EFFECTS OF INDOMETHACIN ON PROSTAGLANDIN E2 IN 7,12-DIMETHYLBENZ(A)ANTHRACENE INDUCED MAMMARY TUMORIGENISIS IN RATS FED DIFFERENT LEVELS OF POLYUNSATURATED FAT

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Masters of Science in Biology

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by

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Accepted by the faculty of the school of Arts and Sciences. Morehead State University, in partial fulfillment of the requirements for the Master of Science degree

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ABSTRACT

EFFECTS OF INDOMETHACIN ON
PROSTAGLANDIN E2 IN 7,12-DIMETHYLBENZ(A)ANTHRACENE
INDUCED MAMMARY TUMORIGENESIS IN RATS FED DIFFERENT
LEVELS OF POLYUNSATURATED FAT

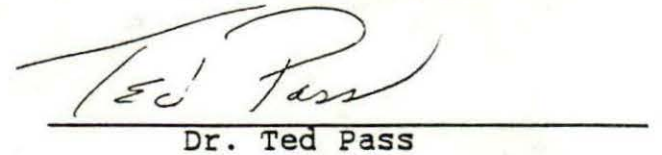
Candace S. Smith
Morehead State University, 1988

The relationships between DMBA-induced mammary cancer, polyunsaturated fatty acids, and PGE2 as well as the effect of prostaglandin inhibitor indomethacin (IM) were examined. Those animals fed a diet containing 20% corn oil (HF) exhibited higher tumor burden, more rapid tumor growth and higher PGE2 levels than the rats fed 4% corn oil (LF). The addition of IM to the animals drinking water in the HF group caused a reduction in PGE2 levels. However, IM had little effect on their tumor growth. The addition of IM to the LF group reduced the percentage of tumor bearing rats by 23% to that of the LF group. Yet, the average tumor volume per rat was higher in all groups that received IM. Therefore, the conditions of this research demonstrated a mild role of PGE2 in the promotion of tumorigenesis in those groups not given IM treatment.

Accepted by:

Dr. David Magrane

Dr. David Saxon



Dr. Ted Pass

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Investigations have identified a number of risk factors for the development of breast cancer, including early age at menarche, late age at first child birth, late age at menopause, and a family history of breast cancer. However, these factors do not explain the marked differences in breast cancer among women of different ethnic background around the world (1). The fact that one out of 10 American women develope breast cancer (2), and this type of cancer is the most frequent cause of cancer related death in these women brought about many studies in the last decade in the possible causes for the discrepancies (3). It was quickly determined that dietary fat played a major role. The United States has one of the highest dietary fat intakes per capita in the world and it has been estimated that the average American diet is composed of 20% fat (4).

It has been shown that diet effects mammary tumors at two levels--the rate at which they form and the rate at which they grow (3). Yet, the mechanisms by which these rates are effected are still unclear. Several animal studies using a variety of both

spontaneous, translatable, and induced mammary tumors have demonstrated a direct correlation between a high fat diet and the growth of mammary tumors (4-17).

A possible mechanism being widely studied is the role of prostaglandins. Prostaglandins are lipid derived, therefore it was assumed that an increase in dietary fat would lead to an increase in prostaglandin production. Several laboratories have demonstrated this concept (4-31). It was through the use of prostaglandin inhibitors that a correlation between a reduction in the level of prostaglandins and tumor growth was first exhibited in several studies (4,5,11) while a study by Feldman and Hilf was not as successful (21).

Diet and Cancer

The accepted mechanism for cancer development is that the cancer is first initiated by some factor (i.e. chemical, radiation, virus) followed by a longer term promotional phase. Lipids have been suggested to be a tumor promoter.

After it was demonstrated that a high fat diet enhanced tumorigenesis, further study was needed to determine if some types of lipid were more effective at enhancing tumor growth than others. There appeared to be a tendency for diets high in polyunsaturated fat to

have higher tumor yields than saturated fat diets (1). It was further observed that a combination of a large amount of polyunsaturated fat with a small amount of saturated fat produced more mammary tumors than either source of fat (1). In general, diets rich in unsaturated fats (e.g. most vegetable oils) are more efficacious than diets rich in saturated fats (e.g, many animal fats) in the enhancement of the promotional stage of mammary tumorigenesis (9). Recent studies suggest that diets rich in saturated fatty acids, in contrast to unsaturated fatty acids, may be more important in the initiation stage of this tumorigenic process (9). Evidence now indicates that only a small amount of polyunsaturated fat is required to enhance the yield of mammary tumors. As little as 3% polyunsaturated fat when fed in a high saturated fat diet promoted tumor development as effectively as a diet containing four times as much (10).

In a study by Hillyard and Abraham on the cellular elavation of tumors, it was determined that there was no difference in G1,S,G2,M, or the total cell cycle time between the two diets. The same study reported that only about 40% of the tumor cells were dividing at any given time, and there was probably a constant interchange between the proliferating and

quiescent cell populations (15). These results show that the difference in tumor weights between the two groups could not be accounted for by an effect on the fraction of cells dividing or a difference in cell cycle time. The size difference in the tumors were determined by the balance between tumor cell lysis and those tumor cells proliferating (15). The tumor cells provided a diet of saturated fat die faster than those provided a diet of polyunsaturated fat. Another possible explanation is that the cells promoted by a polyunsaturated fat diet started growing earlier. This is not the case. It was determined by Hillyard and Abraham that rapid tumor growth occurred at the same time if the animal received a fat free or polyunsaturated fat diet (6). The increase in polyunsaturated fat increases fluidity of the cell membrane, but this does not influence the susceptibility of the target cells to complement-mediated lysis (14).

Corn oil, contains 60% linoleic acid (11). Linoleic acid is the only pure polyunsaturated fatty acid known to promote mammary tumor growth (13). Rats with diets containing either 0.5% or 20% corn oil showed a tumor incidence of 20 to 28% with the low fat and 72 to 76% with the high fat diet (12). Fish oil which also contains polyunsaturated fatty acids doesn't

have similar tumorigenic effects (10). Another fatty acid isolated from the seed oil of the columbine, is an 18-carbon fatty acid with three double bonds. It contains two cis bonds at carbons 9 and 12 similar to linoleic acid and, in addition, it possesses a double bond with a transconfiguration at the 5 position (13). This fatty acid with its configuration so similar to linoleic acid would be expected to enhance tumor growth to some extent but it does not. Rats fed this fatty acid do not convert it to linoleic acid to any appreciable degree (13). Stearate which contains 18 carbons but is saturated and oleate which contains a single double bond in the cis 9 position also do not enhance tumor growth (13). It was concluded from this data that 18 carbon fatty acids require two double bonds, as does linoleic acid, to be effective promoters.

Work from independent laboratories have shown an increase in the level of free fatty acids in tumor bearing animals and most of which is believed to be utilized by the tumor as a source of energy (7). The cholesterol levels, free and esterified, were also increased in the plasma of tumor bearing rats (7). A later study determined that the amount of linoleic acid in the total serum lipids closely reflects that of the diet (5). However this was not unexpected since

linoleic acid is an essential fatty acid. Another study demonstrated that the total serum lipid levels were higher in animals fed a low fat diet while it was the reverse in the high fat diet. A possible explanation is that a high fat diet would inhibit endogenous lipid synthesis (5). It is well known that most animals convert linoleic acid to dihomo-γ-linolate and arachidonate (see Fig. 1), precursors of prostaglandins (13). Although dietary linoleic acid correlates closely with total serum and tumor linoleic acid levels it does not equate with its metabolic product arachidonic acid in either tumor or serum lipids (5).

If you use a ratio of linoleic acid to arachidonic acid (A.A.) as a rough index to the degree of conversion of linoleic to A.A., it is highest in low incidence groups and low in the high incidence groups (corn oil). Also when pure A.A. was added to the diet of tumor bearing rats it did not produce a significant increase in tumor mass (5,13). This would imply no direct relationship between dietary linoleic acid, A.A. and prostaglandins. It is possible that dietary A.A. does not enter the same tissue pool as A.A. synthesis from linoleate (13).

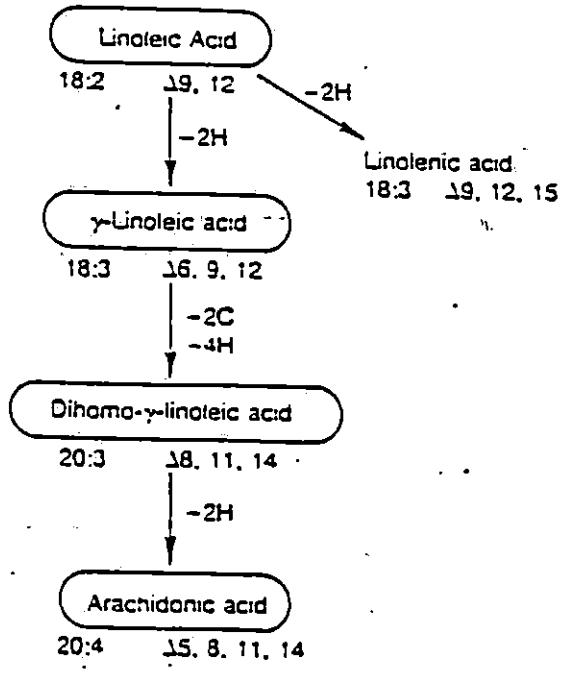

Figure 1. Biosynthesis of prostaglandin precursors from linoleic acid. Taken from Martin (32).

7,12-dimethylbenze[a]anthracene (DMBA)

The effects of dietary fats are strictly promotional. They may assist in the initiation phase but some type of carcinogen must be present for the induction of cancer. The two commonly used methods of induction of mammary cancer are radiation and hydrocarbons (16).

Polycyclic aromatic hydrocarbons (PAH) are widely distributed in environmental pollutants that have been linked to human tumorigenesis at a variety of anatomic sites. In animals, PAH are potent carcinogens

(8). In particular, the synthetic PAH, DMBA, is used to induce tumors of the mammary gland in experimental animals. Although DMBA is less widely distributed in the environment as compared to other related PAHs such as benzo(a)pyrene, chrysene, and fluorunthene, its overall metabolism is similar (16).

A high incidence of testicular cancer was observed in chimney sweeps in the early 1800's. It was determined by Percivall Pott (1808) that it was due to a carcinogen found in the soot. After a series of research that encompassed four countries and required a 162 years, aromatic hydrocarbons were isolated and synthetic forms began being produced (16).

DMBA is one of the most potent carcinogens. Its structure is shown in figure 2. Carcinogenic hydrocarbons are flat molecules with conjugated double bonds systems and usually posses substituent groups of a special sort or an additional ring at a salient position on the molecule (16).

There are three cancer prone areas in relation to DMBA: (1) mammary acinar, which line the lactiferous ducts (17), (2) hemopoietic tissue, and (3) fibroblasts (16). This results in several types of cancer: mammary adenocarcinoma, mammary fibroadenoma, leukemia, and ear duct cancer (16). However, mammary cancer is the most

prevalent of the group (table 1).

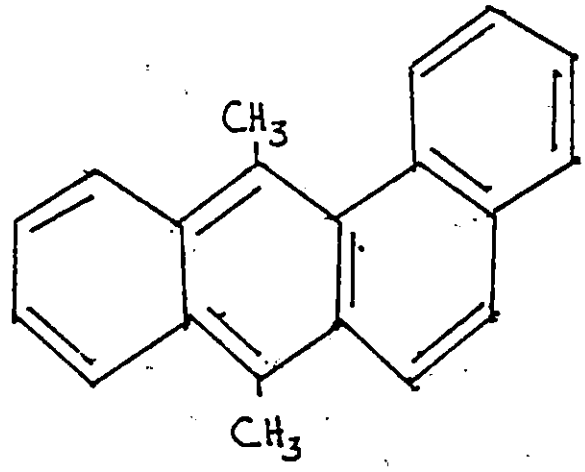

Figure 2. The structure of DMBA. Taken from Huggins (16).

Adenocarcinomas consist of acini lined with many layers of epithelial cells arranged to form gland like structures with papillary projections (16). These tumors can arise any where in the mammary gland. In the rat they have been found to occur from the neck to perineum. These tumors rarely metastasize and they bring about death by attaining gigantic size and invading adjacent tissue with consequent hemorrhage, necrosis, and ulceration (16).

Table 1. Incidence of neoplasms in rats fed DMBA. Taken from Huggins (16).

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

DMBA is taken up by the liver metabolized and excreted into the bile or exported. Mammary tissue also has an ability to metabolize DMBA to a limited extent. The metabolic pathways consist primarily of oxygenation of the methyl groups and to a small extent of the ring structure (16). The main products of the metabolism of DMBA by rat liver homogenates are the isomeric monohydroxymethyl derivatives: 7-hydroxymethyl-12-methyl-BA and 7-methyl-12-hydroxymethyl-BA. Small amounts of phenols, diols, epoxides, and dihydrodihydroxy compounds as well as 7,-12-epidioxy-7,12-DMBA were detected (16).

The electrophilic intermediates of the metabolism of DMBA are what bind to nucleophilic regions of DNA. The bay-region of syn- and anti-3,4-

dihydrodiol-1,2 epoxides are considered to be the ultimate carcinogenic forms of DMBA (8). These bind covalently to deoxyadenosine and deoxyguanosine residues of DNA (8).

It was determined in 1971 that the number one chromosome of the rat was the most vulnerable, to DMBA (16). This high susceptibility to DMBA is exhibited in late interphase and prophase (16). In only 24 hours after injection of DMBA one half of the cells in metaphase in bone marrow have chromosomes with breaks and gaps (16).

The tumors developed due to DMBA are excellent for scientific study of breast cancer. DMBA is not only metabolized in a similar fashion to naturally occurring PAHs. the superficial position of the tumors assures easy palpations, the end point is sharp, and they rarely metastasize (16).

INDOMETHACIN (IM)

Numerus laboratory and clinical studies have demonstrated that prostaglandins (PG) are involved in the initiation, development, and progression of some malignant diseases(5,12 14 20-31). Prostaglandin levels in both human and experimental carcinomas are consistently higher (19). Recent studies in animal

tumor models suggest that inhibitors of arachidonic acid (A.A.) metabolism such as indomethacin (IM) may inhibit tumor growth (5,12,20-28) although in some studies no antitumorigenic effects were observed (21). IM and other aspirin like compounds were recently studied by Karmali and Marsh (19). As seen in table 2, all of these compounds resulted in a reduction of tumor growth.

Most chemopreventitive drugs have effects at one of three stages in tumor development (figure. 3). A.A. metabolic inhibitors work as suppressing agents (19). This has been demonstrated by the use of IM in several studies (5,8,11,12,15,20,22-31), while others who had a reduction in PGs saw no drop in tumor burden (21). In fact, the presence of IM in the diet has been shown to suppress tumor growth in animals fed a high fat diet, to 1/3 the size of tumors of high fat control rats (11). It was demonstrated that IM does not effect mammary tumor cell proliferation <u>in vitro</u> whereas it reduces the growth rate of the same neoplasms <u>in vivo</u> (15). It is therefore evident that the products of A.A. metabolism help in tumor promotion. One of the products of the pathway inhibited by indomethacin (cyclooxygenase) are PGEs. Their role in the bodies immunological system warranted research to determine their function in the relationship between a high fat

diet and mammary cancer.

IM treatment which inhibits the conversion of A.A. to PGs; could be expected to cause an accumulation in PG precursors in the membrane. This however, is not the case, as demonstrated in a recent study by Feldman and Hilf (21). In addition to the direct activity of IM on prostaglandins synthesis, this inhibitor of the cyclooxygenase pathway can lead to increased synthetic activity of the lipoxygenase pathway which has products that are reported to stimulate natural killer (NK) cell activity (27). The products of the lipoxygenase pathway are leuoktrienes so named because the compounds were first detected in leukocytes. (34).

TABLE 2. Mammary tumor growth in rats treated with cyclooxygenase inhibitors. Taken from Karmali and Marsh (19).

Treatment	Tumor weight in grams
Control	2.49
Indomethacin	1.96
Ibuprofen	1.96
Flurbiprofen	1.71

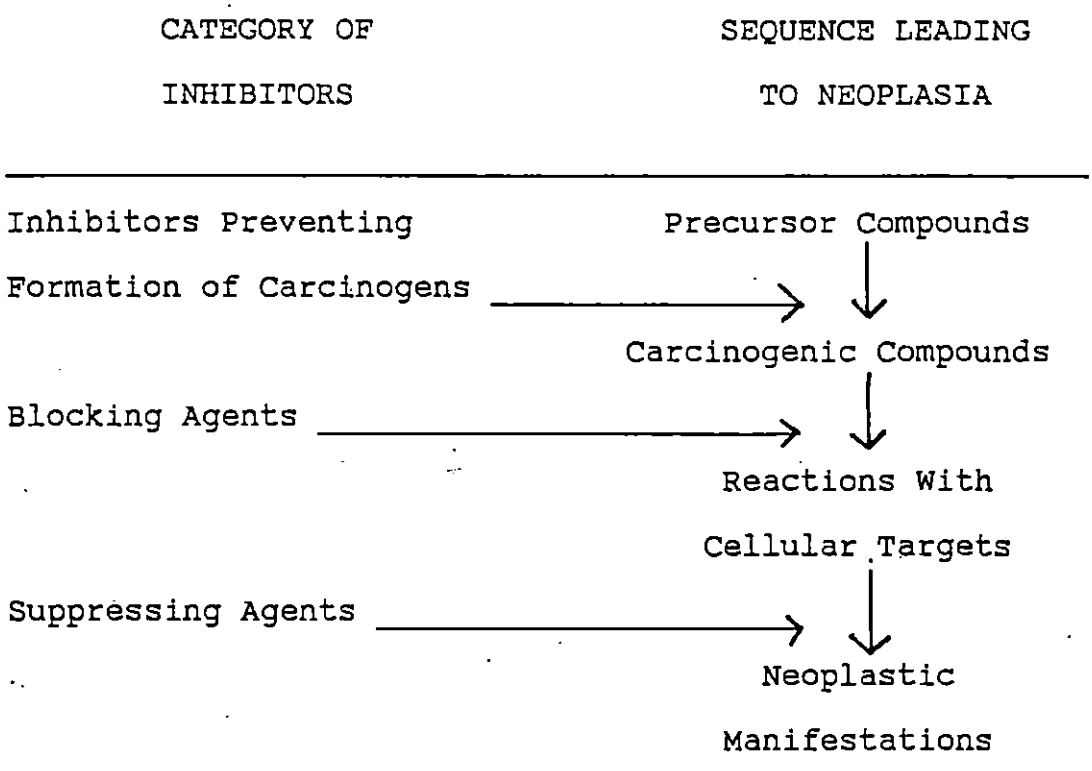

FIGURE 3. Classification of chemopreventive agents. Taken from Wattenberg (18).

Prostaglandin E2

The major rate limiting factor for PG synthesis is the availability of the fatty acid precursor (32). Cells usually contain substantial quantities of enzymes in the active form, but they sequester the fatty acid in the membrane phospholipids and in cholesteroyl esters, triacylglycerols, and lipoprotiens. Phospholipases, cholesterol esterases, triglycerides, lipases, and lipoprotein lipases that catalyze hydrolysis of the lipids can be activated by numerus "non-specific"

factors. These include mechanical pressure, hypoxia, temperature changes and mild trauma (32).

The activation of these enzymes stimulate the release of a 20 carbon fatty acid, arachidonic acid, which is metabolized into a variety of active mediators. The initial enzymes, cyclooxygenase and lipoxygenase, that convert A.A. to its mediators are constitutively active within cells (33). Thus three main groups of derivatives- the prostaglandins, the thromboxanes, and the leukotrienes- are formed (34). Prostaglandins and thromboxanes are formed via the cyclooxygenase pathway (figure 4). It is this pathway that is inhibited by aspirin and aspirin like drugs such as indomethacin (IM). These non-steroidal anti-inflammatory drugs inhibit PGs and thromboxanes by either binding to or covalently modifying the protein region near the heme unit of the cyclooxygenase enzyme (35).

A cyclooxygenase enzyme binds to a cytochrome b-like hemoprotein cofactor (mw=70,000), which is readily lost, and in addition requires a nonheme iron which catalyzes the formation of PGG2 from A.A. (35). Prostaglandin G2 is a highly unstable endoperoxide that is rapidly converted to PGH2. The cyclooxygenase also catalyzes the second reaction, and the enzyme is therefore known as the "prostaglandin synthetase

system". The cyclooxygenase additionally catalyzes its own destruction and thereby limits PGG production after a time. The fate of PGG depends on the cell type. In most parts of the body a PGE isomerase catalyzes the formation of PGE (32). It is this particular PG that cancer researchers believe to be involved in the enhancement of cancer.

The actions of PGs are executed through specific membrane bound receptors (36), which interact in complex ways with other regulators. These include cAMP, cGMP, Ca++, calmodulin, phospholipids, neurotransmitters, growth-promoting hormones, and kinase C (32). Investigators have shown that PGs, particularly of the E-series, stimulate membrane bound adenylate cyclase in mammary tissue, therefore elevating cAMP levels (36). The same mechanism by which retioniods are believed to inhibit tumor growth (37). Thus, the concentration of the cAMP may play a role in determining the effect brought about. PGEs have a short half life. Structurally, they have a ketone at carbon 9 and an alcohol at position 11 (figure 5).

It has become increasingly apparent that among human peripheral blood mononuclear cells only the monocyte-macrophage and not T cells or B cells have the ability to produce these PGs (38). It is believed that

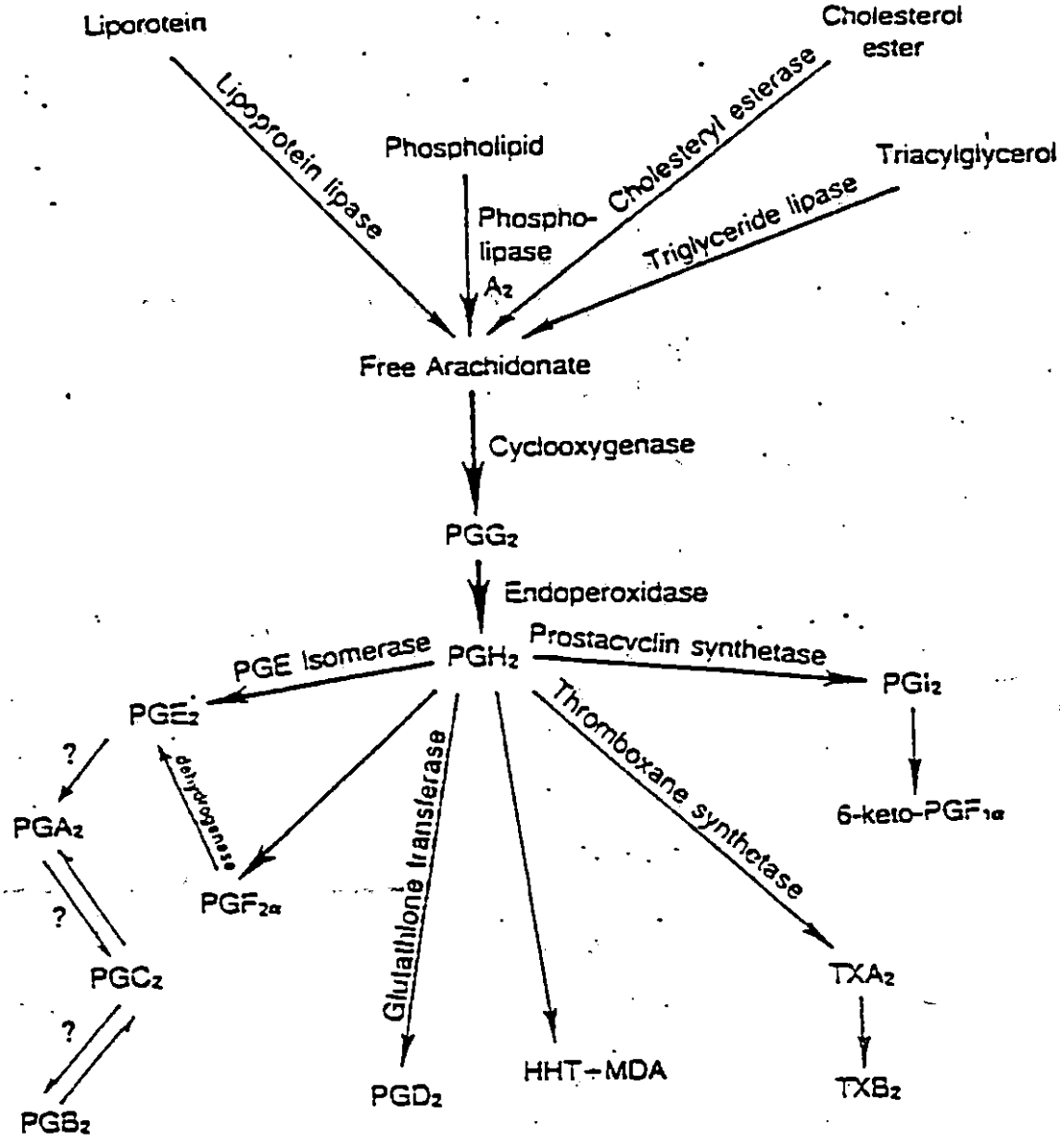

Figure 4. Sources of arachidonate and metabolites of the cyclooxygenase pathway. Taken from Martin (32)

the PGEs secreted by natural killer suppressor cells of the monocyte-macrophage lineage inactivate natural killer cells (23-25,38). If this is true, host macrophages may promote metastasis by PG mediated inactivation of natural killer cells (23).

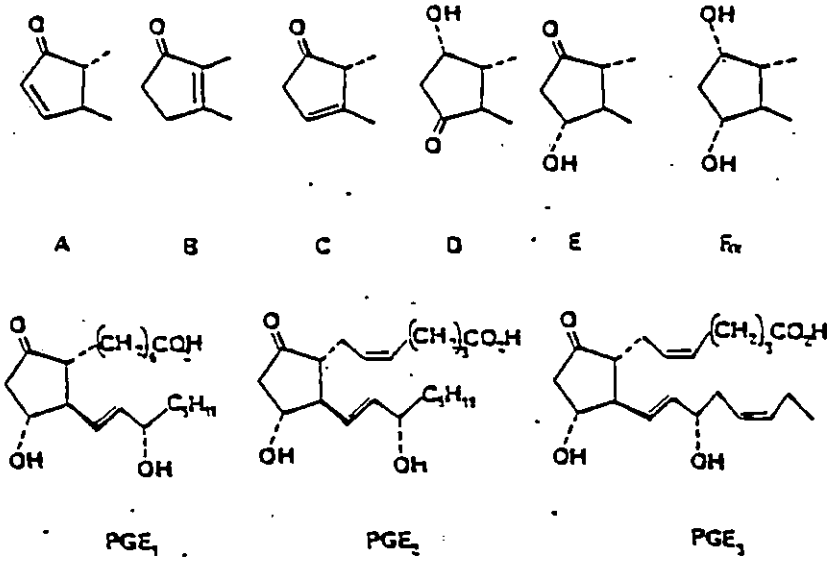

Figure 5. Structure and nomenclature of the classical prostaglandins. Taken from Caton and Hart (36).

This suppression may have its effect at one or all of the following steps: (1) development of the lytic function (activation process); (2) the recycling function of the activated cells, and (3) the killer function of the activated cells. PGs have also been demonstrated to suppress antibody formation, lymphokine production and T cell cytotoxicity (25).

There appears a strong correlation between PG levels and metastasis. Whatever the size of the lesion, a high PG level was found associated with a marked increase in axillary lymph node metastasis in human breast cancer (26) and in animal models (27).

While T cells, activated macrophages and NK cells have all been considered to play a role in metastasis, the role of the latter two cell classes appears particularly appealing because they do not require the fine antigenic specificity or memory response obligatory for T cell function and thus may be able to respond to a broad spectrum of tumors and may be more amenable to therapeutic manipulations (23).

It has recently been reported that during development of transplanted or spontaneous tumors in mice, the NK activity declines with increasing tumor burden. It appears that NK cells are inactivated not reduced in number. This inactivation may be mediated via PGs (23). Although significant inhibition of cytotoxicity by PGs was observed in experiments a complete suppression of NK activity was never obtained (25). This finding may reflect the existence of a subpopulation of NK cells that lack PG receptors, and are therefore resistant to PG mediated inhibition (25).

IM and its antiaggratory action would prevent

tumor cells form sticking to the endothilial wall of the circulatory system and as such reduce the formation of new tumors. In this respect it is also important to consider the direct aggregatory of tumor cells on platelets (by means of release of thromboxane) and on vasoconstriction (27,39). The concept that PGs and related metabolites of A.A. are local regulators of the microcirculation is one that has been investigated extensively for the past several years. It is based on the observations that the adminstration of locally synthesized PGs are capable of profoundly changing microvascular tone and various vascular beds (39). In this relation PGEs are vasodilators therefore it is easy to see how PGEs could enhance metastasis.

The effect of PGs on the immune response appears to follow a bell shaped dose response relationship. Low levels of PGs are required to initiate the immune response. This was demonstrated by the fact that adding anti-PGE2 inhibited cell mediated immunity (21). When the PGE2 concentration was high it inhibited the same response (21). The question still remains whether it is the absolute level of PGE or the proportional decrease (21). As more and more research data are brought forward it will become more clear how PGEs and dietary fat increase tumor growth. Yet, some areas are still

inconclusive. How PGs control the immune system? Why dietary fat and the formation of A.A. are not correlated? How they increase metastasis? These and several other questions will have to be answered before the relationship between dietary fat and mammary cancer can be understood.

Research Objectives

In this study young virgin female rats were fed diets of different levels of polyunsaturated fat and then given IM to reduce PGE levels. Testing of the following hypothesis was accomplished by examination of tumor number, tumor burden, percentage of tumor bearing rats per group, PGE2 levels, and tumor occurence.

Diet

A high level of polyunsaturated fat in the diet and its relationship to mammary cancer has been observed in several studies (4-16). Its role in the increase of PG levels is still being widely studied.

It was hypothesized that those animals fed a high fat diet would show an increase in tumor number, tumor burden, percentage of tumor bearing rats, PGE2 levels, and have a later time of occurence.

Indomethacin

This nonsteroidal anti-inflammatory agent has not only been demonstrated to reduce PGE2 levels, but also tumorigenesis (12-13).

It was hypothesized that those animals given IM would show a decrease in PGE2 levels, tumor number, percentage of tumor bearing rats, tumor burden, tumor size andhave a earlier time of occurence.

PGE2

The effects of PGE on the immune system of tumor bearing rats have only recently been demonstrated (4-31). These changes in the immune system would cause a decrease in survival chances.

It was hypothesized that animals with high PGE2 levels would show an increase in tumor burden, tumor number, percentage of tumor bearing rats and have an earlier time of tumor occurence.

MATERIALS AND METHOD

Animal Treatment

Female Sprague-Dawley rats were obtained from Harlen Sprague-Dawely Inc., Indianapolis, Indiana. The rats were housed in stainless steel cages with one rat per cage in a temperature and light controlled room. Food and water were available ad libitum. The rats were assigned to one of four groups --two experimental: IM high fat (HFI) and IM low fat (LFI) and two control:high fat (HF) and low fat (LF). The animals were then placed on either a high or low polyunsaturated fat diet containing 20% or 4% fat diet respectively. The common components in both diets were in percentage by weight and both diets were equal in calories (table 3). The casein, cellulose, minerals, vitamins, methionine, and choline were purchased from U.S. Biochemical, Cleveland, Ohio. The corn oil and sugar were purchased locally. IM (Sigma Chemical Company, St. Louis, MO) was dissolved in 10 ml of ethanol and diluted with water to give a final concentration of .025mg/ml.

At 50 days of age all animals received a single

10mg dose of DMBA in 1ml of corn oil by intragastric administration. One month later all rats were being palpated weekly. At day 89 all of the animals were decapitated and at the time of sacrifice blood was collected in polypropylene tubes and clotted blood was centrifuged at 3000xg for 10 minutes, serum was removed, and stored at 0 degrees centigrade. All of the tumors were measured in 3 dimensions (LxWxH) with calipers. One tumor was dissected from each tumor bearing rat, frozen in dry ice-acetone mixture, and stored at -80 degrees centigrade.

Table 3. Diet concentration by percentage

COMPONENT	HIGH FAT DIET	LOW FAT DIET
Casein	21.6%	21.7%
Sucrose	21.9%	57.0%
Corn 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

Tumors were homogenized 1 to 5 (g/ml) in Kerbs Ringer Bicarbonate Glucose and centrifuged at 3500xg at 4 degrees centigrade. The homogenates and plasma samples were reduced to pH 4 with 1N HCL. The prostaglandins were then extracted 1:10 with ethyl acetate (39). The samples were then dried with a vacuum pump and resuspended 1:1 in assay buffer. PGE2 concentrations were determined with a prostaglandin E2 [125I] RIA kit (Dupont Biomedical Products, Boston, MA). Buffer and antiserum were added to each sample and incubated 24 hours. The precipitating solution was added and the samples were centrifuged at 2000xg at 4 degree centigrade. The pellets were resuspended in 5ml of Ready Safe Scintillation Fluid and read in a Beckman Scintillation Counter model LS 801.

Statistical Analysis

An un-paired t-test was used to determine the significance of the results ($P>.05$).

RESULTS AND DISCUSSION

Rats exposed to DMBA at day 50 developed tumors 89 days later on all dietary regimes (table 4). A lower percentage of rats developed tumors in both LFI and LFC when compared to their experimental groups. The indomethacin did not reduce the percentage of rats developing tumors in the groups receiving the HF diet, but was somewhat effective in reducing the number of rats with tumors from 73% to 50% on low fat diets. The indomethacin rats also developed tumors later than their controls (table 4).

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

GROUP	BODY WT.	PERCENTAGE	FIRST DAY OF OCCURRENCE
LFC	249	73%	76
HFC	240	82%	68
LFI	222(a)	50%	85(b)
HFI	230(a)	91%	71(b)

a Statistically different from others, $p>0.05$.
b Statistically different from others, $p>0.05$.

A comparison of body weights at death can be seen in table 4. Those rats in the experimental groups had body weights that were significantly lower. This could be due to IMs toxic effects in the gastric system (4,20,23). A study done by McCormick and Wilson demonstrated that these effects can be counteracted by certain antioxidants like butlyated hydroxytolune (20).

Total tumor number per dietary group and tumor number per rat were reduced in the LFC and LFI groups (table 5). Tumor number per tumor bearing rat was lower only in the LFC group (table 5). The data presented in tables 4 and 5 disagree with the hypothesis statement that the effects of indomethacin would be demonstrated equally in both experimental groups. Its effects were more profound in the low fat group.

A distinct difference can be seen in relationship to type of diet, total tumor burden, and average tumor burden per rat (figures 6 and 7). In both cases the low fat diets were lower, which agrees with the hypothesis that the low fat diet will reduce tumorigenesis at all phases of the study. The comparisons between the control groups and the experimental groups were not significant, but the experimental groups were somewhat higher.

Table 5. Comparison between groups, total tumors, tumor number per rat and tumor number per tumor bearing rat.

GROUP	TOTAL TUMORS	TUMOR # PER RAT	TUMOR # PER TUMOR BEARING RAT
LFC	11(a)	1	1.4
HFC	23	2.1	2.5
LFI	15(a)	1.3	2.5
HFI	21	1.9	2.1

a Statistically different from high fat diet, $p>0.05$.

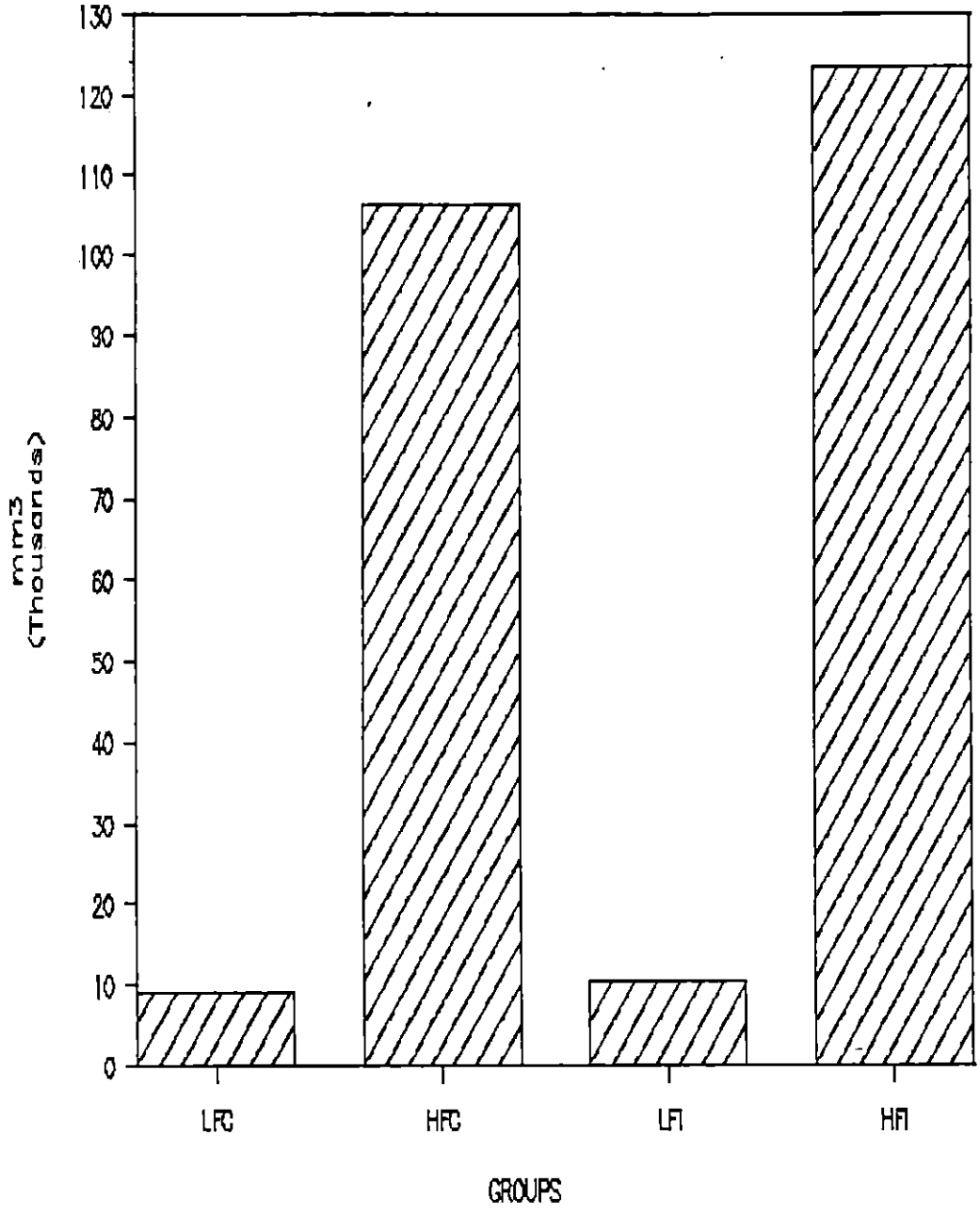

Figure 6. Total tumor burden per group. Showing a significant difference between the two diets, $p>0.05$.

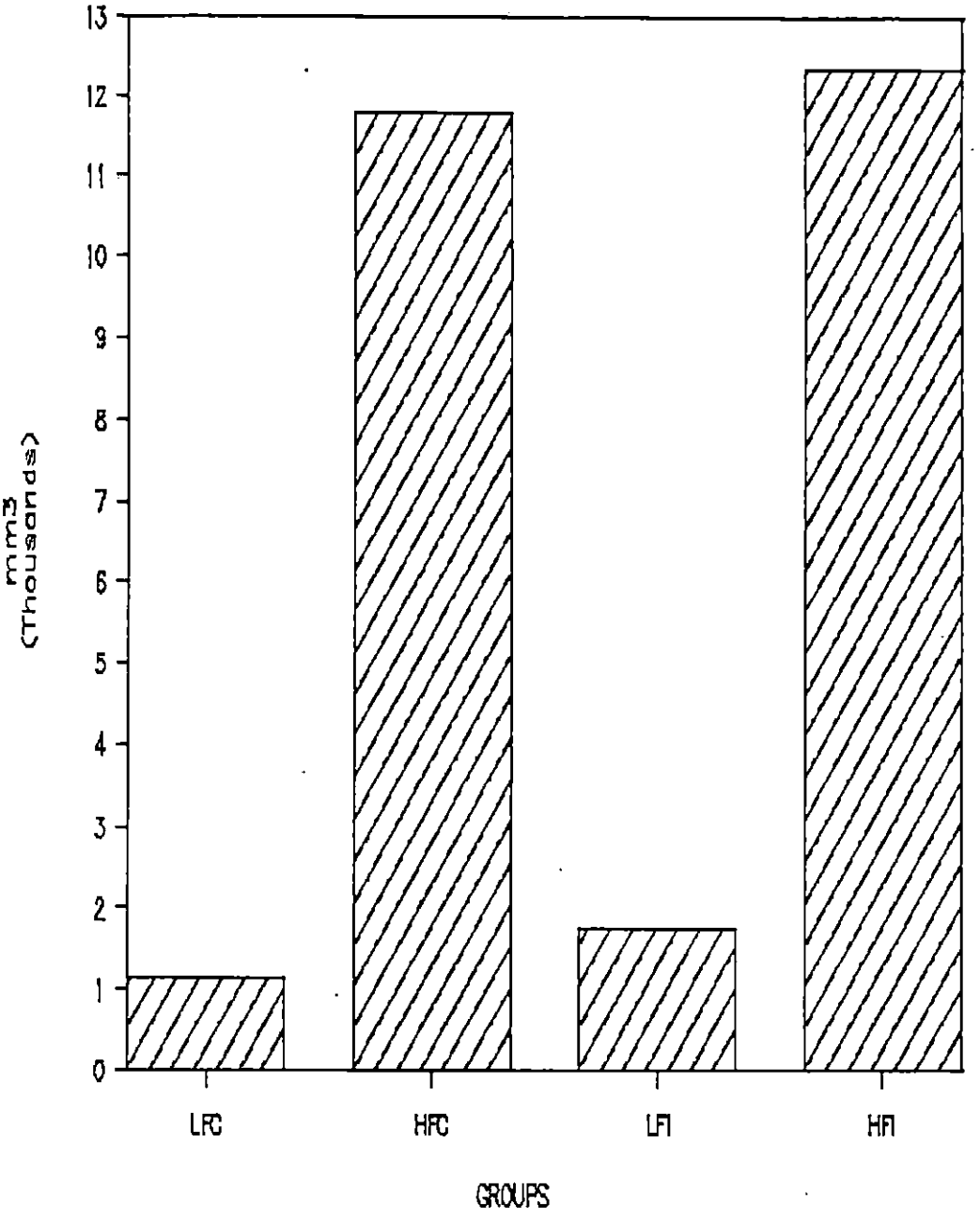

Figure 7. Average tumor burden per rat. Showing a significant difference between the two diets, $p>0.05$.

The PGE2 assay was inconclusive. Yet, certain trends could be seen in the average normalized percent of PGE2 [I125] bound. Since the assay required the use of a competitive binding, a inverse relationship exists between percent bound and PGE2 levels. Thus, the higher the level of PGE2 [I125] bound the lower the concentration of PGE2 in the sample (table 6). In the plasma samples the LFC group had higher PG levels than the LFI group. A similar tend could be seen in the comparison of the HFC and HFI groups. The PGE2 concentrations in the tumors appeared higher than those in the plasma samples but this could be due to a discrepancy in protocol. The same trends were observed in relation to experimental and control groups as seen in the plasma samples. IM was again successful in the reduction of PGE2 levels.

In summary, the low fat diet reduced the percentage of tumor bearing rats, deterred tumor formation by a number of days, reduced the total tumors per group and per rat, and lowered the tumor burden (tables 4 and 5, figures 6 and 7). These reductions may have been brought about by the effects of lower PGE2 levels in those rats fed a low fat diet (table 6).

IM treatment also deterred tumor formation, decreased the percentage of tumor bearing rats, and

lowered animal body weights at death (table 4). This treatment had no significant effect on total tumor numbers or tumor burden (table 5, figures 6 and 7).

Table 6. Normalized percent bound of PGE2 [I125]

SAMPLE	LFC	HFC	LFI	HFI
TUMORS	5.5	4.7	10.7(a)	6.4
PLASMA	21.8	13.4	35.0(b)	16.4

a Statistically different from the other groups, p>.05.
b Statistically different from the other groups, p>.05.

Therefore, it was demonstrated that indomethacin reduces the number of tumor bearing rats, but does not reduce the size of the tumors which do occur. A study on human breast cancer and PG concentration indicated that PG production was higher in small tumors and in tumors with a high cellular density (26). That was not demonstrated in this study. The LFC group had lower tumor number per tumor bearing rat (figure 6) and lower average tumor volume (figure 7) than the HFC, but the LFC tumor PGE2 levels were lower.

It would be interesting to see the influence of anti-inflammatory steroids on DMBA induced mammary tumors. These steroids act by inhibiting the release of

A.A., they prevent formation not only of PGs and thromboxanes, but also leukotrienes (34). Corticosteriods have side effects, but a new dual 5-lipooxygenase/cyclooxygenase inhibitor "L-652,343" is being studied for use in hypersensitivity disorders, does not (41). The outcome of a study using this new molecule and mammary cancer would give an answer to the question of the role of lipoxygenase pathway in this type of cancer.

In the battle, to determine the concentration at which PGEs initiate or deter the immune system, PGE agonist like 16-dimethyl PGE2 could be used (42,43), while the role of leukotrienes could be determined by using there antagonist and agonist (44). Experiments using these molecules would help define the roles of PGEs and leukotrienes in the relationship between a high fat diet and mammary cancer

Although a large amount of research has been performed linking PGE2 and mammary cancer it will require several more years before the true mechanism, by which PGE2 and dietary polyunsaturated fat cause mammary cancer, will be completely understood. Until that time IM will probably be widely used as a chemopreventative drug either by itself or in combination therapy. It is not completely known if the drug works via PG reduction

or increasing the levels of leukotrienes, but what is important to cancer research is the IM reduces tumorigenesis in relation to certain types of diet, particularly a diet low in polyunsaturated fatty acids as demonstrated here.

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