Salmonella* typhimurium TA 98 was used, and the background (20–30 colonies) was subtracted from all data prior to analysis. (A) preparation A, data presented as mean ± SEM where the SEM is larger than the symbol. Assays were conducted in triplicate except for the control where 6 replicates were performed. Because of limited material, toxicity was assessed by examining the bacterial lawn, which appeared normal on all plates. The amount of modulator tested in this experiment was not precisely quantified and is therefore given as gram-equivalents of original ground beef starting material. (B) preparation B, data presented as means of duplicate determinations except for the control for the mutagenesis assay, where three determinations were performed and the result plotted as the mean ± SEM. The specific activity of this preparation (based on that amount necessary to inhibit mutagenesis by 50%, which is defined as one unit) (6) was calculated to be 9.5 units/mg. (C) preparation C, data presented as means of duplicate assays. The specific activity of this preparation was calculated to be 31 units/mg (6).

![Figure 2](image2.png)

**FIGURE 2.** Inhibition of initiation of mouse epidermal tumors by three different preparations of modulator; (●) positive control; (○) modulator-treated mice (6).
acetone alone according to this schedule). The group receiving the modulator 5 min. prior to DMBA appeared to be more inhibited but this has not yet been confirmed in other experiments and for this reason, the results from the two treatment times were combined in calculating the data for Figure 2.

The data of Figure 2B are particularly interesting, since each modulator-treated mouse was treated with 20 mg of preparation B which, by extrapolation (6) was derived from only 20 g of ground beef (weight prior to frying).

Previously we reported (4,5) that the modulator seems to act in part through a direct effect on microsomal enzymes although the nature of this hypothetical interaction is not yet known. Very recently we found that the modulator inhibits the metabolism of 3H-DMBA by rat liver microsomes (Table 1). Further, liver microsomal preparations from untreated or phenobarbital-treated rats were much more sensitive to inhibition by the modulator than were microsomes from 3-methylcholanthrene-treated rats. Interestingly the formation of the DMBA-3,4-diol (a precursor to the ultimate carcinogenic bay region diol epoxide) was substantially more sensitive to inhibition than was formation of other metabolites, suggesting that the modulator acts selectively on certain forms of cytochrome P-450 and notably on form(s) producing high levels of the 3,4-diol (Table 1).

Role of Net Energy in Enhancement of Carcinogenesis by Dietary Fat

The possibility that net energy might be involved in the enhancement of carcinogenesis by dietary fat was first proposed by Boutwell et al. (12). Using data generated by Forbes and co-workers (13–16), Boutwell et al. (12) concluded that the enhancement of epidermal carcinogenesis by dietary fat could be accounted for by the more efficient utilization of fat calories, as compared with calories from carbohydrates. One may imagine the following model: food energy enters the body, and some of it is retained primarily through the synthesis of fat and protein. The rest is lost from the animal as heat or in excreta. Forbes and co-workers (13–16) established that as the amount of fat in the diet increases, the amount of energy lost as heat decreases. The result is a net gain in energy retained per unit of energy intake within the carcass of the animal, as protein and/or fat. It should also be recognized in this context that body weight gain is not synonymous with body energy accumulation (17). For example, a heavier, muscular rat may contain less energy than a lighter fat rat.

The molecular basis for the increased efficiency of utilization of fat is not understood, but it has been confirmed in many species of animals, including fowl (18). It is due in part to the need to synthesize fat from carbohydrate under conditions of low dietary fat (13–16), and fat also increases intestinal transit time which may permit greater absorption of nutrients (18).

The conclusions of Boutwell et al. (12), while intellectually provocative, were not widely appreciated or accepted apparently due in part to criticism by Silverstone and Tannenbaum (19). The criticism centered on the interpretation of that part of the diet that should be considered necessary for maintenance of body functions, and that part that should be considered supplemental and available for energy gain. However, the enhancement observed by Boutwell et al. (12) over that which could be accounted for by calories alone was quite small (20) and hence was consistent with either the interpretation of Boutwell et al. (12) or Silverstone and Tannenbaum (19).

Given the uncertainties surrounding this issue, we have undertaken a re-examination of the effect of relative net energy in terms of the enhancement of car-

Table 1. Effects of the modulator (preparation B) on metabolism of [3H]DMBA by liver microsomes from untreated rats (control) and rats pretreated with phenobarbital (PB-induced) and 3-methylcholanthrene (MC-induced).

| Liver microsomes | DMBA metabolite, pmole/mg/min<sup>a,b</sup> | 5,6-Diol | 8,9-Diol | 3,4-Diol | 7HOMMBA<sup>c</sup> | 12HOMMBA<sup>c</sup> | Total<sup>d</sup> |
|----------------|------------------------------------------|----------|----------|----------|----------------|----------------|-----------|
| Control        |                                          |          |          |          |                |                |           |
| - modulator    |                                          |          |          |          |                |                |           |
| + modulator    |                                          |          |          |          |                |                |           |
| PB-induced     |                                          |          |          |          |                |                |           |
| - modulator    |                                          |          |          |          |                |                |           |
| + modulator    |                                          |          |          |          |                |                |           |
| MC-induced     |                                          |          |          |          |                |                |           |
| - modulator    |                                          |          |          |          |                |                |           |
| + modulator    |                                          |          |          |          |                |                |           |

*Specific activities for all metabolites were determined at 0–30 min for control microsomes, 0–10 min for PB-induced, and 0–5 min for MC-induced microsomes, using the HPLC procedure of Cristou et al. (11).

<sup>a</sup>Values in parentheses denote percent inhibition.

<sup>b</sup>7 HOMMBA: 7-hydroxymethyl-12-methylbenz[a]anthracene 12 HOMMBA: 12-hydroxymethyl-7-methylbenz[a]anthracene.

<sup>c</sup>Includes phenols and polyoxygenated products.

<sup>d</sup>ND, below limits of detection.
Table 2. Effect of dietary relative net energy value (NEV) on breast tumor incidence.\textsuperscript{a,b}

| Diet  | Average daily intake | Body weight, g | Tumor incidence | Yield |
|-------|----------------------|----------------|-----------------|-------|
|       | g/day kcal/day RNE   |                |                 |       |
| HF    | 7.9 ± 0.6 40.7 1.06  | 226 ± 17 73%   | (9/15)          | 2.4   |
| LF    | 11.0 ± 0.9 42.5 1.00 | 203 ± 15 45%   | (6/14)          | 1.2   |
| HFPF  | 6.8 ± 0.7 34.8 0.91  | 196 ± 13 7%   | (1/14)          | 1.0   |

\textsuperscript{a} Weanling female F344 rats were obtained from a commercial supplier and fed \textit{ad libitum} a refined diet (20) containing 5% corn oil (LF). At 52 days of age they were given a single dose of DMBA (65 mg/kg) by stomach tube and divided into three groups. One group was continued on the LF diet, and a second group was fed \textit{ad libitum} a diet containing 30% corn oil (HF). The concentrations of protein, vitamins, minerals, and fiber were balanced to account for differences in the caloric densities of the diets. The third group of rats was given the HF diet, but pair-fed to the LF group in terms of net energy as determined using the interpretation of Boutwell et al. (12) of the data of Forbes and coworkers (13-16) (HFPF group). Note the direct relationship between RNE and tumor incidence.

\textsuperscript{b} Data from Boissonneault et al. (21).

\textsuperscript{c} Different from LF and HFPF groups (p < 0.05).

Carcinogenesis by dietary fat.

The results obtained in a recent experiment, summarized in Table 2, demonstrate that the low fat (LF) group exhibited greater food intake in terms of grams/day and kilocalories/day than did the high fat (HF) group. However, when corrected for relative net energy value (RNE) as interpreted by Boutwell et al. (12), it was apparent that the HF group consumed more net energy, which is also consistent with the fact that the HF group exhibited greater body weight. The food intake of the high-fat pair-fed (HFPF) group was less than what was offered and for this reason the HFPF group consumed less net energy than the LF group. However, the body weights of the LF and HFPF groups were virtually identical. The tumor incidence and yield was in the order HF > LF > HFPF. Notably, there was a direct relationship between tumor incidence and RNE of the diet. Tumor incidence did not correlate with either food intake (kcal/day) or body weight.

Discussion

Modulators of carcinogenesis in the diet may be relevant to human health through either inhibition or enhancement of neoplasia (2,3). This report is focused on two modulators actively studied in this laboratory: a mutagenesis modulator from beef that inhibits initiation of mouse epidermal tumors by DMBA, and the role of net energy in the enhancement of carcinogenesis by dietary fat.

To our knowledge, this is the first report on an anticarcinogenic activity associated with beef. Inhibitors of carcinogenesis have been reported in other foods, notably edible plants, and are postulated to mitigate the potentially deleterious effects of low levels of mutagens and carcinogens that are ingested with food on a daily basis (2,9). Modulator-mediated inhibition of carcinogenic activity is of great importance in view of the fact that a number of PAH carcinogens, such as benzo(a)pyrene, may be found in beef cooked under some conditions (1). Case-control epidemiologic investigations of the possible association of beef and other meats with cancers of the colon-rectum and breast are at present unclear (22). One might speculate that the modulator reported herein can act to moderate the effects of low levels of carcinogens that may be present in cooked beef, leading to epidemiologic findings that are equivocal.

The role of net energy in the enhancement of carcinogenesis by dietary fat has broader public health implications. For example, the public is currently being advised to lower the amount of fat in the diet, from 40–42% to 30% of total calories (23). This advice is based in part on the assumption that the quantity of dietary fat \textit{per se} is critical in tumor enhancement. However, our data indicate that rats can consume a high fat diet and yet develop fewer tumors than rats fed a low fat diet.

The key seems to be restriction of net energy intake rather than fat or even calories \textit{per se}. Reinforcement of this suggestion based on further experimental evidence may alter the way that we think about dietary fat and carcinogenesis and, ultimately, the advice that is given to the public.

\textbf{Note added in proof:} The data shown in Table 1 were obtained using preparations that had not been purified by chromatography. More recent experiments with material of greater purity indicate that the modulator inhibits all forms of cytochrome P-450.

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