# EFFECTS OF DIETARY CORN OIL AND MENHADEN OIL ON RAT MAMMARY TUMORIGENESIS AND PROSTAGLANDIN E2 LEVELS

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by

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ABSTRACT

EFFECTS OF DIETARY CORN OIL AND MENHADEN OIL ON RAT MAMMARY TUMORIGENESIS AND PROSTAGLANDIN E2 LEVELS

Melissa L. Philley
Morehead State University, 1988

Director of Thesis: 

Promotion of mammary tumors by dietary lipids has been established by experimental and epidemiological research. Menhaden oil, a fish oil, however, has been shown to have an inhibitory effect upon mammary tumorigenesis in rodent models. This inhibition may be attributed to the biochemical effects of the omega-3 fatty acids in menhaden oil as opposed to the omega-6 fatty acids of corn oil, a known promoter of mammary tumors.

This study was undertaken to evaluate the effects of different levels of dietary menhaden oil (MO) and corn oil (CO) on tumorigenesis and prostaglandin (PG) levels. At 50 days of age, 120 female Sprague-Dawley rats were given 10 mg of 7,12-dimethylbenz(a)anthracene by gastric intubation. Twenty rats were assigned to one of six isocaloric diets: 1) 3.9% CO, 2) 3.9%

MO, 3) 19.4% CO, 4) 19.4% MO, 5) 15.5% CO:3.9% MO, and 6) 15.5% MO:3.9% CO. The animals were sacrificed on day 127.

Results showed that the 3.9% MO diet was not inhibitory to tumorigenesis. However, rats fed diets containing 19.4% MO exhibited lower tumor burden, less rapid tumor growth and lower tumor PGE2 levels. When rats fed high menhaden oil (15.5%) were supplemented with 3.9% corn oil, tumor inhibition was abolished even though the total lipid levels were the same. Body weights and tumor data of rats on this diet (15.5% MO:3.9% CO) were non-significantly elevated when compared to all other groups. The only rats significantly inhibited in all evaluations were those fed high menhaden oil.

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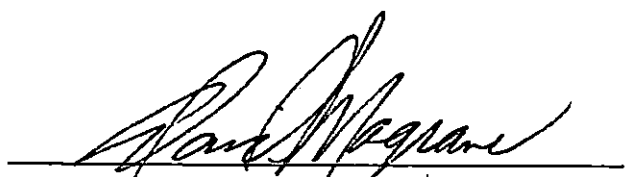

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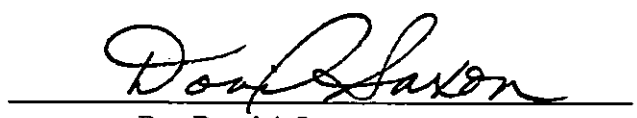

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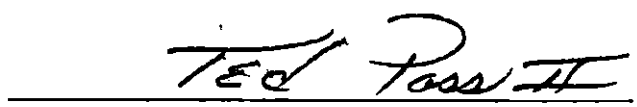

Dr. Ted Pass

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# INTRODUCTION

In the United States one of ten women develop breast cancer, the most frequent cause of death among women (1). There is also evidence of the importance of the early onset of puberty in increasing the risk of breast cancer. Other risks include the family history of the disease, nulliparity, as well as first child after age thirty (2). However, dietary influences have long been suspected as an important cause of breast cancer (3). Epidemiological and laboratory studies suggest a positive correlation between dietary fat intake and cancer of the breast, colon, and prostate (4).

## Diet and Cancer

In experimental animals, both the amount and type of fat in the diet have been shown to influence development of carcinoma of breast, colon, and prostate (5). Some evidence suggests that saturated fat may influence the initiation stage of mammary tumorigenesis and that polyunsaturated fatty acids act on the promotional phase (2,6). Initiation, an early and irreversible stage in the carcinogenic process, is produced by a single or very limited application of a carcinogen. Promotion is the stimulation of development from an initiated cell to a tumor.

Some dietary modifications have been shown to be capable of altering each stage of tumor development (2). Many studies with rats and mice have shown that unsaturated fats, mainly those in the linoleic family, are the most effective in the enhancement of the promotional phase of mammary tumorigenesis (6,7). In 1985, Ip *et al.* observed that mammary tumor development appeared to increase in a step-wise fashion with increasing fatty acid levels in the diet up to 4.4%. Increases in fatty acids above this level appear to have minimal effects (7). A number of mechanisms have been proposed to explain the enhancement of mammary neoplasia. These include:

(1) suppression of immune responses,
(2) inhibition of intercellular communication,
(3) increase in secretion of mammotropic hormones,
(4) alteration of membrane structure and properties, and
(5) increase in production of cyclooxygenase products of arachidonic acid (8).

Vegetable oils, such as corn oil, contains high levels of lenoleic acid (18:2n-6), the precursor of arachidonic acid (20:4n-6) and ultimately dienoic eicosanoids or prostaglandins of the 2-series (9). These dienoic eicosanoids have been implicated by several investigators as the stimulator of the promotional phase of mammary tumorigenesis (10).

7, 12- dimethylbenz(a)anthracene (DMBA)

DMBA (Figure 1) is a synthetic polycyclic aromatic hydrocarbon (PAH) commonly used in the initiation of the rat mammary tumors.

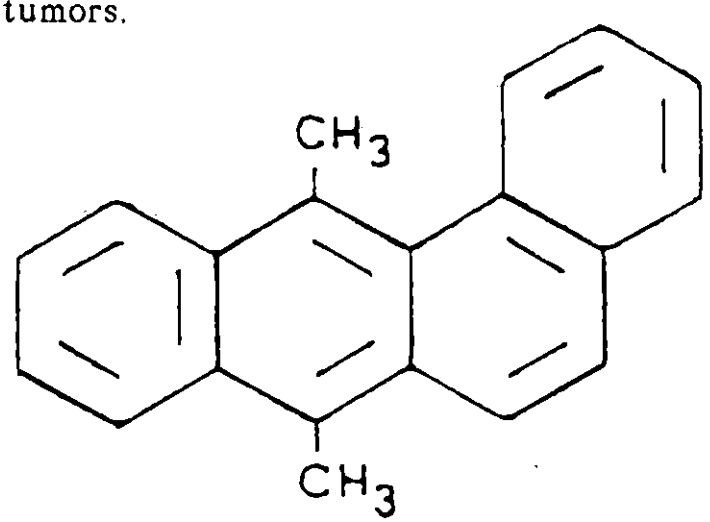


Figure 1. The structure of DMBA. Taken from Huggins (11).

DMBA works consistently and rapidly in a single 10-milligram dose (12). Huggins reported that after a single 20-milligram dose of 7,12-DMBA, the maximum number of mammary tumors observed in a rat was 21 (13). Certain conditions under which mammary cancers are initiated by DMBA include the nature of the hydrocarbon, dosage, species of the rat, strain, age, and hormonal status of the rat (13). Huggins discovered that mammary tumors induced by DMBA in female Sprague-Dawley rats are similar to human breast tumors in that they are easily

palpable and hormone responsive. Also, since they do not metastasize to new tumor sites, the DMBA-induced model is useful in experimentation. (13). The effectiveness of DMBA as an initiator of tumorigenesis is illustrated in Table 1. Mammary tumors, primarily adenocarcinomas consist of mammary acini lined with many layers of epithelial cells arranged to form gland-like structures with papillary projections. This type of tumor can occur from neck to perineum and usually cause death through hemorrhage, necrosis, and ulceration due to enormous size rather than metatasis (11).

Table 1. Incidence of neoplasms in rats fed DMBA. Taken from Huggins (11).

| TUMORS | PERCENT |
|---|---|
| Mammary cancer | 100 |
| Fibroadenoma | 89 |
| Leukemia | 3 |
| Ear duct cancer | 5 |

Carcinogens can be divided into two classes based on the electrophilic hypothesis of carcinogenesis, which states that most, if not all, chemical carcinogens are metabolized to biologically

reactive electrophils that exert their carcinogenic activity (2). DMBA is taken up by the liver, metabolized, and excreted into the bile or exported. Mammary tissue can metabolize DMBA to a slight extent. DMBA is primarily metabolized by the oxidation of the methyl groups and, less completely, by the ring structure (11).

The main electrophilic intermediates, the bay-region of syn- and anti-3,4-dihydrodiol-1,2 epoxides, are thought to bind to DNA to exert carcinogenic activity (15). These epoxides bind covalently to deoxyadenosine and deoxyguanosine residues of DNA (15).

In 1971, researchers discovered that DMBA bound preferentially to the number one chromosone in rats and was expressed in the late interphase and prophase of the cell cycle (11). Within 24 hours of injection with DMBA, one half of the cells in metaphase in bone marrow had chromosomes with breaks and gaps (11).

## Essential Fatty Acids

Dietary polyunsaturated fatty acids are the only source of essential fatty acids (EFA) required by man and other mammals. EFA covers a wide range of 18-, 20-, and 22-carbon chain length polyunsaturated fatty acids (PUFA) with 2 to 6 methylene interrupted double bonds with the *cis* configuration. Neither of

the two classes, the linoleic (omega-6) and linolenic (omega-3), can be synthesized by mammalian organisms (16). There are two main EFAs that are manufactured by plants and not by mammals- the *cis:cis*-linoleic acid (18:2 n-6) and the *cis:cis:cis*-alpha-linolenic acids (18:3 n-3). These are metabolized in the body as shown in Figure 2. EFAs of the n-6 and n-3 type appear to compete for the same enzyme systems, with the n-3 compounds being preferentially metabolized (17). In order for a fatty acid to have EFA activity, all its double bonds must be in the *cis* configuration. Even one bond in the *trans* form leads to loss of EFA activity and to competitive inhibition of the metabolism of all *cis* EFAs. Many commercially available oils contain substantial amounts of *trans* acids and the factor should be taken into account (17).

The parent EFA can undergo chain elongation and desaturation to produce long-chain derivatives of 20- and 22-carbons with 3 to 6 double bonds (16). As seen in Figure 2, linoleic acid is desaturated to form gamma-linoleic acid which is, in turn, elongated to give rise to dihomogammalinoleic acid, the precursor of the series one prostaglandins (PGE1 and PGE1 alpha). Dihomogammalinoleic acid can be desaturated also to arachidonic acid, the precursor of the series two prostaglandins (PGE2 and PGE2 alpha), thromboxanes and leukotrienes. Of the omega-3 fatty acids, alpha linolenic acid is desaturated and elongated to create eicosapentaenoic acid (EPA), the precursor of the series

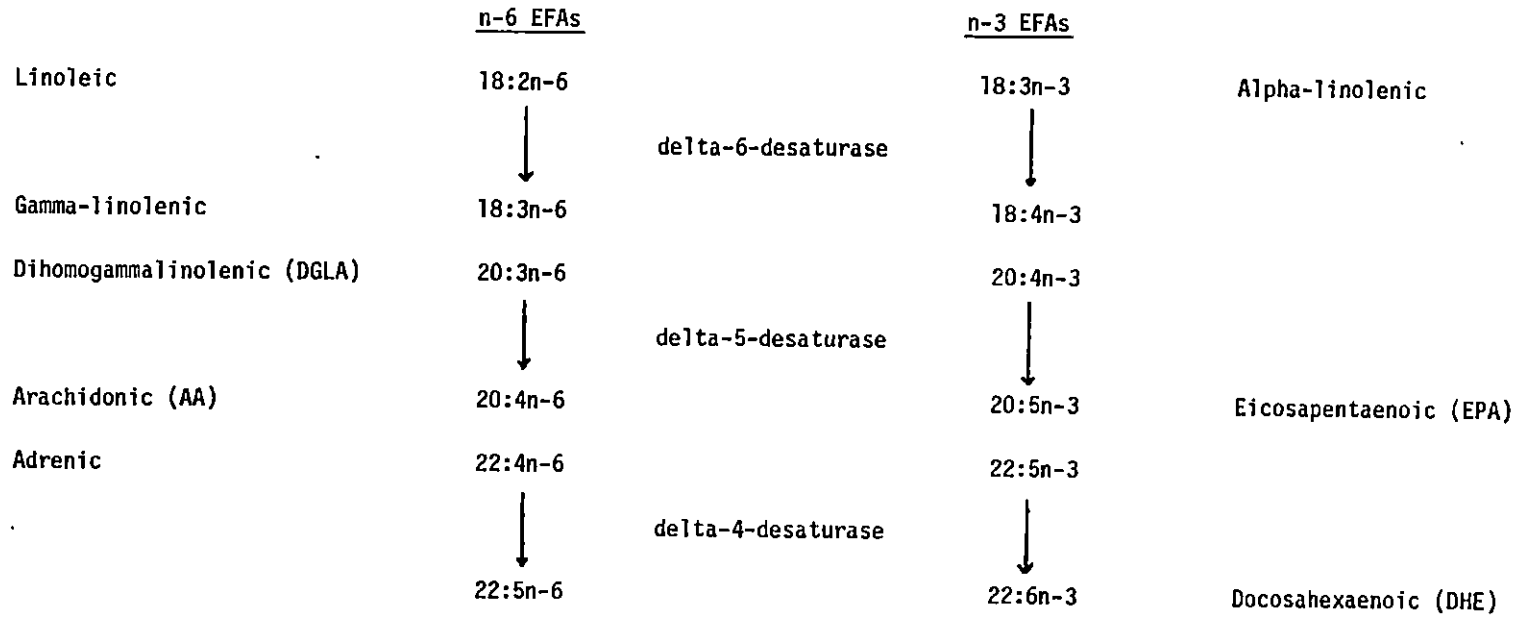


Figure 2. Metabolism of two main dietary EFAs, linoleic acid (18:3n-6) and linolenic acid (18:3n-3). Taken from Reddy(17).

three prostaglandins (PGE3 and PGE3 alpha). EPA is further converted to docosahexaenoic acid (DHA). The resulting fatty acids are fuctional components of cell membranes. They determine membrane fluidity and flexiblity and modulate permeability and the functioning of membrane-bound enzymes and receptors (17).

Because of their polyunsaturation, the EFAs are susceptible to oxygen attack with the formation of peroxidized fatty acids which are highly toxic and may be involved in the metabolic activation of carcinogens. Peroxides may be formed from EFAs prior to consumption or within the body itself. Animals have developed complex mechanisms for protecting themselves against peroxides, both by reducing formation and increasing removal of them (17).

Essential fatty acids are essential nutrients that play a major role in the normal development of the mammary glands and are also constituents of all cell membranes (18). Therefore, it is possible that malignant tumors derived from mammary glands require EFAs for growth and as with any essential nutrient, it will be possible to demonstrate that deprivation of EFAs will inhibit tumor growth and that provision of the nutrients will stimulate growth (17). Animal studies done by Ip *et al.* sugggest that 1 to 2% of the total calories in the form of linoleic acid is the minimum required to prevent EFA deficiency and that mammary

development in rodents may fail at EFA levels which do not maintain normal growth. This suggests that more linoleic acid, about a minimum of approximately 4% of total calorie intake, may be required for the mammary gland (7).

The main conclusions from Cohen's series of studies involving animal mammary tumors and essential fatty acids are:

1) Animals on a high-fat diet develop more tumors earlier than animal on a low-fat diet.

2) Animals on a EFA-deficient diet have a reduced risk of tumor formation.

3) The fat effect is not related to any action on the absorption of lipid-soluble carcinogen (DMBA) or on its uptake into mammary tissue.

4) A certain minimum level of EFA intake is required for maximum tumor development. About 2 to 3% of total caloric intake as EFAs seems to be the critical figure. Provided that this is present in the diet, high intakes of any type of fat, saturated, monounsaturated, and polyunsaturated are effective in further increasing tumor growth. Either n-6 or n-3 EFAs seem to be able to fulfill the minimum requirement. Since n-3 EFAs inhibit prostaglandin formation from arachidonic acid, this argues against prostaglandin formation being critical.

5) Administering a high-fat intake prior to the carcinogen with a normal or low-fat intake afterwards seems to have no

effect on tumor growth. In contrast, many weeks after giving DMBA, adding fat or withdrawing it from the diet is able to modulate tumor growth.

6) And as an exception to the rule that fatty acids promote tumorigenesis, rapeseed oil, which contains erucic acid, can inhibit the conversion of linoleic acid to arachidonic acid and it inhibits the lipoxygenase enzyme which converts PUFAs to hydroxy-acids and leukotrienes (17).

Menhaden oil

Fish oils contain high concentrations of omega-3 fatty acids. Current interest in omega-3 fatty acids has been spawned from the observations that despite their high-fat, high cholesterol, low carbohydrate diet, Greenland Eskimos rarely die of cardiovascular disease and Alaskians have a 10.3% death rate due to cardiovascular disease whereas approximately 50% of all deaths in the mainland United States are due to cardiovascular disease. These omega-3 fatty acids of interest are eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids. They are found almost exclusively in seafood (19). Whereas corn oil, with its main constituent of linoleic acid, has been shown to greatly enhance tumorigenesis (2-7,14,17,18), several recent studies have demonstrated the inhibition of menhaden or fish oil on DMBA-

induced mammary cancer (8,9,16,20) and in transplanted mammary tumors (21-23). Table 2 taken from Abou-El-Ela *et al.* illustrates the fatty acid components of both corn oil and menhaden oil (9).

Table 2. Fatty Acid Composition of Oils (%) (9).

| Fatty acid | Corn oil | Menhaden oil |
|---|---|---|
| 14:0 | -- | 8.35 |
| 16:0 | 11.2 | 15.17 |
| 16:1 | -- | 11.62 |
| 16:2 | -- | 2.37 |
| 16.3 | -- | 1.96 |
| 16:4 | -- | 1.73 |
| 18:0 | 2.1 | 2.67 |
| 18:1 | 25.0 | 9.50 |
| 18:2n-6 | 59.9 | 1.81 |
| 18:3n-6 | 0.5 | -- |
| 18:3n-3 | -- | 1.82 |
| 18:4 | -- | 3.47 |
| 20:1 | -- | 1.32 |
| 20:4n-6 | -- | 2.30 |
| 20:5n-3 | -- | 16.03 |
| 22:5 | -- | 3.92 |
| 22:6n-3 | -- | 10.83 |
| others | 0.1 | 4.37 |

In 1984, Karmali *et al.* used a MaxEPA marine oil to test the effect of omega-3 fatty acids on transplanted mammary tumors (20). They concluded that EPA and DHA replace the omega-6 fatty acids in the tissue lipid, plasma, and platelets membranes of their experimential animals, and that EPA and DHA should limit the synthesis of the 2-series of prostaglandins. These results suggested that the mechanism underlying tumor growth may be linked to arachidonic acid metabolism and may be similar to the effect of EPA and DHA in lowering platelet responsiveness in the Eskimos. They surmised that omega-3 fatty acids may exert their effects by competing with arachidonic acid for the cyclooxygenase enzyme, thereby reducing the synthesis of prostaglandin E2 from arachidonic acid (20).

A study by Jurkowski and Cave in 1986 showed that while a 20% dietary fat level was effective in reducing tumor development, this was less apparent in 0.5% and 3% dietary fat level (22). Also, the animals given a diet of 0.5% menhaden oil developed more tumors than the 0.5% corn oil control group.

The following year, Gabor and Abraham experimented with mixed diets of menhaden and corn oil (21). They observed that a diet of 10% menhaden oil and a mixed diet of 9% mehaden and 1% corn oil were effective in inhibiting tumorigenesis, while a mixed diet of 7.5% menhaden oil and 2.5% corn oil was not effective in significantly inhibiting tumorigenesis.

Menhaden oil has also been shown to inhibit NMU-induced mammary tumors (22), L-azaserine-induced preneoplastic lesions in rat pancreas (24), IX transplantable mammary adenocarcinomas (23), azomethane-induced colon tumors (25), and lung tumors (26). Current evidence suggests that further efforts are needed to establish the optimal dietary ratios of omega-3 and omega-6 fatty acids and the actual mechanism of action.

Prostaglandin E2

Prostaglandin is a term applied to a series of compounds derived enzymatically and nonenzymatically from twenty carbon fatty acids such as arachidonic acid and eicosahexaenoic acid. Research over the past twenty years has identified prostaglandins as products of almost every cell, although the amount and class of prostaglandin produced varies with cell type (27). The dienoic eicosanoids are formed from arachidonic acid which can be obtained only from dietary sources of omega-6 EFA (Figure 3). In normal mammary fat pads, levels of PGE2 (Figure 4) were not changed by increasing linoleate content of diet (9). The production and secretion of arachidonic products, particularly PGE2, is increased several fold in a large number of animal (9) and human (28, 29) tumors.

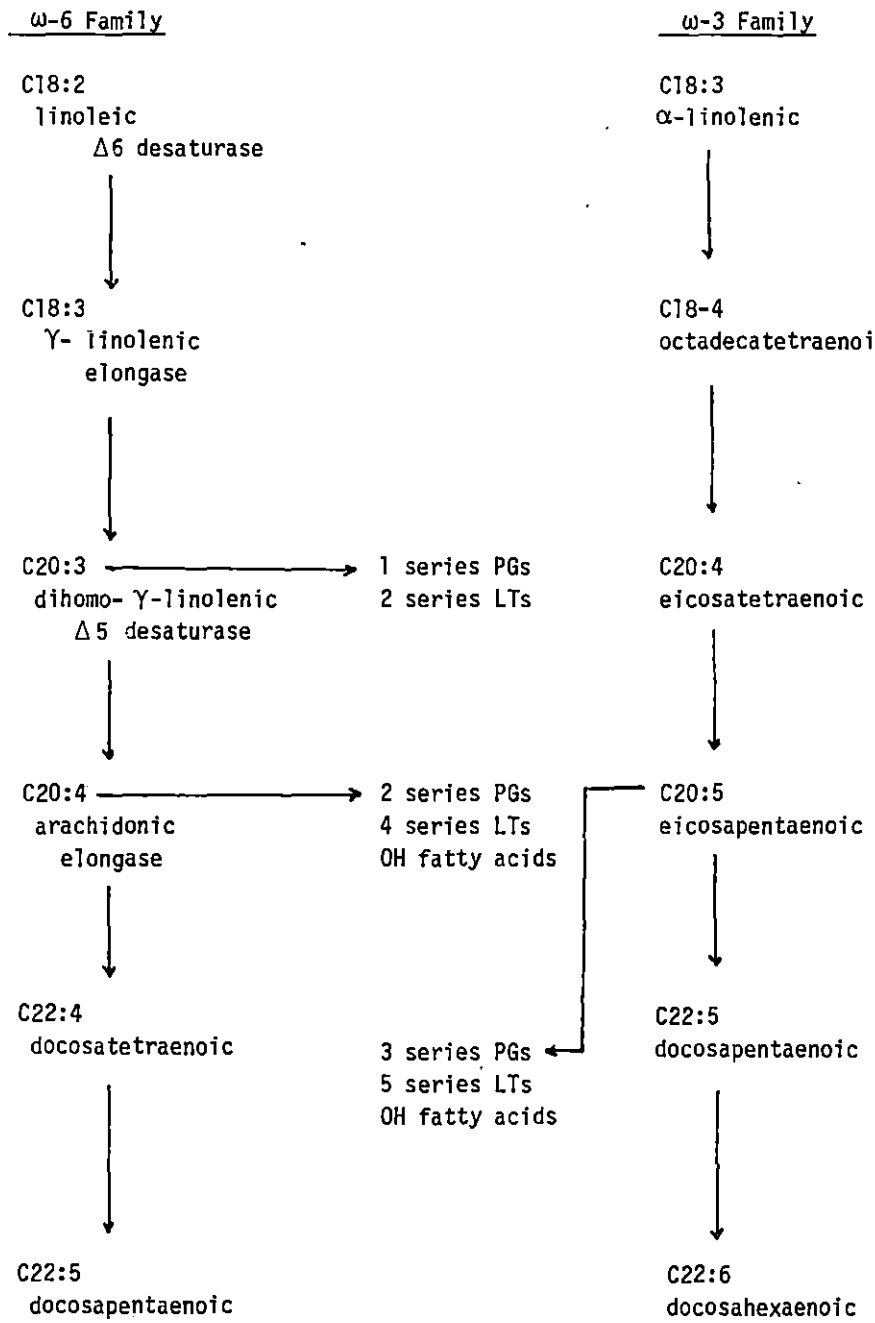


Figure 3. Formation of Eicosanoids. Taken from Karmali (8).

Further support for the prostaglandin-high-fat-diet-mammary tumorigenesis hypothesis comes from reports suggesting that prostaglandin inhibitors such as indomethacin are effective in suppressing mammary tumorgenesis in rats fed diets high in unsaturated fatty acids. Indomethacin works by inhibiting the cyclooxygenase component of the prostaglandin synthetase enzyme system (10).

Along with the inhibitory effect on mammary tumor growth, diets rich with fish oil may also be able to control tumor cell metastasis. EPA and DHA have the ability to alter platelet function though the reduction of thromboxane A2 synthesis and by these means can possibly interfere with tumor cell platelet interaction, an important mechanism in tumor cell metastasis (31).

However, when analyzing prostaglandins it is essential to process tumors as soon as possible due to the lability of the enzymes associated with prostaglandin synthesis. Substantial amounts of prostaglandins may be generated in response to trauma and variation in degree of tissue trauma might be expected to be associated with differences in prostaglandin levels (28).

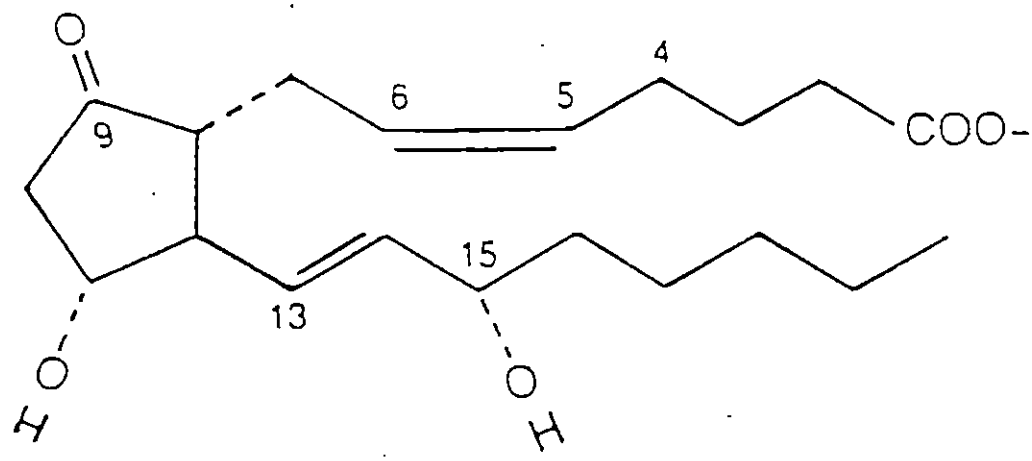


Figure 4. Structure of prostaglandin E2. Taken from Martin (30).

Research Objectives

In this study, the effects of dietary menhaden oil, rich in omega-3 fatty acids, were studied on tumor incidence, tumor number, tumor burden per tumor bearing rat, and tumor PGE2 levels. High- and low-fat diets as well as mixed diets of menhaden and corn oil were compared. It was hypothesized that rats fed a high-fat diet of menhaden oil would show a decreased tumor incidence, tumor number, tumor burden, and PGE2 levels when compared to high-fat corn oil. The effect of mixed diets on tumorigenesis is controversial and preliminary effects are unknown (9,22-24).

# MATERIALS AND METHODS

## Animal Treatment

One hundred and twenty female Sprague-Dawley rats, 36 to 40 days of age, were obtained from Harlen Sprague-Dawley Inc., Indianapolis, Indiana. The rats were housed in stainless steel cages, one per cage, in a temperature- and light-controlled room (14L:10D). Food and water were given *ad libitum*. At 50 days of age, each animal was lightly etherized and given a 10-milligram dose of DMBA in 1ml of corn oil by intragastric intubation. Twenty rats were then assigned to one of six groups: 1) 3.9% corn oil (3.9%CO), 2) 3.9% menhaden oil (3.9%MO), 3) 19.4% corn oil (19.4%CO), 4) 19.4% menhaden oil (19.4%MO), 5) 15.5% corn oil & 3.9% menhaden oil (15.5%CO:3.9%MO), and (6) 15.5 % menhaden oil & 3.9% corn oil (15.5%MO:3.9%CO). These percentages reflect low and high concentrations of lipids. The components of all diets were expressed in percentage by weight and all diets were isocaloric (Table 3). All diets were prepared to contain 3.51 kilocalories per gram. The casein, cellulose, minerals, vitamins, methionine, choline, and menhaden oil were purchased from United States Biochemical Corporation, Cleveland, Ohio. The sucrose and corn oil were purchased locally. One rat in the 3.9% corn-oil group died in the ninth week due to unrelated causes.

Five weeks following initiation, all rats were palpated weekly for tumor growth. At day 77, all rats were sacrificed by decapitation and their total number of tumors measured in three dimensions with calipers. Tumor position and dimensions were recorded on a rat diagram.

Each tumor from each tumor-bearing rat was dissected, placed in polypropylene tubes containing 0.5 milliliters of indomethacin at a concentration of 10 micrograms per milliliter (31). The tumors were then frozen in a mixture of dry ice and acetone and stored at -20 degrees Celsius.

Table 3. Diet concentrations by percentage.

| COMPONENT | HIGH FAT DIET | LOW FAT DIET |
|---|---|---|
| Casein | 21.6% | 21.7% |
| Sucrose | 21.9% | 57.0% |
| Oil | 19.4% | 3.9% |
| Cellulose | 31.8% | 12.1% |
| Minerals | 3.8% | 3.8% |
| Vitamins | 1.0% | 1.0% |
| Methionine | 0.3% | 0.3% |
| Choline | 0.2% | 0.2% |

PGE2 Extraction and Determination

PGE2 extraction was performed according to the procedure of Abou-El-Ela *et al.* (9). Tumors were finely minced at 4 to 6 degrees Celsius and placed in Krebs Ringer Bicarbonate Glucose solution in a concentration of 1 gram per 5 milliliters. They were then incubated of 37 degrees Celsius for one hour in 95% oxygen/5% carbon dioxide environment. The tumor samples were homogenized for 15 seconds at 8 amperes with a Branson sonifier (Branson Instruments Inc., Stanford, CT) and acidified to a pH 3 with 0.8M phosphoric acid. The prostaglandins were extracted with four volumes of ethyl acetate with vigorous shaking. The organic phase was removed and 50 microliters of 0.1M Tris HCl buffer (pH 7.5) was added. The organic phase was then evaporated under nitrogen and the residue was reconstituted with an appropriate aliquot of assay buffer (9). PGE2 concentrations were determined using a prostaglandin E2 [125 I] RIA kit (Dupont Biomedical Products, Boston, Massachusetts). Antiserum and radioactive tracer were added to each sample and these were incubated for 16 to 24 hours at 2 to 8 degrees Celsius. Following the appropriate time, the precipitating reagent was added and the samples were centrifuged at 1000 to 2000 x g at 4 to 6 degrees Celsius. The excess fluid from each tube was removed and the tubes inverted for one minute prior to a one-minute count in a gamma counter.

Statistical Analysis

Analysis of variance and Duncan's multiple range test were used to determine the significance of the results at $p < .05$.

## RESULTS AND DISCUSSION

Each group, except 19.4% menhaden oil, developed tumors by the end of the fifth week (Table 4). Only one tumor in the 19.4% menhaden oil group was found and not until the time of autopsy. High concentrations of menhaden oil (19.4%) also greatly reduced the percentage of rats with tumors in comparison to the other groups. However, more than half of the rats receiving 3.9% menhaden oil developed tumors. This suggests that low levels of fish oil do not inhibit tumorigenesis. Since 60% of the rats receiving diets of 15.5% menhaden oil and 3.9% corn oil were tumor positive, high levels of the menhaden oil mixed with low levels of corn oil also were not inhibitory. This suggests that minimal levels of corn oil mixed with high levels of menhaden oil eliminated the tumor inhibition by the fish oil alone even though the percentage of lipids was the same in both diets.

A comparison of body weights at death can be seen in Table 5. Rats in the 19.4% menhaden oil had body weights that were significantly lower than all groups. Also, rats on diets of 3.9% CO, 3.9% MO, and 19.4% CO had body weights that were significantly reduced compared to the 15.5%MO:3.9%CO group. This addition of 3.9% corn oil to the diets significantly elevated the body weights compared to the rats receiving 19.4% menhaden oil alone. O'Conner *et al.* states that while a decrease rate of body

weight gain is associated with decreased tumor development, it is erroneous to think that the associated weight development is the single cause for decreased tumorigenesis when the diet causes a rich diversity of other physiological and biochemical effects (24).

Table 4. Comparison between groups, percentage of rats with tumors and first day of tumor occurrence in relation to palpation schedule.

| GROUP | PERCENTAGE | FIRST DAY OF OCCURRENCE |
|---|---|---|
| 3.9%CO | 42% | 41 |
| 3.9%MO | 55% | 41 |
| 19.4%CO | 35% | 41 |
| 19.4%MO | 5% | 77 |
| 15.5%CO:3.9%MO | 45% | 41 |
| 15.5%MO:3.9%CO | 60% | 41 |

Analysis of data between all groups was somewhat difficult due to the fact that only one tumor was found in the 19.4% menhaden oil group (Table 6). While 19.4% menhaden oil group had only one tumor, the 3.9% MO and 15.5%MO:3.9%CO groups had high numbers of tumors. It is unknown why the 19.4% CO had the same number of tumors (13) as the 3.9% CO, in light of published reports that high corn oil diets generally yield

greater tumor numbers than low corn oil diets. The low-fat corn oil group, at 2.3% linoleic acid, is below the linoleic acid level (4%) required for mammary tumorigenesis and the high-fat corn oil diet, at 11.6% linoleic acid, is much above this level (7). Therefore, the higher fat diet should have produced a significantly higher number of tumors.

Table 5. Analysis of variance of body weights at time of death.

| Source | DF | SS | MS | Fcal 5.14 > Ftab 2.29 |
|---|---|---|---|---|
| Factor | 5 | 28541 | 5708 | |
| Error | 113 | 42594 | 377 | |
| Total | 118 | 71135 | | |

| GROUP | N | MEAN | STD. DEV. |
|---|---|---|---|
| 3.9%CO | 19 | 229 (a) | 23.5 |
| 3.9%MO | 20 | 231 (a) | 23.5 |
| 19.4%CO | 20 | 224 (a) | 16.5 |
| 19.4%MO | 20 | 195 (b) | 17.8 |
| 15.5%CO:3.9%MO | 20 | 233 | 16.2 |
| 15.5%MO:3.9%CO | 20 | 245 | 17.5 |

a significantly different from 15.5%MO:3.9%CO at $p<.05$.
b significantly different from all groups at $p<.o5$.

Table 6. Comparison between groups of total tumors, average tumor number per rat, and tumor number per tumor bearing rat.

| Group tumor | Total tumors | Average tumor # per group | Tumor # per bearing rat |
|---|---|---|---|
| 3.9%CO | 13 | .68(a) | 1.6 |
| 3.9%MO | 23 | 1.15 | 2.1 |
| 19.4%CO | 13 | .75(a) | 1.9 |
| 19.4%MO | 1 | .05(a,b) | 1.0 |
| 15.5%CO:3.9%MO | 18 | .90 | 2.0 |
| 15.5%MO:3.9%CO | 32 | 1.60 | 2.7 |

a significant at $p<.05$ from 15.5%MO:3.9%CO.
b significant at $p<.05$ from 3.9%MO and 15.5%CO:3.9%MO.

Average tumor number per group was significantly lower in the 19.4% MO group. Tumor number per tumor-bearing rat was also much lower in the 19.4% menhaden oil group and highest in the 3.9% MO and 15.5%MO:3.9%CO groups. These effects in the 3.9% MO group has been observed by several researchers and implies that additional biochemical features of the lipids must be involved (22,32). The effects of mixed dietary ratios of corn oil and menhaden oil are inconclusive. This research suggests that the 3.9% menhaden oil added to the 15.5% corn oil does not suppress tumorigenesis, but that the 3.9% corn oil added to the

15.5% menhaden oil overcomes the inhibition of 19.4% menhaden oil alone.

Figures 5 and 6 illustrate total tumor burden per tumor-bearing rat per group and average tumor volume per tumor-bearing rat, respectively. Although there was no significant difference among the five statistically analyzed groups, in both figures the obvious difference is the low tumor volumes of the 19.4% menhaden oil group. The average tumor volume per group in Figure 7 again illustrates this difference. The general trend from Figures 5, 6, and 7 is that rats fed diets of either low menhaden oil alone or high menhaden oil plus low corn oil have non-significantly elevated tumor data.

Although there was no significant difference among the comparable groups, there was a noticeable difference in level of PGE2 in the 19.4% menhaden-oil group (Table 7). This lower picogram amount correlates with the findings of the majority of researchers that a high-fat dietary level of menhaden oil is effective in depressing tumorigenic activity in induced mammary tumors through the inhibition of PGE2 synthesis.

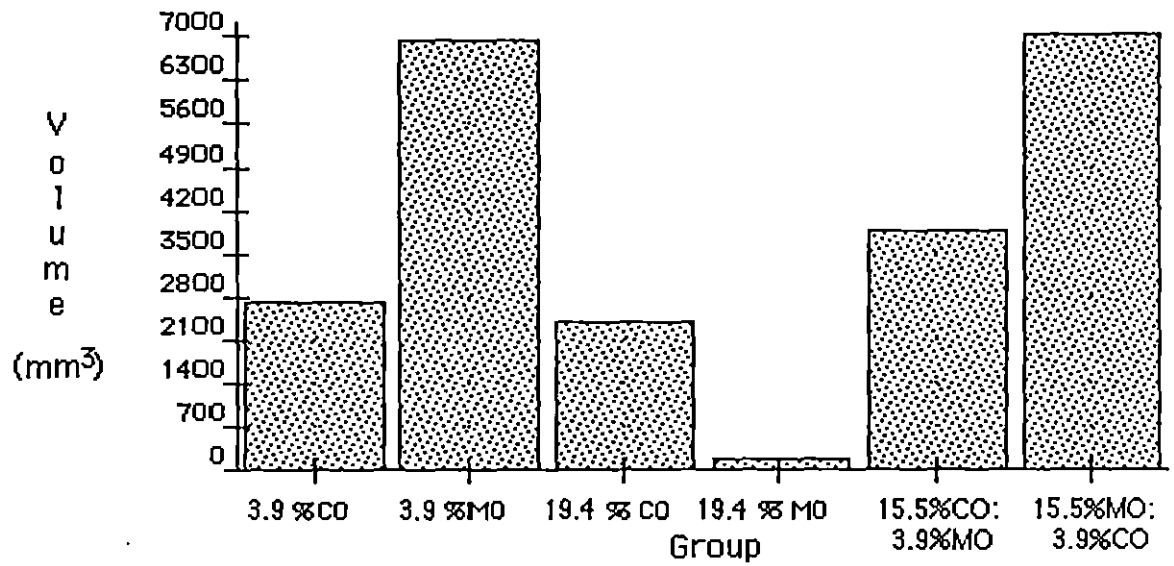


Figure 5. **Total Tumor Volume per Tumor Bearing Rat per Group**

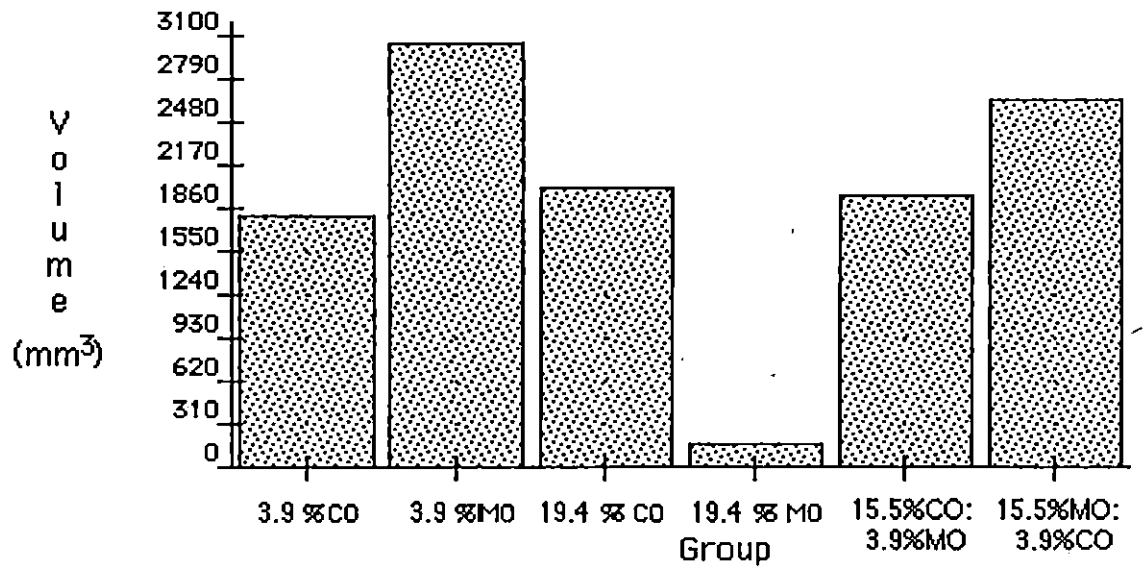


Figure 6. **Average Tumor Volume per Tumor Bearing Rat per Group**

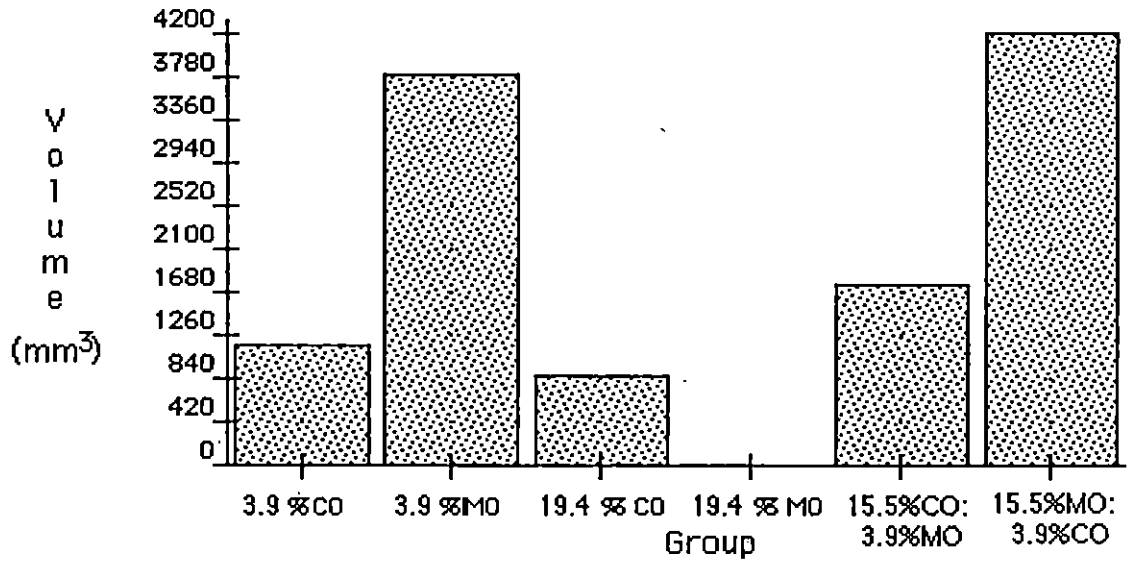


Figure 7. **Average Tumor Volume per Group**

Table 7. Picograms of prostaglandin E2 per dietary group.

| GROUP | PICOGRAMS | STD.DEV. |
|---|---|---|
| 3.9%CO | 6.6 | 4.2 |
| 3.9%MO | 6.1 | 2.2 |
| 19.4%CO | 5.1 | 2.5 |
| 19.4%MO | 3.8 | - |
| 15.5%CO:3.9%MO | 6.7 | 3.8 |
| 15.5%MO:3.9%CO | 6.1 | 2.0 |

In summary, although the mixed diets proved inconclusive and that a 3.9% diet of menhaden oil enhanced tumorigenesis, the obvious conclusion is that a high-fat diet of only menhaden oil was distinctly effective in inhibiting tumorigenesis. The exact mechanism by which this happens is as of yet elusive; however, these data are supportive of the hypothesis that the omega-3 fatty acids (EPA and DHA) contained in menhaden oil are inhibitory to the enzymes of prostaglandin E2 synthesis. However, it is possible that the inhibition by 19.4% menhaden oil is a result of the lowering of body fat and lack of essential fatty acids of the n-6 series. A comparison of body weights, tumor incidence, and tumor weights showed significant reduction in rats

menhaden oil compared to the 15.5% menhaden oil / 3.9% corn oil group. These reductions may be due to low energy reserves in the 19.4% rats, a hypothesis that has been reported by Kritchevsky *et al.* (34).

However, caution must be exerted when evaluating these benefits for human use. Much more experimentation needs to be done to evaluate the ratios at which omega-3 fatty acids are effective. As shown in this study, a low-fat diet of menhaden oil and a mixed diet high in menhaden oil were actually stimulatory in their effectiveness in tumorigenesis.

Fish oil capsules now available contain 300 to 500 milligrams of omega-3 fatty acids per capsule. Therefore, 15 to 30 capsules must be taken per day to equal the amount used in many experimental studies. At this dosage, it would add 570 to 1130 kJ per day to the diet. If the diet were not adjusted to accommodate the increase, then a gain of 11 kg of fat tissue would occur annually. Other adverse side effects would include vitamin A and D toxicity and vitamin E difficiency.

Superfically, omega-3 fatty acids appear to be a great hope for the prevention of dietary fat related cancers. At this moment the actual benefits versus adverse side effects make any human use of fish oil a possible hazard and research is conflicting. However, with more research and other possible treatments,

many cancer tragedies through simple dietary modifications may be prevented.

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