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EFFECTS OF DIETARY FAT ON MAMMARY TUMOR DEVELOPMENT AND GROWTH AND RELATION TO THE ENDOCRINE SYSTEM

By

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ABSTRACT

EFFECTS OF DIETARY FAT ON MAMMARY TUMOR
DEVELOPMENT AND GROWTH AND RELATION
TO THE ENDOCRINE SYSTEM

By

Charles Frederic Aylsworth

1. Rats fed the 20.0% high fat (HF) diet consistently consumed less of their diet than rats fed the 4.5% control fat (CF) diet. After 4 days of dietary treatment, no differences in daily caloric consumption or body weight were observed during the 4 week treatment period. It was concluded that the effects of HF diets on mammary tumor development are not mediated by an increased caloric intake in rats fed the HF diet.

2. The effects of HF diet consumption on serum prolactin levels during the estrous cycle of intact female rats and during the progesterone-induced surge of prolactin in ovariectomized, estrogen-primed rats were determined. No differences in serum prolactin levels were observed at any time during the estrous cycle or in the induced surge of prolactin in rats fed either the HF or the CF diet. It was concluded that the mechanism by which HF diet consumption

stimulates mammary tumor development does not involve alterations in serum prolactin levels.

3. The influence of estrogen and prolactin upon the stimulatory action of high dietary fat during mammary tumor development was examined. Female Sprague-Dawley rats, 55 days of age, were injected with DMBA, placed on either the CF or the HF diet, and subjected to various drug and endocrine manipulations to maintain serum estrogen and prolactin at controlled levels. Sham operated and ovariectomized rats treated with both haloperidol and estradiol benzoate which were fed the HF diet showed a significant stimulation of mammary tumor development when compared with similarly treated rats fed the CF diet. These results demonstrate that HF diets can increase mammary tumor development in rats which have controlled levels of estrogen and prolactin, and suggest that HF diets may act directly on the incipient mammary tumor tissue to sensitize it to circulating hormone levels.

4. Promotional aspects of stimulated mammary tumorigenesis by HF diet were examined. Female Sprague-Dawley rats were injected with DMBA and placed on high fat and low fat diets for variable lengths of time at different periods during early tumorigenesis. Rats fed the HF diet for equal time intervals, but at different times during tumorigenesis, showed similar development of mammary tumors. Increasing the duration of HF diet treatment resulted in increased mammary tumor development, suggesting a time dose-response

relationship. Removal of the HF diet treatment reversed the stimulatory effects of the HF diet on mammary tumor development. These results suggest that dietary fat may act as classical promotors to enhance mammary tumorigenesis.

5. The effects of a high fat diet on the growth of established mammary tumors were studied. Female rats with established DMBA-induced mammary tumors were placed on a HF and CF diet approximately 12 weeks after DMBA administration. Mammary tumor growth was significantly stimulated in rats fed the HF diet. Furthermore, in rats fed the HF diet, there was an increased number of newly palpable tumors at the end of the 5 week treatment period. These data suggest that a HF diet can stimulate mammary tumor development and growth even during the later stages of tumorigenesis.

6. The effects of a HF diet on specific prolactin binding to DMBA-induced mammary tumors were investigated. Isolated membranes from mammary tumors obtained from rats fed either a HF or a CF diet were measured for specific prolactin binding by a radioreceptor assay. No differences in specific prolactin binding was observed in rats fed either a high or low fat diet. Therefore, the effects of HF diet on mammary tumor development do not appear to depend on changes in prolactin binding.

DEDICATION

I dedicate this dissertation to my loving wife, Janie; who, along with our families, gave me the necessary encouragement, inspiration, and love in my research endeavors.

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INTRODUCTION

It has been suggested that most cancers which occur in humans are causally related to environmental factors and conditions. In recent years, nutrition repeatedly has become one of the most intriguing and extensively examined environmental factors in cancer research. The incidence of mammary carcinomas among women has been related specifically to various nutritional components, particularly to dietary intake of protein and fat (Carroll, 1975).

Approximately 50% of the cancers developing in women of Western cultures are believed to be related to nutritional factors (wynder, 1976). Positive correlations have been established between per capita consumption of dietary fat and protein, and the incidence of breast cancer in women (Carroll, 1975). Generally, women belonging to Western cultures, whose members consume greater quantities of dietary fat, exhibit a higher incidence of breast cancer than their counterparts in Eastern cultures who consume less dietary fat. Further support for the role of dietary factors in the etiology of human breast cancer is provided by epidemiological studies which reported that breast cancer mortalities increased among second generation Japanese migrants to the

United States. A primary behavioral modification inherent in the assumption of a Western lifestyle by these individuals was the consumption of diets containing larger amounts of fat and protein.

Epidemiological evidence has been substantiated by widespread reports that high fat diets stimulate mammary tumor development in many rodent mammary systems, including spontaneous, transplantable and carcinogen-induced tumors. Animals fed high fat diets develop a greater number of mammary tumors, more rapidly and with a higher incidence, than in similarly treated rats fed a lower fat diet (Carrol, 1975).

The influence of dietary fat treatment on mammary tumor development does not appear to involve a general caloric effect, since the caloric intake of rats fed high and low fat diets were similar even though the diets differed in caloric content (Gammal et al., 1967). Tannenbaum (1945) demonstrated that when the caloric intake was equally reduced in mice fed high fat and low fat diets, the stimulatory effect of the high fat diet was still observed.

Although it has been conclusively demonstrated that the consumption of high fat diets can stimulate murine mammary tumorigenesis, no adequate mechanism has been proposed to wholly account for these observed effects. Since most of the murine mammary tumor models are highly hormone dependent and responsive, particularly with regard to estrogen and prolactin, many of the investigations concerned with elucidating the mechanisms by which high fat diets stimulate

mammary tumor development have primarily focused on the possible mediation of these effects through the endocrine system. Prolactin, in particular, has been implicated in the mediation of the influence of dietary fat on mammary tumor development. Chan and Cohen (1974) observed that the differential effects between high and low fat diets on mammary tumor development could be completely eliminiated by chronic suppression of anterior pituitary prolactin secretion. Inhibition of the influence of estrogen by chronic administration of an anti-estrogen drug reduced mammary tumor development, but could not remove the differential effects of high and low fat diets on mammary tumor development. The role of prolactin in the stimulation of mammary tumorigenesis by dietary fat was further suggested by reports that high fat diets elevated circulating levels of prolactin at various times during the estrous cycle. These observations led to the view by Chan et al. (1975) that the enhancement of mammary tumorigenesis by high fat diets was mediated indirectly via the hypothalamic-hypophyseal system, to increase pituitary prolactin secretion rather than by a direct action of dietary lipids on the mammary tissue. More recent reports, however, have determined that these putative effects of high fat diets on serum prolactin levels may not be valid or have minimal significance in the mediation of enhanced mammary tumorigenesis by high fat diets (Cave et al., 1979; Ip et al. 1980). Further investigation into the effects of dietary fat on anterior pituitary prolactin secretion, and other

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endocrine involvement in the stimulation of mammary tumorigenesis by dietary fat appears to be warranted.

The Berenblum hypothesis, or the initiation-promotion theory of tumorigenesis, has been used to describe the effects of high fat diets on mammary tumor development. Carroll and Khor (1970) have reported that the type of diet consumed following, but not prior to, carcinogen administration, is important in its influence on mammary tumor development. Dietary fat does not appear to have an effect on the metabolism, distribution, or uptake of the carcinogen by the mammary gland during initiation (Gammal et al., 1968). It appears, therefore, that high fat diets stimulate mammary tumor development by acting during the promotional stage of mammary tumorigenesis, but additional aspects of tumor promotion by high fat diets need to be explored.

Upon consideration of the research studies already reported on the influence of high fat diets on mammary tumorigenesis, it was of interest to further assess the role of the endocrine system, with particular emphasis on prolactin, but also on estrogen, and to further evaluate the promotional aspects of high fat diets on mammary tumor development.

LITERATURE REVIEW

General Theories of Tumorigenesis

The observable phenomenon of tumorigenesis has spawned a multiplicity of research designs, provoking the advancement of various theories describing the processes involved in tumor development. As the development of most tumors appears to be mediated by similar factors, integration and synthesis of research observations have generated the formation and proposal of theories of tumorigenesis. Three of the most prominent and widely accepted theories of tumorigenesis will be considered here. These include the Tumor Progression Theory as proposed by Leslie Foulds, the Berenblum Hypothesis and the Somatic Mutation Theory originally advanced by Theodor Boveri. In the discussion which follows, the basic principles on which these theories are established and their relation to mammary tumorigenesis will be explored.

Tumor Progression Theory of Tumorigenesis

Tumor progression, as defined by Foulds (1969) asserts that tumors develop by the acquisition of sequential, heritable, qualitative changes in one or more of the biological properties of the cells contained in the tumor. This tumor

progression concept attempts to explain these qualitative changes that occur during the development of tumors. Suggesting that the process of tumor progression is a multistage phenomenon, Foulds classified such development as the initiation, intermediate, and advanced phases of tumorigenesis (Foulds, 1969). As an incipient neoplasia progresses through these phases of tumorigenesis, irreversible qualitative changes are produced whereby lesions with increasing degrees of neoplastic capacity may or may not be observed.

Upon exposure to an initiator, cells in the area of exposure undergo a series of irreversible changes in their biological composition which may or may not be accompanied by histological or proliferative alterations, thus establishing a potential for tumor development. In the region exposed to the initiating agent, a state of incipient neoplasia is established possessing a finite capacity for neoplastic development. The induced incipient neoplasia persists for the lifetime of the animal and may or may not at any time undergo the qualitative changes of tumor progression. Lesions which develop from the region of incipient neoplasia during the initiation phase have been classified by Foulds as Group A lesions. This lesion-type represents either side effects of the initiating agent or dead-end hyperplasias which are not neoplastic by histological examination and are nonparticipants in further neoplastic development. Conversely, the region of incipient neoplasia has the potential to progress through other phases

tumorigenesis, thereby evolving lesions with greater neoplastic potentials.

An analysis of Foulds' second, or intermediate, phase of tumorigenesis demonstrates a characteristic emergence of the earliest recognizable non-malignant, precancerous (Group B) lesions representing various degrees of neoplastic severity. Group B lesions may undergo one of four fates: complete regression to the original state of incipient neoplasia, an extensive static existence without qualitative or quantitative changes, growth without qualitative changes in structure or neoplastic potential, or finally, qualitative progression into lesions having greater neoplastic capacity. Thus, during Foulds' intermediate stage, Group B lesions may be derived directly from an incipient neoplasia or from other Group B lesions having a lesser degree of neoplastic severity.

Foulds' third phase of tumor progression is the advanced phase, characterized by the presence of clinically defined, malignant (Group C) lesions. Most Group C lesions have the capacity to be derived directly from the region of incipient neoplasia or indirectly from other Group B or Group C lesions with lesser degrees of neoplastic and malignant qualities. An exception is the highly malignant "Type C_3" lesion in which no counterpart has been observed in humans and results only after extended tranplantation of tumors in experimental animals. Type C_3 lesions appear to have sole derivation from other Group C lesions exhibiting a lesser degree of

malignancy, and thus are derived directly from the region of incipient neoplasia.

Estrogen-induced and spontaneous mammary neoplasia occurring in high incidence strains of mice have provided a basis for understanding the early stages of mammary tumor progression. Each group of lesions defined by Foulds is observable in a single animal suggesting that progression occurs independently in different tumors within the same animal. Group A lesions in the estrogen-induced hyperplasia of the mouse mammary gland are produced by the side effects of estrogen and include changes in the mammary gland resembling cystic disease or an inflammatory response as often observed with mastitis. However, no correlation has been established between the development of such cystic-type lesions and later developing advanced stage mammary tumors. Group B lesions in mouse mammary tumorigenesis are primarily hyperplastic nodules and plaques. It is not thought that these lesions represent consecutive stages along a single course of neoplastic development, but rather are alternate pathways through which the incipient neoplasm may proceed. Both hyperplastic nodules and plaques have the capacity to progress to lesions possessing higher degrees of mammary neoplastic potential. It is thought that hyperplastic nodules and plaques account for the majority, but not all, of the advanced phase, or Group C lesions. Group C lesions are composed of a wide variety of adenocarcinomas and

adenoacanthomas which, in the mouse, are largely hormone independent and unresponsive to endocrine ablation or treatment.

The Berenblum Hypothesis

The Berenblum hypothesis, or the two-step mechanism of tumorigenesis, advances the premise that cancerigenesis is a multistep phenomenon. Berenblum hypothesized that tumorigenesis is composed of two discrete stages, initiation and promotion. The terms initiation and promotion were first suggested by Friedwald and Rous (1944) from studies involved with tumors arising from rabbit ear epidermis treated with the carcinogen, benzypyrene. Berenblum (1954, 1969) derived his hypothesis primarily by focusing upon the mouse integument tumor system (Berenblum & Shubik, 1947).

Similar to the Tumor Progression Theory, initiation according to the Berenblum Hypothesis is a rapidly occurring process in which permanent changes in the cellular phenotype are induced in the tissue involved. These permanent changes are most probably invoked by mutation-like alterations in the genome of the cell. Such a hypothesis is supported by the observations that a wide range of initiating agents have been shown to specifically bind covalently to nucleic acids, particularly DNA, in many tissues sensitive to their carcinogenic action (Brooks & Lawley, 1964; Goshman & Hadelberger, 1967; Prodi et al., 1970). Depending on the type and dose of the initiator used, tumors can be produced

either by application of the initiator alone, as in the case of a large dose of a complete carcinogen, or by treatment with a subthreshold dose of an incomplete initiator and subsequently treated with a promoting agent. In the latter case, limited treatment with an initiator produces "dormant" tumor cells which require further stimulation by promoting agents to proliferate into tumors. Furthermore, according to the Berenblum hypothesis, a prolonged interval between the initiation and promotion events does not decrease the resulting tumor yield. Berenblum and Shubik (1947) reported that an interval up to 43 weeks between initiation with benzpyrene and promotion with croton oil did not decrease the yield of skin tumors in mice.

In contrast to initiation, promotion in tumorigenesis is a slow process in which repeated administration of relatively large doses of the promoting agents are usually required. Also, unlike initiation, the effects of promotion can largely be reversed when the application of the promoting agent is discontinued (Berenblum & Shubik, 1947). It has been demonstrated that promotion occurs only when the promoting agents are applied after, but not prior to, initiation.

Unlike the mechanism of initiation, promotion does not induce permanent changes in the genome but rather influences the expression of pre-existing alterations induced by the initiator. It has been suggested by Berenblum (1974) that promoting agents may act by binding to various regulatory

proteins to exert their effect. Furthermore, promotors do not appear to act by simply inducing non-specific hyperplasia of the initiated tissue, but rather may induce specific hyperplasia and/or delayed maturation of altered undifferentiated stem cells. This concept is particularly plausible when considering the epidermal tumor models.

Berenblum's classic example of initiation-promotion is based upon the benzpyrene-croton oil model using the mouse integument tumor system (Berenblum, 1941). In these studies, administration of subcarcinogenic doses of the initiating agent, benzpyrene, resulted in the development of few or no epidermal tumors. However, when the promoting agent, croton oil, was administered following the subthreshold treatment with benzpyrene, tumor incidence was increased and the latency period of tumor development was decreased, indicating enhanced tumorigenesis. When croton oil was given alone or prior to the subcarcinogenic dose of benzpyrene, few or no tumors developed. These results would indicate that croton oil alone has little or no carcinogenic activity and that the promoting activity of croton oil is only effective when applied after initiation.

Although the concepts of the initiation-promotion hypothesis of mammary tumorigenesis were derived from, and are best applicable to the mouse integument and rabbit epidermis tumor systems, the hypothesis also appears to be relevant to many rat mammary tumor models. Hormones, particularly estrogen and prolactin, have often been classified

as promotors of carcinogen-induced mammary tumorigenesis. The role of hormones in mammary tumorigenesis will be explored in a subsequent section of this literature review. Promotion of mammary tumorigenesis has also been reported using the classical systemic promotor, phorbol (Armuth & Berenblum, 1974). In this study, rats treated twice weekly with intraperitoneal injections of phorbol for 10 weeks following DMBA administration showed significantly augmented mammary tumor incidence when compared to rats treated with DMBA alone. However, in contrast to the effect of most promotors of skin tumorigenesis, administration of phorbol had no effect on the average latency period of mammary tumor development.

Somatic Mutation Theory

The permanent nature of the initiating event during tumorigenesis provided the premise upon which the Somatic Mutation Theory was evolved. Boveri (1914) suggested that alterations within the hereditary material of the cell (a "mutation" thereof) were responsible for the initiation of tumorigenesis. Credence to this hypothesis was not forthcoming for many years due to the failure to demonstrate that many carcinogenic substances could also cause mutations in various microbial and cell test systems. Maher et al. (1968) and Miller and Miller (1966) reported that "activated forms" of non-mutagenic carcinogens, such as aminoacetyl-fluorine (AAF) were, in fact, able to cause mutations in bacterial

test systems. Furthermore, it has been determined that most carcinogens are metabolized to an activated, mutagenic form in vivo by cytochrome P450-dependent-microsomal-mixed-function oxidases which then act on the tissue to produce transformation. Subsequently, it has been shown that there is a positive correlation between the carcinogenicity and mutagenicity as determined by the Ames Salmonella/microsome test in which the enzymes required for metabolic activation of the carcinogens were provided (McCann et al., 1975).

Further evidence that somatic cell mutations may be responsible for the transformation of normal cells into cancer cells resulted from the observation that most carcinogenic compounds bind to DNA and could thereby induce tumorigenesis. Brooks and Lawley (1964) observed a correlation between the carcinogenicity of a compound in vivo and its ability to bind to DNA. Binding to RNA and cellular protein, however, were not correlated to the carcinogenic potency of the substance.

Finally, if one assumes that somatic cellular mutations are the primary factors involved in the induction of cancer, then those cells deficient in enzymes required for their DNA repair mechanisms should have a higher incidence of transformation than cells possessing normal DNA repair mechanisms. Cells deficient in DNA repair enzymes have an increased susceptibility to transformation by chemical carcinogens and ultraviolet radiation (Maher et al., 1976; Maher et al., 1977). Individuals with deficiencies in DNA repair mechanism, as in xeroderma pigmentosium patients, have an

extremely high incidence of skin cancer when exposed to ultraviolet radiation. Therefore, the relationship between the cells' ability to repair errors in DNA and cancer incidence, suggests that mutations cause tumorigenesis.

The relationship between the somatic mutation theory and mammary tumorigenesis has been based primarily on the observation that many carcinogens shown to induce mammary tumors in rats also bind selectively to DNA in mammary cells and can transform mammary cells in vitro (Janss et al., 1972; Dao, 1970).

Models for Studying Mammary Tumorigenesis

The selection of a model for the study of human breast cancerigenesis should consider the following criteria. Initially, tumor induction should proceed with a relatively high frequency in a reasonable period of time utilizing laboratory animals suitable for such study with respect to size, ease of handling, and expense. Developing tumors should resemble human breast cancer both morphologically and in biological characteristics relating to local invasiveness and metastasis to distant sites.

Chemical Carcinogens

Aminofluorenes

Chemically induced mammary tumors have provided much information contributing to an understanding of the endocrine effects on mammary tumorigenesis and growth. The

first chemicals to be successfully used in the induction of mammary tumors in experimental animals were the aminofluorenes. Wilson et al. (1941) first reported that 2-aminofluorene could initiate tumors in mammary tissue when administered in the diet. Bielschowsky (1944) showed that female Wistar rats fed aminoacetylfluorene (AAF) developed mammary tumors, primarily adenocarcinomas. Also reported was the observation of reduced incidence of AAF-induced mammary tumors in ovariectomized female or intact male rats. These observations suggest that the development of AAF-induced mammary tumors is dependent on an ovarian influence. However, ovariectomy had little effect on the growth of established AAF-induced mammary tumors (Bielschowsky, 1944). Symeonidis (1954) reported that the susceptibility to carcinogenesis by AAF was greatest in immature female rats, and decreased with age.

Polycyclic Aromatic Hydrocarbons

By far, the most commonly cited class of chemical carcinogens that has been used in the study of mammary tumorigenesis are the polycyclic aromatic hydrocarbons. Shay et al. (1949) found that repeated intragastric administrations of 3-methylcholanthrene (MC) resulted in the development of mammary adenocarcinomas in female Wistar rats. Shay et al. (1952) also reported that mammary tumors could be induced in male rats and that the number and type of tumors that developed was largely influenced by hormone treatment.

Huggins et al. (1958) reported that most of the carcinomas induced by MC were hormone sensitive tumors observing that ovariectomy resulted in a delay in tumor induction, whereas hypophysectomy completely prevented tumor induction. This suggests that both ovarian and pituitary factors influenced mammary tumor development induced by MC. Ovariectomy also was reported to cause rapid regression of established MC-induced mammary tumors.

Seven-twelve Dimethylbenz(a)anthracene (DMBA) has become the most widely used carcinogen for the induction of mammary tumors in experimental animals in the last 20 years. Huggins et al. (1959) reported that administration of DMBA induced largely hormone dependent mammary tumors with a relatively short latency period. Huggins et al. (1961) further showed that a single intragastric administration of 20 mg of DMBA was as effective in inducing mammary tumorigenesis as repeated doses of MC. The optimum age for induction of mammary tumorigenesis by DMBA has been observed to occur between 50 and 65 days of age, after which time the mammary gland becomes relatively unresponsive to the carcinogen (Huggins et al., 1961). Huggins (1965) also showed that a single intravenous injection of smaller doses of DMBA into rats 50 to 60 days of age results in the development of multiple mammary adenocarcinomas in Sprague-Dawley rats. Young et al. (1963) reported that the first tumors to develop after administration of DMBA were largely adenocarcinomas which histologically resembled human breast cancer in that

they were derived primarily from the ductal epithelial portion of the mammary gland. The hormone responsiveness of DMBA-induced mammary tumors also appears to be similar to human breast cancer. However, one important deficiency of the DMBA-induced mammary tumor is its inability to locally invade surrounding tissues and metastasize to distant sites as often observed in human breast cancer (Young et al., 1963).

Polycyclic aromatic hydrocarbons by themselves are precarcinogens and like many other types of carcinogens require enzymatic activation. Metabolism of polycyclic aromatic hydrocarbons to ultimate carcinogens is by arylhydrocarbon hydroxylase (AHH). AHH is a cytochrome P_{450} dependent microsomal mixed function oxidase. AHH oxidizes polycyclic aromatic hydrocarbons to epoxides, which are thought to represent the active forms of these carcinogens.

Nitrosamines

Nitrosamines, such as N-methyl-N-nitrosourea (NMU) have been reported to be potent mammary gland carcinogens in BUF/n and Sprague-Dawley rats (Guillino et al., 1975; Rose et al., 1980). All of the mammary tumors induced by 3-monthly intravenous injections of NMU were adenocarcinomas and papillary carcinomas, most of which appeared to be hormone responsive (Guillino et al., 1975). Recently, McCormick et al. (1981) have shown that a single injection of NMU induces a high incidence of mammary tumors in

Sprague-Dawley rats in a dose-responsive fashion. However, the most important feature of the NMU-induced mammary tumor is its ability to metastasize to some small degree to bone, spleen, and lung tissue (Guillino et al., 1975). Upon such consideration, the NMU-induced mammary tumor may prove to be a better model for the study of human breast cancer than other carcinogen-induced mammary tumor models which do not induce metastatic tumors. However, the degree of metastasis does not approach that occurring in human breast cancer.

Transplantable Mammary Tumors

Many transplantable mammary tumor cell lines have been developed. A major advantage to using the transplantable mammary tumor cell line is that much of the heterogeneity seen with the carcinogen-induced mammary tumors is eliminated. This is especially important for many biochemical investigations since primary carcinogen-induced mammary tumors often have diverse biological characteristics. Two of the most widely used transplantable tumors are the MTW9 and the R3230AC.

The MTW9 transplantable mammary tumor was developed in Wistar/Furth rats bearing a somatomammotropic pituitary tumor (MtT), injected with a sub-carcinogenic dose (10 mg) of 3-methylcholanthrene, and perpetuated in female rats bearing MtT (Kim & Furth, 1960). The MTW9 requires sustained elevated serum prolactin levels and physiological concentrations of ovarian steroids for growth. Elevation of

serum prolactin by either co-implantation of MtT, pituitary isografts to the kidney capsule, or by injection with periphenozine, have been reported to be required for development and growth of MTW9 (Kim & Furth, 1960; MacLeod et al., 1964; Hollander & Diamond, 1978). Ovariectomy at the time of MTW9 transplantation, or after MTW9 has been established, inhibits development and growth of the tumor (MacLeod et al., 1964; Kim & Furth, 1960).

The R3230AC transplantable mammary adenocarcinoma was derived from a spontaneous mammary tumor in the Fisher rat (Hilf et al., 1967). R3230AC is an autonomous tumor, growing equally well in both intact or castrated, male or female rats. Unlike most carcinogen-induced and transplantable mammary tumors, exogenously administered estrogen and/or prolactin inhibited the growth of R3230AC in vivo. Such treatment also appears to stimulate the differentiation of the tumor into a secretory tissue as indicated by the presence of milk proteins such as casein and α-lactalbumin (Hilf et al., 1967; Turkington & Riddle, 1969).

Human Breast Cancer Cell Lines

The cell line MCF-7, derived by Soule et al. (1973) from a pleural effusion in a post-menopausal woman with metastatic breast cancer, is the most universally employed human breast cancer cell line. The MCF-7, unlike many other derived human breast cancer cell lines, has been well characterized as containing human mammary epithelial cells, using

morphological, chromososomal, and biochemical criteria. It has been reported to contain receptors for glucocorticoids, estrogens, progestins. androgens, and insulin, lending further support to its mammary epithelial derivation. These parallels in morphology are also associated with the hormone responsiveness of the cell line. Recently, other human breast cancer cell lines (the ZR-75 cell lines) have been derived from malignant effusions (Monaco & Lippman, 1978). These, like the MCF-7, have been characterized as derived from human mammary epithelial cells and are similarly hormone-responsive.

Role of Hormones in Murine Mammary Tumorigenesis

Neuroendocrine Relationships

Treatments which influence neuroendocrine function dramatically affect murine mammary tumorigenesis. Drugs which act on the central nervous system, particularly the hypothalamus, have long been shown to influence murine mammary tumor development and growth. Lacassagne and Duplan (1959) first showed that tranquilizers, such as reserpine could hasten the development of spontaneous mammary tumors in C_3H mice. Reserpine, a catecholamine storage inhibitor and depletor, also stimulated the growth of DMBA-induced mammary tumors in rats (Welsch & Meites, 1970). Hypothalamic dopamine activity is inversely correlated with mammary tumorigenesis and growth. Inhibition of dopamine activity by dopamine receptor blockers, such as haloperidol

and pimozide, also stimulated DMBA-induced mammary tumor growth (Quadri et al., 1973; Hodson et al., 1978). Other antidopaminergic drugs, such as perphenazine, sulpiride, and methyl-dopa, also stimulated mammary tumor development and growth (Pearson et al., 1969; Pass & Meites, 1977; Quadri et al., 1973).

Drugs which increase hypothalamic dopamine activity inhibit mammary tumor development and growth. L-DOPA, the immediate precursor of dopamine inhibited growth of DMBA-induced mammary tumors (Quadri et al., 1973). Dopamine agonists, such as various ergot alkaloids, piribedil, and iproniazid, suppressed growth of established carcinogen-induced mammary tumors (Cassell et al., 1971; Hodson et al., 1978; Nagasawa & Meites, 1970). An increase in hypothalamic dopaminergic activity by ergot alkoloids such as bromoergocryptine (CB-154) and ergocornine also inhibited mammary tumor development in mice and rats (Welsch & Gribler, 1973; Nagasawa & Morii, 1981.

Treatments with drugs which influence hypothalamic norepinephrine and serotonin activity also affect mammary tumor growth in rats. Hodson et al. (1978) reported that a decrease in brain serotonin activity by parachlorophenylalanine (PCPA) inhibited growth of DMBA-induced mammary tumors. Administration of the α-adrenergic agonist, clonidine, also inhibited DMBA-induced mammary tumor growth, suggesting that increased noradrenergic activity

may have a suppressive effect on mammary tumor growth (Hodson et al., 1978).

Hypothalamic lesions also affect mammary tumorigenesis. Lesions of the median eminence area of the hypothalamus enhanced DMBA-induced mammary tumor growth (Welsch et al., 1969; Klaiber et al., 1969), whereas lesions of the preoptic area of the hypothalamus and the amygdala caused significant regression of these tumors (Welsch et al., 1969). Median eminence lesions also stimulated spontaneous mammary tumor development in rats (Welsch et al., 1970). However, median eminence lesions placed prior to carcinogen administration inhibited mammary tumor development (Klaiber et al., 1969). Bruni and Montemurro (1971) also reported that lesions of the anterior and medial hypothalamic areas stimulated spontaneous mammary tumor development in C302F female mice. Lesions of the posterior hypothalamus had no effect on mammary tumor development.

Hypothalamic releasing factors such as TRH also stimulated mammary tumor growth by increasing serum prolactin levels (Chen et al., 1977). Suppression of endogenous opioid activity with opiate antagonists, such as naloxone or naltrexone, inhibited mammary tumor growth as a result of inhibition of anterior pituitary prolactin secretion (Aylsworth et al., 1979).

Control of Anterior Pituitary Prolactin Secretion

Most of the above mentioned effects of hypothalamic lesions and drug treatment on mammary tumor development and growth are mediated by their action on pituitary prolactin secretion. Secretion of prolactin by the mammalian anterior pituitary is under a tonic inhibitory influence from the hypothalamus. Thus, separation of the anterior pituitary from this inhibitory influence by stalk section or transplantation of anterior pituitaries to ectopic sites resulted in enhanced prolactin secretion and elevated serum prolactin levels (Meites & Nicoll, 1966). Destructive lesions of the median eminence area of the hypothalamus also elevated serum prolactin levels (Meites et al., 1963). Disruption of communication between the hypothalamus and anterior pituitary via the hypophysial portal vessels removes the predominantly inhibitory effect of the hypothalamus on secretion of prolactin by the anterior pituitary. Talwalker et al. (1963) and Pasteels (1963) were first to demonstrate that the hypothalamus contains a factor that inhibited anterior pituitary secretion. Acid hypothalamic extracts were shown to suppress anterior pituitary prolactin secretion in vitro. Pharmacological and physiological conditions, such as the suckling stimulus, estradiol, reserpine, and perphenazine, all known to elevate serum prolactin levels, were shown to decrease hypothalamic PIF activity (Ratner & Meites, 1964; Ratner et al., 1965; Danon et al., 1963). Evidence for a

prolactin releasing factor (PRF) also was demonstrated by Meites et al., (1960) and Mishkinsky et al. (1968). The chemical structures of these putative releasing and release inhibiting factors have not been identified.

Hypothalamic neurotransmitters also are involved in the control of anterior pituitary prolactin secretion. These substances act either directly on the pituitary or indirectly by influencing the putative PIF and PRF peptides. Dopamine (DA) exerts an inhibitory effect on anterior pituitary prolactin secretion. Pharmacological agents that increase hypothalamic dopaminergic activity depress serum prolactin levels. Thus, dopamine agonists such as various ergot alkoloids, apomorphine, and piribedil, reduced serum prolactin levels (Nagasawa & Meites, 1970; Mueller et al., 1976). Conversely, drugs which decrease hypothalamic dopaminergic activity, such as reserpine, chlorpromazine, and haloperidol, increased serum prolactin levels (Lu et al., 1970; Dickerman et al., 1973). Dopamine directly inhibited prolactin release from incubated anterior pituitaries in vitro (MacLeod, 1976). There is considerable evidence that dopamine is the major physiological PIF (MacLeod, 1976; Greibrokk et al., 1975). Shaar and Clemens (1974) reported that removal of catecholamines from hypothalamic extracts completely eliminated all prolactin inhibiting activity of these extracts in vitro. However, when dopamine receptors were blocked by haloperidol, prolactin secretion was still inhibited in a

hypothalamic-pituitary co-incubation system, suggesting that inhibitory factors other than dopamine are present in the hypothalamus (Ojeda et al., 1974).

The role of norepinephrine (NE) in the control of anterior pituitary prolactin secretion is not clear. Injections of DOPS, a precursor of NE, increased prolactin release (Donoso et al., 1971). Inhibition of NE synthesis by disulfram decreased prolactin release (Meites & Clemens, 1972). These observations suggest that the noradrenergic system may exert a stimulatory influence on prolactin secretion. However, other noradrenergic agonists, such as clonadine, depressed serum prolactin levels (Meites et al., 1977; Hodson et al., 1978). NE also directly inhibited prolactin release in vitro (MacLeod, 1976).

Serotonin influences anterior pituitary prolactin secretion in vivo, presumably by its effect on hypothalamic PIF or PRF release. Injection of the serotonergic precursors, tryptophan or 5 hydroxytryptophan, or serotonergic agonists, such as quipazine, elevated serum prolactin levels (Meites & Clemens, 1972; Meites et al., 1977). Conversely, serotonin antagonists, such as methysergide or PCPA, blocked the suckling-induced rise in prolactin in lactating rats (Kordon et al., 1973; Blake et al., 1973). Furthermore, the suckling induced rise in prolactin is associated with increased hypothalamic serotonergic activity (Kordon et al., 1973). Thus, present evidence suggests that serotonin exerts a stimulatory effect on anterior pituitary secretion of prolactin.

Acetylcholine reduced serum prolactin levels (Grandison et al., 1974), an effect thought to be mediated through the adrenergic system (Grandison & Meites, 1976). However, a physiological role for acetylcholine on the control of prolactin secretion has not been established.

More recently, the endogenous opiate peptides have been shown to influence anterior pituitary prolactin secretion. β-endorphin, leucine- and methionine-enkephalin, and other opiates increased serum prolactin levels (Meites et al., 1979). Conversely opiate antagonists, such as naloxone, reduced serum prolactin levels (Meites et al., 1979). The opiates reduced dopamine and increased serotonin activity in the hypothalamus (Meites et al., 1979), actions that would increase prolactin secretion.

Pituitary target tissue hormones also have an important influence on serum prolactin levels. Estrogen was shown repeatedly to increase serum prolactin levels, and can act directly on the anterior pituitary to stimulate prolactin secretion (Meites et al., 1972). However, there also is evidence that the effects of estrogen on anterior pituitary prolactin secretion are mediated in part via hypothalamic mechanisms involving a decrease in PIF (DA) activity and/or an increase in PRF activity (Meites, 1979).

Adrenal glucocorticoids decreased, whereas adrenalectomy increased serum prolactin levels (Chen et al., 1976). Inhibition of prolactin secretion by glucocorticoids appears to be mediated by a direct action on the anterior

pituitary (Leung et al., 1980). Thyroid hormones directly stimulate secretion of prolactin by the anterior pituitary in vitro. Nicoll and Meites (1963) showed that addition of either thyroxine or triiodothyronine to anterior pituitary organ cultures increased prolactin release. However, radioimmunoassay of prolactin in the blood of hypo- or hyperthyroid rats showed no significant change (Chen et al., 1976).

Role of Prolactin in Murine Mammary Tumorigenesis

By influencing the control of anterior pituitary prolactin secretion, mammary tumor development and growth are dramatically affected. In general, treatments and physiological conditions that elevate serum prolactin stimulate mammary tumorigenesis, whereas treatments and physiological conditions that reduce serum prolactin inhibit mammary tumorigenesis (Welsch & Nagasawa, 1977). Boyne et al. (1973) and Hawkins et al. (1976) reported that higher mammary tumor incidence was associated with strains of rats which have comparatively greater basal serum prolactin levels. Hyperprolactinemia induced by grafted pituitaries or median eminence lesions enhanced spontaneous mammary tumor development in rats (Welsch et al., 1970a; Welsch et al., 1970b). Loeb and Kirtz (1937) showed that subcutaneous pituitary grafts increased mammary tumor incidence in mice. Hyperprolactinemia produced in rats after carcinogen administration stimulated mammary tumor development, whereas

hyperprolactinemia induced prior to carcinogen administration inhibited mammary tumorigenesis (Klaiber et al., 1969; Clemens et al., 1968). Also, administration of reserpine or haloperidol to evelate serum prolactin prior to DMBA treatment, decreased mammary tumorigenesis (Welsch & Meites, 1970; Kledzik et al., 1974). It appears that hyperprolactinemia stimulates growth of normal mammary tissue and development of a predominantly lobulo-alveolar system, rendering the mammary gland refractory to the action of the carcinogen (Welsch & Nagasawa, 1977).

Hypophysectomy reduced the incidence of mammary tumors in mice (Korteweg & Thomas, 1946). Reduction of serum prolactin with ergot drugs or L-DOPA either prior to or after carcinogen treatment reduced mammary tumor development in rats (Clemens & Shaar, 1972; Kledzik et al., 1974). In a recent report by Nagasawa and Morii (1981) temporary suppression of serum prolactin with CB-154 between weeks 4 and 11 of life suppressed subsequent spontaneous mammary tumor development in rats.

Neuroendocrine treatments that elevate serum prolactin levels also stimulate growth of murine mammary tumors. These include median eminence lesions (Welsch et al., 1969), pituitary homografts (Welsch et al., 1968; Harada, 1976), and various dopamine antagonists (Quadri et al., 1973; Pearson et al., 1969; Hodson et al., 1978; Pass & Meites, 1977). Physiological conditions which elevate serum prolactin levels, such as pregnancy (McCormick & Moon, 1965) and

pesudopregnancy (Dao, 1959), also stimulated mammary tumor growth. However, during lactation, when serum prolactin levels are increased, most mammary tumors regressed (McCormick & Moon, 1965), probably because of enhanced adrenal glucocorticoid secretion (Aylsworth et al., 1979).

Elevated serum prolactin levels appear to be able to sustain mammary tumor growth, at least for a limited time period, even in the absence of estrogen. Welsch et al. (1969) showed that hyperprolactinemia induced by median eminence lesions could maintain established mammary tumor growth for a limited period of time in the absence of ovarian influence. Administration of large doses of prolactin to ovariectomized rats also stimulated mammary tumor growth (Nagasawa & Yanai, 1970). Manni et al. (1977) reported that elevation of serum prolactin levels by perphenazine could support and restore DMBA-induced mammary tumor growth even if the influence of estrogen was removed by ovariectomy or Tamoxifen adminstration. However, Sinha et al. (1973) reported that the effects of hyperprolactinemia on mammary tumor growth in the absence of ovarian influences were transitory, and that sustained mammary tumor growth required replacement with ovarian steroids. Prolactin can act directly on carcinogen-induced mammary tumor tissue *in vitro* to stimulate growth as measured by incorporation of ^{3}H-thymidine into DNA (Lee et al., 1975; Welsch & Rivera, 1972). However, estrogen combined with prolactin produced

a marked synergistic effect on in vitro mammary tumor growth when compared with treatment using either hormone alone.

Suppression of serum prolactin causes rapid regression of most established hormone-dependent mammary tumors. Hypophysectomy caused regression of most mammary tumors in the rat (Clifton & Sridharan, 1975), and upon replacement of prolactin these tumors resumed growth. Dopamine agonists such as pargyline, L-DOPA, and ergot alkoloids, reduced serum prolactin levels and inhibited rat mammary tumor growth (Quadri et al., 1973; Nagasawa & Meites, 1970).

Involvement of prolactin in the growth of human breast cancer is not as clear as in animal tumor models. Hypophysectomy has been reported to cause regression of breast tumors in humans (Kennedy et al., 1956; Pearson & Ray, 1960; Van Gilder & Goldenberg, 1975). However, regression of breast cancer in women by suppression of serum prolactin with L-DOPA and ergot drugs has rarely been reported (McGuire, 1975; Schultz et al., 1973; Heuson et al., 1972).

Role of Estrogen in Murine Mammary Tumorigenesis

Estrogen has a profound influence on mammary tumorigenesis. Removal of estrogen influence by ovariectomy or by use of anti-estrogen drugs, such as Tamoxifen, prior to or shortly after carcinogen administration inhibited mammary tumorigenesis (Dao, 1962; Jordon, 1976). Talwalker et al. (1964) showed that replacement of estrogen following ovariectomy partially reversed the inhibitory effect of

ovariectomy on mammary tumorigenesis. Administration of low or moderate doses of estrogen increased mammary tumor incidence in mice and rats (Bern & Nandi, 1961; Dao, 1962). Since estrogen increases serum prolactin levels (Meites & Nicoll, 1966), it has been suggested that such a stimulatory effect is mediated, at least in part, through its effects on prolactin secretion (Furth, 1973). Brooks & Welsch (1974), and Welsch et al. (1977) have shown that while chronic treatment with estrogen increased the incidence of mammary hyperplasias and tumor development in C_3H mice, concurrent reduction of serum prolactin levels with CB-154 blocked the stimulatory effects of estrogen on mammary tumorigenesis.

Estrogen also influences the growth of established carcinogen-induced tumors. Removal of estrogen by ovariectomy or Tamoxifen resulted in a rapid regression of most established carcinogen-induced mammary tumors (Huggins et al., 1959; Jordan & Jaspar, 1976). Replacement with estrogen reversed regression of mammary tumors induced by ovariectomy. Administration of low or moderate doses of estrogen to intact DMBA-induced tumor bearing rats also stimulated mammary tumor growth (Huggins et al., 1962). However, estrogen has no effect on mammary tumor growth in the absence of the pituitary (Sterental et al., 1963). Large doses of estrogen inhibited mammary tumor growth and induced tumor regression (Huggins et al., 1962). These inhibitory effects of large doses of estrogen can be reversed by concurrent administration of large doses of prolactin

(Meites et al., 1971). Large concentrations of estrogen also can inhibit the action of prolactin in vitro (Welsch & Rivera, 1972), an effect that can be reversed by increasing the amount of prolactin added to the culture media (Chan et al., 1976). Since there is no evidence that large doses of estrogen inhibit pituitary prolactin secretion, it is likely that estrogen acts on the tumor tissue to antagonize the action of prolactin. Evidence of such an inhibitory effect of pharmacological doses of estrogen on mammary tumor growth was reported by Kledzik et al. (1976). These investigators showed that large doses of estrogen reduced specific prolactin binding to mammary tumor membranes and thereby inhibited the peripheral action of prolactin on the tumor.

Estrogen stimulated incorporation of ^{3}H-leucine into protein in cultures of DMBA-induced tumor tissue (Lee et al., 1975). However, Welsch and Rivera (1972) reported that estrogen over a wide range of concentrations was either without effect or inhibited in vitro DNA synthesis. Estrogen may not be essential for carcinogen-induced murine mammary tumorigenesis under some experimental conditions, but it does appear to have an important stimulatory role in mammary tumor development and growth under most physiological conditions, by acting directly on the mammary tumor tissue and indirectly through its influence on anterior pituitary prolactin secretion.

Role of Insulin in Murine Mammary Tumorigenesis

Insulin appears to have an important effect on murine mammary tumorigenesis and growth. Removal of insulin by alloxan-induced diabetes shortly after DMBA administration inhibited mammary tumor development in rats (Heuson & Legros, 1972). Induction of diabetes by alloxan in rats bearing DMBA-induced mammary tumors caused regression of 90% of the tumors present (Heuson & Legros, 1972). Similarly, Cohen and Hilf (1974) reported that streptozotocin-induced diabetes also caused regression of 60% of the DMBA-induced mammary tumors present, and that replacement with insulin reversed the regression. Administration of relatively large doses of insulin to intact rats stimulated DMBA-induced mammary tumor growth (Heuson et al., 1972). However, the effects of insulin removal or administration on mammary tumor growth depends on the tumor model used. Growth of the R3230AC transplantable, autonomous, but hormone responsive mammary adenocarcinoma, was inhibited by administration of insulin, whereas growth was enhanced in diabetic rats (Cohen & Hilf, 1975). Removal of insulin also blocked the growth of MCF-7 human breast cancer cells transplanted into diabetic athymic nude mice (Shafie, 1980).

Insulin receptors have been identified in both rat mammary tumors and in human breast cancers (Hilf et al., 1978; Holdaway and Friesen, 1977). Recently, Shafie and Hilf (1981) found a positive correlation between insulin

binding and the magnitude of the biological response of the mammary tumor tissue to insulin in vitro.

The presence of insulin is required by most DMBA-induced mammary tumors to proliferate in culture (Heuson et al., 1966). Heuson and Legros (1971) also showed that insulin could stimulate DNA synthesis in most DMBA-induced mammary tumors in vitro. Insulin also increased DNA synthesis in human breast cancer tissue in vitro (Welsch et al., 1976).

It has been suggested that the effect of insulin on mammary tumor growth may be mediated through its effect on prolactin receptors. In support of this concept, Smith et al. (1977) showed that removal of insulin by streptozotocin-induced diabetes decreased prolactin receptors in the regressing, insulin-dependent tumors. This would suggest that insulin deprivation can render the tumors less responsive to circulating prolactin levels and may thereby cause regression of these tumors.

Role of Progesterone in Murine Mammary Tumorigenesis

Progesterone is generally thought to have a stimulatory effect on murine mammary tumorigenesis. Huggins et al. (1962) showed that treatment with progesterone 1 month after DMBA administration shortened the latency period of mammary tumor appearance and increased the number of developing tumors. Jabara (1967) similarly showed that progesterone treatment just prior to and following DMBA treatment enhanced mammary tumor development. Pregnancy induced shortly after

DMBA also enhanced mammary tumorigenesis (Huggins et al., 1962). Increased progesterone secretion during pregnancy is believed to be primarily responsible for stimulation of mammary tumor development. However, progesterone administered 20 days prior to and 20 days following DMBA administration inhibited mammary tumorigenesis (Kledzik et al., 1974). Thus, as with prolactin, increased progesterone prior to carcinogen administration inhibits, whereas increased progesterone after carcinogen administration stimulates mammary tumorigenesis.

The effect of progesterone on the growth of existing mammary tumors is not clear. Huggins et al. (1962) reported that progesterone treatment stimulated growth of established DMBA-induced mammary tumors. Jabara (1967) however, showed that exogenous progesterone had little or no effect on growth of established mammary tumors. Pregnancy stimulated DMBA-induced mammary tumor growth, but whether increased progesterone secretion or some other pregnancy hormone such as placental lactogen was primarily responsible for the enhanced growth, has not been determined (Huggins et al., 1962; McCormick & Moon, 1965). Other tumor models, such as the transplantable rat mammary carcinoma MTW9 and the pregnancy dependent TPD MT-4 mouse mammary tumor appear to be more progesterone dependent and responsive than many of the carcinogen-induced mammary tumor models (Diamond et al., 1980; Matsezawa & Yamamoto, 1975). Progesterone also

appears to be of primary importance in promoting mammary tumorigenesis in dogs (Briggs, 1977).

Role of Growth Hormone in Murine Mammary Tumorigenesis

The effects of growth hormone (GH) in murine mammary tumorigenesis and growth are largely permissive or supplementary to other endocrine influences. Talwalker et al. (1964) reported that GH and prolactin were somewhat more effective than prolactin alone in promoting carcinogen-induced mammary tumorigenesis and growth in ovariectomized rats. Administration of GH was shown to have either no effect or a slight stimulatory effect on the growth of established carcinogen-induced mammary tumors in intact rats (Nagasawa & Yanai, 1970; Li & Yang, 1974). Injections of GH overcame the inhibitory effects of protein deficiency on mammary tumor growth, but were without effect on mammary tumor growth in rats fed normal protein rations (Welsch & Meites, 1978). Median eminence lesions which suppress GH, but increase prolactin secretion by the anterior pituitary, caused rapid growth of mammary tumors.

Iturri and Welsch (1976) showed that GH caused a slight stimulation of carcinogen-induced rat mammary tumors *in vitro*. However, these effects were much less than the stimulatory effects of prolactin. Also, Welsch et al. (1978) found that GH was ineffective in stimulating incorporation of ^{3}H-thymidine into DNA in organ cultures of human breast

tumors. Thus, GH does not appear to have an important role in mammary tumor growth.

Role of Adrenal Glucocorticoids in Murine Mammary Tumorigenesis

Large doses of adrenal glucocorticoids inhibit mammary tumor growth in vivo and in vitro. The inhibitory effects of glucocorticoids on mammary tumor growth in vivo may be by a direct action on mammary tumor tissue, or by an indirect mechanism through inhibition of anterior pituitary prolactin secretion (Schwinn et al., 1976). Chen et al. (1976) showed that adrenalectomy stimulated mammary tumor growth and elevated serum prolactin levels, whereas glucocorticoid replacement inhibited mammary tumor growth and reduced serum prolactin levels. Hilf et al. (1965) noted that exogenous administration of glucocorticoids to intact rats inhibited the growth of transplantable mammary tumors. Brennan (1973) and Hayward (1970) have shown that administration of glucocorticoids decreased mammary tumor growth in women. Elevated adrenal glucocorticoid secretion also appears to be involved in regression of established mammary tumors during post-partum lactation, since adrenalectomy after parturition blocked the regression and restored mammary tumor growth equal to that of non-lactating intact controls (Aylsworth et al., 1979). Aylsworth et al. (1979) showed that the synthetic glucocorticoid, dexamethasone, inhibited established DMBA-induced mammary tumor growth even in the presence of elevated serum prolactin levels,

suggesting that glucocorticoids may act directly on mammary tumor tissue to inhibit growth.

Glucocorticoids appear to be necessary for prolactin to maximally stimulate DNA synthesis in vitro (Lewis & Hallowes, 1974). However, high concentrations of glucocorticoids inhibited growth of DMBA-induced rat mammary tumors and human breast cancer cells in vitro (Koyama et al., 1972; Osborne et al., 1979). Therefore, glucocorticoids may have a permissive role in mammary tumorigenesis, but in high doses are inhibitory to this process.

Nutrition and Mammary Cancer

Effects of Dietary Fat on Mammary Tumorigenesis

A nutritional factor related to mammary tumors that has generated much interest in recent years is dietary fat. One of the first reports that linked increased fat consumption to enhanced mammary tumor development was the classic study by Tannenbaum (1942) who showed that high fat diets fed to C_3H and DBA mice resulted in an increased incidence of spontaneous mammary tumors. Diets which contained high levels of olive or corn oil also increased the incidence of spontaneously developing mammary fibroadenomas in Sprague-Dawley rats (Benson et al., 1956; Davis et al., 1956). Dunning et al. (1949), Engel and Copeland (1951) and Gammal et al. (1967) showed that diets containing high levels of fat (corn oil) enhanced development of mammary tumors

induced by diethylstilbesterol (DES), AAF, and DMBA. More recent evidence has shown that high fat diets stimulated mammary tumorigenesis in many different carcinogen-induced and transplantable tumors in both mice and rats (Hillyard & Abraham, 1979; Carroll & Khor, 1975). Contributing to the increased interest in studying the effects of dietary lipids on breast carcinogenesis is the strong positive correlation found between breast cancer incidence and dietary fat consumption among women (Carroll & Khor, 1975).

Initiation Versus Promotional Action of Dietary Fat on Mammary Tumorigenesis

Since mammary adipose tissue has been proposed to act as a depot or storage site for administered hydrocarbon-type carcinogens (Dao & Sunderland, 1959), it was suggested that the effects of high fat diets may be mediated through the adipose surrounding the susceptable mammary gland. This hypothesis to explain stimulation of mammary tumorigenesis by high fat dietary intake appears unacceptable, since most reports have shown that the level of fat intake after carcinogen administration is more important in determining mammary tumor development than the level of dietary fat intake before carcinogen administration (Carroll & Khor, 1970; Hopkins et al., 1976). In contrast, Ip (1980) showed that a delay of initiation of high fat diet treatment by up to 20 weeks after DMBA still resulted in enhanced mammary tumor development. Carroll and Khor (1975) reported that a

high fat diet treatment initiated 1-2 weeks after DMBA enhanced mammary tumorigenesis. However, in contrast to Ip (1980), if they delayed the dietary treatment by 4 weeks after DMBA, little or no enhancement of mammary tumorigenesis was observed. Furthermore, Gammal et al. (1968) showed that uptake and clearance of ^{3}H-DMBA by the mammary gland and its fat pad is not altered to any appreciable degree by dietary fat consumption, and concluded that the effects of dietary lipids on mammary tumorigenesis is not likely to be mediated by such a mechanism. However, Janss et al. (1972) showed that DMBA binds to DNA in mammary parenchymal cells and can persist there for up to 2 weeks after DMBA administration. It has been suggested therefore, that high fat diets may still have a direct effect on carcinogen activity at the mammary gland even after most of the DMBA has been cleared from the circulation. Since Berenblum (1969) has defined a promoter as an agent that augments tumor induction when administered after completion of the initiating action, but not when administered before initiation, most of the evidence would indicate that high fat diets act as promoters in mammary tumorigenesis rather than as initiators.

Influence of Type of Dietary Fat

The type of fat consumed in high fat diets has a significant effect on the tumor enhancing ability of the diet. Gammal et al. (1967) demonstrated that high fat diets containing corn oil, which consists mainly of unsaturated fatty

acids, stimulated mammary tumorigenesis to a greater degree than a similar high fat diet that contained coconut oil which consists mainly of saturated fatty acids. Carroll and Khor (1971) used 10 different fats and oils in their high fat diets, and determined that in general, high fat diets that contained primarily unsaturated fatty acids stimulated mammary tumorigenesis to a greater extent than equally high fat diets that contained primarily saturated fatty acids. Hopkins and Carroll (1979) used high fat diets containing different amounts of unsaturated and saturated fatty acids to show that a certain amount of unsaturated fat, as well as high levels of dietary fat, are required to enhance mammary tumorigenesis. They reported that a diet containing 3% sunflower oil (which contains primarily unsaturated fatty acids) and 17% tallow or coconut oil (which contain primarily saturated fatty acids) was just as effective in stimulating mammary tumor development as a 20% sunflower oil diet, and was much more effective in stimulating mammary tumorigenesis than diets containing either 3% sunflower oil or 17% tallow or coconut oil.

Recent evidence has implicated linoleic acid as the most important unsaturated fatty acid involved in stimulation of mammary tumorigenesis by high fat diets. Hillyard and Abraham (1979) suggested that linoleic acid is mainly responsible for stimulating mammary tumor growth by showing that 0.1% linoleic acid added to a fat free diet was as effective in stimulating mammary tumor growth as a 15% corn

oil diet. These results appear to contradict the report of Hopkins and Carroll (1979), that both certain amounts of unsaturated fatty acids and high levels of dietary fat were required to enhance tumorigenesis. Tinsley et al. (1981) used multiple regression statistical tests to isolate the effects of single fatty acids on mammary tumor development. They reported that increasing levels of linoleic acid in the diet was most strongly associated with increased mammary tumorigenesis (i.e., linoleic acid had the highest regression coefficient of all fatty acids tested). Further indication that linoleic acid is of primary importance in stimulating mammary tumorigenesis and growth was shown by Wicha et al. (1979). These investigators showed that normal and neoplastic mammary cell growth could be stimulated in vitro by addition of unsaturated fatty acids, especially linoleic acid, and inhibited by addition of saturated fatty acids.

Proposed Mechanisms by Which High Fat Diets Stimulate Mammary Tumorigenesis

General caloric effect of high fat diets. Since most of the experimental diets used to investigate the effects of dietary lipids on mammary tumorigenesis have a higher caloric content than the comparable control or low fat diets, it has been suggested that these differences may be the means by which high fat diets stimulate mammary tumor development. However, Tannenbaum (1945) showed that even though

caloric restriction inhibited mammary tumorigenesis in mice, at any specific degree of caloric restriction, high fat diets were less inhibitory than similarly restricted diets that contained lower amounts of fat. Thus, the effects of high fat diets on mammary tumorigenesis appears to be due to the specific action of the fat rather than increased caloric intake. Furthermore, Gammal et al. (1967) and other investigators showed that rats fed a low fat diet consume greater amounts of the diet than similar rats fed high fat diets, resulting in similar caloric consumption of the rats fed low and high fat diets. Also, these and other investigators have reported that the growth rates and body weight gains of rats fed diets containing different levels of fat were similar. Therefore, a general caloric effect of high fat diets is not thought to be the mechanism by which high fat diets stimulate mammary tumorigenesis.

Mechanisms involving the endocrine system. Since estrogen and prolactin are two of the most important hormones involved in murine mammary tumorigenesis (Meites, 1972), most of the investigations of endocrine involvement in the effects of dietary fat on mammary tumorigenesis have focused on these hormones. Chan and Cohen (1974) were first to implicate prolactin in stimulation of mammary tumorigenesis by high fat diets. They reported that when serum prolactin levels were chronically decreased by CB-154 (bromoergocryptine), mammary tumor development was decreased and

the differential effects of high fat and low fat diets on mammary tumorigenesis were eliminated. Also reported was the observation that when Nafoxidine, a specific estrogen receptor blocker, was chronically administered to rats fed high fat and low fat diets, mammary tumor development was decreased, but the differential effects of the diets were not eliminated. These findings led to the suggestion that the enhancement of mammary tumorigenesis by high fat diets was mediated through alterations in circulating levels of prolactin, but not estrogens. Further evidence in support of this hypothesis was provided by Chan et al. (1975, 1977), who reported that serum prolactin levels during the afternoon of the proestrous-estrous stage of the estrous cycle was elevated in rats fed high fat diets, and suggested that the enhancement of mammary tumor development by high fat diets are mediated indirectly by increased prolactin secretion via the hypothalamic-pituitary system rather than by a direct action of the fat on the mammary gland itself. One must note however, the small sample size and large variability present in the prolactin values reported by these authors. Conversely, Cave et al. (1979) found no consistent elevation of serum prolactin levels in rats fed high fat diets and no increase in prolactin synthesis _in vitro_ in pituitaries from rats fed high fat diets. Thus, the effects of dietary fat on serum prolactin levels is not clear and is a problem which is addressed in the experimental section of this thesis.

Ip et al. (1980) attempted to clarify the role of prolactin in the promotion of mammary tumorigenesis by high fat diets using median eminence lesions to elevate serum prolactin levels in rats fed high and low fat diets. Ip reported that although serum prolactin levels were similar in both the high fat and low fat median eminence lesioned rats, tumor incidence in the rats fed a high fat diet was still greater than in the rats fed a low fat diet. The authors concluded that although prolactin may partially mediate the effects of high fat diets on mammary tumorigenesis, other factors must be involved. High fat diets also stimulated growth of the hormone responsive transplantable mammary adenocarcinoma R3230AC (Hillyard & Abraham, 1979) which is inhibited by elevated serum prolactin levels (Hilf et al., 1971). This would imply that an increase in serum prolactin levels as hypothesized by Chan et al. (1975) is not involved in mediating the effects of high fat diets on the growth of this type of mammary tumor.

Chan et al. (1977) also reported small increases in circulating estrogen levels at certain stages of the estrous cycle in rats fed high fat diets, but do not attribute this effect to stimulation of mammary tumorigenesis by high fat diets. Furthermore, Carroll and Khor (1975) reported that ^{3}H-estradiol distribution and clearance from the mammary gland is not different in rats that were fed high fat and control fat diets. Thus, it would appear that estrogen secretion and metabolism are not appreciably altered by high

dietary fat and do not provide an adequate explanation for the stimulatory effects of high fat diets on mammary tumorigenesis.

It is possible that the mammary gland and incipient mammary tumor tissue of rats on high fat diets may be more sensitive to circulating levels of prolactin and estrogen and could thereby show enhanced tumorigenesis. Mammary glands from rats fed high fat diets showed more histological evidence of hypertrophy and secretory activity in response to implant DES than rats fed a lower fat diet (Dunning et al., 1949). This stimulated response of the mammary glands in rats fed high fat diets was also correlated with increased incidence of mammary tumors. Recently, Cave et al. (1981) reported that mammary tumors from rats fed a high fat diet showed an increase in lactogenic hormone binding activity when compared to mammary tumors from rats fed a low fat diet. Thus, mammary glands and tumors from rats fed high fat diets may be more responsive to normal circulating levels of estrogen and prolactin, and thereby cause enhanced mammary tumorigenesis.

Recent reports have also implicated the possible involvement of prostaglandins in the mediation of the effects of high fat diets on mammary tumorigenesis. Inhibition of prostaglandin synthesis by administration of indomethecin prevented the growth promoting effects of high unsaturated fat diet consumption on a transplantable mammary tumor in BALB/c mice (Hillyard & Abraham, 1979).

Mechanisms involving the immune system. The immune system plays an integral role in tumorigenesis (Chirigos, 1977), and has been implicated in mediating the effects of high fat diets on mammary tumorigenesis. There is ample evidence that polyunsaturated fatty acids are capable of suppressing immune function. Offner and Glauson (1974) showed that polyunsaturated fatty acids can inhibit antigen-induced proliferation of lymphocytes in vitro. Kollmorgen et al. (1979) reported that the conconavalin A-induced blastogenesis of spleen lymphocytes from rats fed a high fat diet was inhibited as compared to spleen lymphocytes from rats fed low fat diets. Also, nonspecific stimulation of the immune system inhibited stimulation of mammary tumorigenesis by high fat diets (Kollmorgen et al., 1979). Polyunsaturated fatty acids increased the survival of skin allografts in rodents (Ring et al., 1974) and are an effective immunosuppressive therapeutic agent following renal transplantation in humans (Uldell et al., 1975). PGE_1 and PGE_2 inhibited immune responses in lymphocyte test systems (Plecia et al., 1975). Since polyunsaturated fatty acids such as linoleic acid are precursors of prostaglandins, mediation of the immunosuppressive effects of high unsaturated fat diets by prostaglandins is possible. As mentioned above, inhibition of prostaglandin synthesis by indomethecin blocks the stimulation of mammary tumorigenesis by high fat diets (Hillyard & Abraham, 1979). Thus, high fat diets containing large amounts of polyunsaturated fatty acids, may

themselves or via prostaglandins, suppress cell mediated immune response systems and thereby enhance mammary tumorigenesis.

Direct mechanisms by which high fat diets stimulate mammary tumorigenesis. In addition to indirect mechanisms involving the endocrine and immune systems, direct effects of high fat diets on the mammary gland and tumor tissue may explain enhanced mammary tumorigenesis by high fat diets. Tannenbaum (1945) postulated a direct metabolic stimulation of potential tumor cells by dietary fat. Dunning et al. (1949) showed that high fat diets may cause an increased sensitization of mammary glands and tumors to administered DES. Gammal et al. (1967) showed that the composition of mammary tissue reflects the type of fat that is consumed in the diet. Thus, rats which are fed diets containing high levels of corn oil show increased levels of unsaturated fatty acids in their mammary tissue, particularly linoleic acid, the principle fatty acid of corn oil. Membranes with such altered fatty acid composition have different biophysical characteristics than membranes containing lesser amounts of polyunsaturated fatty acids. DeKruyff et al. (1973) showed an increased equilibrium flux of ^{14}C-erythritol across the membranes of Acholephasma Laidlawaii cells grown on linoleic acid enriched media. Thus, the passage of erythritol across these membranes appeared to be enhanced by incorporation of linoleic acid into the membranes. It is

possible that the passage of other substances such as hormones or nutrients into mammary gland or tumor cells may also be enhanced by incorporation of polyunsaturated fatty acids into these membranes of animals fed high fat diets and thereby provide more favorable conditions for mammary tumors to develop and grow.

Polyunsaturated fatty acids are converted to lipid peroxides by normal free radical reactions involved with fatty acid metabolism. Since lipid peroxidation has been associated with carcinogenesis (Shamberger et al., 1973), the possibility that high fat diets exert their effects on mammary tumorigenesis through such a mechanism has been proposed recently. King et al. (1979) reported that antioxidants such as butylhydroxytoluene (BHT) decreased the stimulation of mammary tumor development by saturated and unsaturated high fat diets. High fat diets which were deficient in selenium, an antioxidant, caused further stimulation of mammary tumorigenesis when compared to high fat diets containing normal amounts of selenium (Ip & Sinha, 1981). Chan and Dao (1981), and Carroll (1975) reported that rats fed a non-purified laboratory rat chow showed decreased DMBA-induced carcinogenesis when compared to purified diets containing equal amounts of fat. It is worthy of note that antioxidants such as BHT are routinely added to commercially prepared non-purified rat chow, and may therefore account for the difference observed.

Relation to the Human Condition

Experimental evidence from animal mammary tumor studies investigating the effects of dietary fat on mammary tumorigenesis provides important insight in an attempt to explain international and intercultural differences in breast cancer incidence. In general, Western countries and cultures that consume greater amounts of fat in their diets have a higher incidence of breast cancer than Eastern countries and cultures which consume smaller amounts of dietary fat (Carroll & Khor, 1975). Epidemiological studies by Lea (1966) and others have shown a high positive correlation between dietary fat intake and mortality from breast cancer. Carroll (1975) also demonstrated a strong positive correlation ($r = +0.935$) between total dietary fat intake and age-adjusted breast cancer mortality. However, when the intake of vegetable fat was considered alone, little or no correlation with breast cancer mortality was seen. Furthermore, adipose tissue from Americans was shown to contain less linoleic acid than adipose tissue from Japanese (Insull et al., 1969). Thus, the importance of unsaturated fatty acids as seen in experimental mammary tumor models may not be relevant when considering human breast cancer.

Buell (1973) reported that the incidence of breast cancer increased by five times among Japanese women who immigrated to the United States when compared with breast cancer incidence in native Japanese women. Also Hems (1978) found that changes in breast cancer rates in 20 countries

were positively correlated with similar changes in total fat and animal protein intake. Increases in breast cancer mortality in Japan over the past 20 years have been associated with a marked shift toward a Western lifestyle and an increase in dietary fat in Japan (Reddy et al., 1980).

Therefore, although such epidemiological studies cannot define causative relationships between dietary fat and human breast cancer, the strong correlations between dietary fat consumption and incidence of human breast cancer are relevant to experimental animal mammary tumor studies. Elucidation of the mechanisms by which high fat diets stimulate mammary tumorigenesis in animal models may also provide further understanding of the etiology of human breast cancer.

Effects of Caloric Restriction on Mammary Tumorigenesis

Caloric restriction is another aspect of nutrition that has been shown to have a profound effect on mammary tumorigenesis. In general, restriction of food intake increases the latency period of development and decreases the incidence and growth rates of many types of spontaneous, transplanted and carcinogen-induced cancers in mice and rats (White, 1961). Tannenbaum (1940) reported that diets which were restricted to one-half the normal caloric amounts decreased spontaneous mammary tumor incidence in DBA mice. A prolongation of the latency period required for spontaneous mammary tumors to appear was also observed in food restricted animals (Tannenbaum, 1942). Huseby et al. (1945), reported a decrease in

mammary tumor incidence with a 33% caloric restriction in C_3H mice. Dunning et al. (1949) showed that caloric restriction reduced the development of diethylstilbestrol (DES)-induced mammary tumor development. Conversely, C_3H mice that were made obese by treatment with thioglucose developed spontaneous mammary tumors with a shorter latency period than non-treated controls (Waxler et al., 1953). Clayson (1975), using data from the work of Tannenbaum (1940), has shown that an increase in the incidence of spontaneous mammary tumorigenesis in C_3H and DBA mice is positively correlated with an increase in caloric intake. Also, a correlation between body weight and tumorigenesis in mice with varying degrees of caloric restriction has been established (Tannenbaum & Silverstone, 1953). However, such a correlation could not be established between body weight and incidence of spontaneous mammary tumors in mice fed full rations. Recently, Tucker (1979) reported that the development of spontaneous mammary tumors, as well as other types of tumors, were inhibited in rats and mice treated by restricting their food intake by 20% over 2 years. Also noted in this study was an increase in the latency period of tumor appearance. The 20% caloric restriction also increased the longevity of the restricted animals when compared with the full-fed control animals. Sylvester et al. (1981) showed that restriction of food intake by 50% from 1 week prior to DMBA administration to 30 days following DMBA administration permanently inhibited mammary tumorigenesis even though the

rats were returned to full-fed rations for the remainder of the experiment.

Caloric restriction has also been noted to have an inhibitory effect on the growth of established mammary tumors. Tannenbaum (1942) demonstrated an inhibitory effect of caloric restriction on spontaneous mammary tumors in DBA mice. Tarnowski et al. (1955) showed that the growth of many types of transplantable mammary carcinomas could be inhibited in a dose related manner by caloric restriction. Welsch and Meites (1978) and Leung et al. (1980) reported that caloric restriction inhibited the growth of established DMBA-induced mammary tumors.

Finally, the death rate from breast cancer in humans was demonstrated to be positively correlated with caloric intake (Carroll & Khor, 1975). However, when compared to the correlation seen for high fat diets ($r = +0.935$), the correlation between caloric intake and mortality from human breast cancer is not as strong ($r = +0.737$).

The inhibitory effects of caloric restriction on mammary tumorigenesis appear to be mediated not through the initiation, but the promotional stage of mammary tumorigenesis. Tannenbaum (1942) showed that spontaneous mammary tumorigenesis culd be inhibited when caloric restriction was initiated at 2, 5, or 9 months of age. The diversity of different types of tumors that can be affected by caloric restriction (White, 1961) suggests that a common mechanism is involved. Carroll (1975) suggested simply that the lack

of availability of nutrients required for tumorigenesis to take place accounted for the inhibition of mammary tumorigenesis by caloric restriction. Caloric restriction also suppressed the secretion of most anterior pituitary hormones (Campbell et al., 1977) and induced a state of pseudohypophysectomy (Mulinos et al., 1940). It has therefore been suggested that an endocrine mechanism may be involved in mediation of the effects of caloric restriction on tumorigenesis. Huseby et al. (1945) showed that the normal mammary gland development of C_3H mice was inhibited by caloric restriction. Also noted in this study were changes in the structure and function of the ovaries and uteri analogous to changes associated with hypophysectomy. Such observations led to the inference by Huseby (1945) that the apparent decrease in hormonal stimulation of the mammary gland may be associated with the inhibition of mammary tumorigenesis by decreased food intake.

More direct evidence for an endocrine involvement in the inhibition of mammary tumorigenesis was provided by Sylvester et al. (1981). These investigators showed that supplementation with estrogen or a combination of estrogen, prolactin, and growth hormone, reversed many of the effects of caloric restriction on DMBA-induced mammary tumorigenesis. Also, Welsch and Meites (1978) and Leung et al. (1980) showed that the inhibition of established DMBA-induced mammary tumor growth caused by caloric restriction was associated with reduced serum prolactin and estrogen levels,

and could be blocked by daily injections of haloperidol which elevated serum prolactin levels. Boutwell (1948) noted that adrenal hypersecretion may contribute to the inhibitory effect of caloric restriction on mammary tumorigenesis. Adrenal glucocorticoids inhibit growth of established mammary tumors (Aylsworth et al., 1979; Hilf et al., 1965), yet an inhibitory role for adrenal glucocorticoids on mammary tumor development has not been established.

Effects of Dietary Protein Intake on Mammary Tumorigenesis

The effects of dietary protein on mammary tumorigenesis are not as definitive as the effects of other dietary components such as caloric restriction or dietary fat, and thereby make construction of generalizations difficult. Tannenbaum and Silverstone (1949) reported that spontaneous mammary tumorigenesis was stimulated in mice fed diets containing levels of protein either greater than (45% protein) or less than (9% protein) the control level (18% protein), even though the caloric intake was regulated in each group. Conversely, White and Andervont (1949) found that diets which were severely restricted in protein content (4% casein) and deficient in cysteine prevented formation of spontaneous mammary tumors in C_3H mice. However, such treatment also caused a reduction in body weight gains (similar to caloric restriction), and aberrations in estrous cycles. Engel and Copeland (1952) demonstrated an inverse relationship between dietary protein intake and induced

mammary tumorigenesis observing that high protein levels in the diet were associated with reduced mammary tumor development. Furthermore, Gilbert et al. (1958) reported that rats fed diets containing high levels of casein (77%) had a lower incidence of spontaneously developing mammary fibroadenomas when compared with rats fed diets containing 12-15% protein. Shay et al. (1964) showed that a semi-synthetic 64% casein diet increased mammary tumor development when compared to a commercially prepared diet containing 27% protein. However, the many differences that exist between the semi-synthetic and commercially prepared diets preclude assigning any definitive conclusions concerning the effects of high protein diets on mammary tumorigenesis. More recently, Clinton et al. (1979) showed that reduced protein intake prior to DMBA administration stimulated mammary tumor development, whereas similar treatment after DMBA administration had no effect. Conversely, increased protein intake before DMBA administration reduced mammary tumorigenesis, but had no effect when administered after DMBA treatment. These results suggest that dietary protein can influence the initiation phase, but not the promotional phase of mammary tumorigenesis. Because of the inconsistent evidence attempting to ascertain the effect of dietary protein on mammary tumor development, more extensive investigation is needed.

Evidence from examining the effect of reduced dietary protein intake on the growth of established mammary tumors

is more consistent. White and Belkin (1945) have shown that transplanted adenocarcinomas grow at a reduced rate in severely protein deficient mice when compared to control mice consuming normal amounts of protein. Also the growth of established DMBA-induced mammary tumors was inhibited when rats were fed a protein deficient (6% protein) diet (Welsch & Meites, 1978).

The incidence of breast cancer in women appears to be associated with intake of protein in the diet. Carroll and Khor (1975) found a strong positive correlation between breast cancer mortality and animal protein consumption. However, no correlation was observed with vegetable protein consumption and death due to breast cancer. In an epidemiological study controlled for height, weight, and age of menarche, Gray et al. (1979) also positively correlated breast cancer incidence and consumption of animal protein.

The effects of dietary protein on mammary tumor development may involve an altered metabolism of carcinogens induced by changed in dietary protein intake. Clinton et al. (1979) have shown that aryl-hydrocarbon-hydroxylase (AHH) activity at the time of DMBA administration is elevated when protein intake is increased. AHH is the principle mixed function oxidase involved in the conversion of polycyclic aromatic hydrocarbon-type carcinogens to more hydrophillic and readily excretable compounds. Thus, the attenuated mammary tumorigenesis seen with high protein consumption prior to carcinogen administration may be a result of reduced

carcinogenic response of DMBA due to a more rapid detoxification caused by increased AHH activity. An endocrine mechanism has also been suggested to explain the inhibition of growth of established mammary tumors resulting from consumption of a protein deficient diet. Administration of growth hormone overcame the inhibition of mammary tumor growth in protein deficient rats bearing DMBA-induced mammary tumors (Welsch & Meites, 1978). These investigators suggest that the effect of growth hormone on mammary tumor growth is probably mediated indirectly through conservation of body proteins rather than direct stimulation of mammary tumors.

Effects of Vitamin A and Its Derivatives on Mammary Tumorigenesis

Another nutritional component influencing mammary tumorigenesis that has generated interest in recent years has been Vitamin A and associated retinoid compounds. The principle function of Vitamin A is maintenance of the integrity of epithelial tissues. Additionally, Vitamin A and its derivatives may participate in the stabilization and maintenance of permeability characteristics of cellular and intracellular membranes (Harper, 1979). A deficiency in Vitamin A resulted in the replacement of certain secretory epithelium with a keratinized epithelium similar to that induced by some carcinogens (Harris et al., 1972).

Recent reports suggest that administration of retinoic compounds inhibits murine mammary tumorigenesis. However, earlier work by Brandis and Anton (1966) showed that administration of retinoid compounds enhanced mammary tumorigenesis induced by cytoxen. Also, Schmahl et al. (1972) reported that retinyl palmitate had no effect on DMBA-induced mammary tumorigenesis. More recent investigations by Moon and his co-workers (1976) have shown conclusively that exogenous administration of retinyl derivatives such as retinyl acetate can inhibit mammary tumorigenesis. Daily administration of 2.5 mg of retinyl acetate beginning 7 days after treatment with DMBA decreased both the incidence and the number of developing benign and malignant tumors (Moon et al., 1976). Retinyl acetate also inhibited mammary tumorigenesis induced by N-methyl-N-nitrosucrea (NMU), (Moon et al., 1977). Rettara et al. (1975) reported that growth of transplantable mammary adenocarcinomas in mice could be inhibited by retinyl acetate. Most recently, Welsch et al. (1980) reported that suppression of serum prolactin levels by CB-154 potentiated the inhibition of tumorigenesis induced by retinyl acetate.

Retinoid compounds appear to inhibit mammary tumorigenesis both at the initiation and promotional phases. McCormick et al. (1980) found that short-term treatment with retinyl acetate from 2 weeks before to 1 week after DMBA was nearly as effective in permanently inhibiting mammary tumorigenesis as treatment with retinyl acetate for up to 30 weeks after DMBA. This would suggest that retinyl

acetate can act in the initiation and promotional phases to inhibit mammary tumor development.

The mechanism by which retinyl compounds inhibit mammary tumorigenesis is not clear. Because the major biological function of Vitamin A is to induce proper differentiation of epithelial tissues, it has been suggested that retinoids may act by reversing carcinogen-induced anaplasia. However, Thompson et al. (1979) reported that short-term treatment with retinyl acetate after carcinogen treatment temporarily suppressed mammary tumor development and continuous administration of retinyl acetate was required to inhibit mammary tumorigenesis induced by NMU. These results suggest that retinyl acetate inhibits the progression of mammary tumor development instead of reversing the carcinogen-induced anaplasia.

Hill and Shih (1974) demonstrated that retinoic acid inhibits liver mixed function oxidases that are responsible for the activation of many polycyclic aromatic hydrocarbons such as DMBA, benzo(a)pyrene, and methylcholanthrene, thereby reducing the initiation capacity of these compounds. Since the uptake and binding of many carcinogens such as DMBA and NMU to cellular proteins and DNA are completed within 2 weeks of administration (Janss et al., 1972), such a process would probably not be affected by administration of retinyl acetate after this time (Moon et al., 1976). Therefore, while inhibition of mixed function oxidases could explain the decrease in initiation of mammary tumorigenesis,

it would not explain the inhibition of mammary tumor development by retinoids during the promotional stage. The toxic effects of large doses of retinyl compounds do not appear to totally account for their inhibitory effects on mammary tumorigenesis. No changes in body weight gains or in liver function and structure were associated with suppression of mammary tumorigenesis by retinyl acetate (Moon et al., 1976, 1977). Also, no changes in estrous cycle activity in rats treated with retinyl acetate is necessary for inhibition of mammary tumorigenesis to occur (Moon et al., 1976). Therefore, it would not appear that the effects of retinyl acetate are mediated by any major alteration in endocrine or reproductive function. However, more careful examination of the effects of retinyl compounds on the endocrine system is required to properly assess the role of hormones in the inhibition of mammary tumorigenesis by retinoids. There is evidence that retinoic acid may inhibit mammary tumorigenesis via the immune system by stimulation of thymus-derived killer cell induction (Lotan & Dennert, 1979). Finally, retinoid binding proteins have been identified in NMU-induced mammary tumors, suggesting that retinyl compounds may act directly at the mammary cell to inhibit tumor development and growth (Mehta & Moon, 1979).

Effects of Antioxidants on Mammary Tumorigenesis

Antioxidants such as butylated hydroxanisole (BHA), butylated hydroxytoluene (BHT) and alpha tocopherol

(Vitamin E) are among the most widely used compounds added to commercially prepared foods consumed by both humans and laboratory animals. Because consumption of these antioxidants are frequently encountered in the human diet, these compounds have been of interest, particularly in relation to their possible role in carcinogenesis (Wattenberg, 1978).

Antioxidants inhibit a wide variety of chemically induced neoplasms (Wattenberg, 1978). Wattenberg (1972) reported that DMBA-induced mammary tumorigenesis could be inhibited by addition of either BHA or BHT to the diets consumed by rats. Furthermore, diets supplemented with BHT resulted in a decrease in the incidence of mammary tumors induced by AAF. Harman (1969) reported that alpha-tocopheral suppressed mammary tumorigenesis induced by DBA. However, Wattenberg (1972) reported no such effect of alpha-tocopheral in DMBA-induced mammary tumorigenesis.

Antioxidants are thought to exert their inhibitory effects on mammary tumorigenesis via their effects on the mixed function oxidases involved in the metabolism of carcinogenic compounds. BHT increased the detoxification of AAF by enhancing its conjugation to glucoronic acid and excretion (Granthram et al., 1972), thereby lowering the availability of the carcinogen for activation reactions. BHA altered the metabolism of benzo(a)pyrene by mixed function oxidases such that there was a decrease in the epoxidation and increase in the detoxification reactions. Such actions of BHT and BHA could account for their

inhibitory effect on the induction of mammary tumors by carcinogens.

Selenium is also involved in the endogenous anti-oxidation reactions and affects mammary tumorigenesis. Selenium administered in the drinking water of C_3H mice over a 15-month period decreased the incidence of spontaneous mammary tumors from 83% to 10% (Schrauzer & Ishmed, 1974). Harr et al. (1973) showed that selenium deficient diets stimulated mammary tumor development when fed to AAF-treated rats and that this effect could be reversed by selenium supplementation. Medina and Shepart (1980) reported that selenium inhibited spontaneous mammary tumorigenesis in BALB/cf C_3H (MuMTV-positive) mice without alterations in normal reproductive function or growth processes. Ip and Sinha (1981) reported that selenium deficient, high polyunsaturated fat diets enhanced DMBA-induced mammary tumorigenesis to a greater extent than high polyunsaturated fat diets which contained normal levels of selenium. In humans, increased incidence of breast cancer has been associated with geographic locations in which selenium is known to be lacking (Schrauzer & Ishmed, 1974).

The biological function of selenium important to its role in mammary tumorigenesis is its effect on glutathione peroxidase activity (Griffin, 1979). Selenium itself is not an antioxidant, but rather functions indirectly as an antioxidant through the selenium containing enzyme glutathione peroxidase. Glutathione peroxidase converts

potentially harmful hydrogen peroxide and hydroperoxides generated from lipid metabolism to water. Thus, removal of the peroxides by glutathione peroxidase prevents oxidation and subsequent damage to various cellular components and proteins by the peroxides. Selenium also appears to have an effect on mixed function oxidases which metabolize many types of carcinogens. Marshall et al. (1979) and Ip and Sinha (1981) reported that selenium may impede activation and/or accentuate detoxification of AAF and DMBA. Medina and Shepard (1980) reported that selenium had little or no effect on pre-neoplastic mammary tumor outgrowth lines maintained in BALB/c (MuMTV-negative) mice or on the growth of transplanted primary mammary tumors. These results suggest that selenium may act through either inhibition of the carcinogen-induced transformation of normal mammary tissue or through inhibition of the promotion of previously transformed cells.

MATERIALS AND METHODS

Research Animals

Animals used in studies described here were female Sprague-Dawley rats obtained from Harlan Research Animals, Indianapolis, IN, and Charles River Research Animals, Wilmington, MA. Animals were housed in metal suspension cages and maintained in a temperature controlled (25±°C) room on a 14 hour light (0500-1900 hr)/10 hour dark illumination regimen. All animals were fed ad libitum a diet of tap water and either laboratory rat chow in pelleted form (Ralston Purina Co., St. Louis, MO), or a semi-synthetic variable fat diet as specified.

Tumor Induction

Mammary tumors were induced in animals by the method of Huggins (1965). Virgin female Sprague-Dawley rats, 55-60 days of age, were given a 1 ml lipid emulsion containing 5 mg of 7,12-dimethylbenz(a)anthracene (DMBA) by tail vein injection under light ether anesthesia. The DMBA emulsion was kindly provided by the Upjohn Co., Kalamazoo, MI. Most tumors became palpable 1-3 months after DMBA injection.

Tumor Measurements

Tumors were palpated and measured at weekly intervals, beginning 1 month after administration of DMBA. Palpable mammary tumors were measured with a vernier caliper and the two largest perpendicular diameters were recorded and averaged. Weekly tumor measurements were totaled for each rat, and expressed as "the sum of the average tumor diameter per rat" for each treatment group. In addition, mammary tumor development was assessed by the average latency period of tumor appearance, which was calculated for all tumors in each treatment group. This value represents the period of time (in weeks) between DMBA administration and the initial appearance of tumors as determined by palpation.

In the studies examining the effects of high fat diets on the growth of established mammary tumors, tumors were palpated and measured immediately prior to and at weekly intervals after initiation of treatments. Tumor growth or regression was expressed as the percent change in average tumor diameter for each rat when compared to the initial tumor measurements. Tumors developing after the initiation of treatments were also measured and recorded.

Blood Sampling and Radioimmunoassays

Blood was sampled in the tumor induction and growth experiments by the orbital sinus puncture technique, using light ether anesthesia. All blood samples were taken between 1000 and 1200 hr to minimize variability in serum prolactin levels.

In the studies examining the effects of high fat diets on serum prolactin levels, blood was sampled via an implanted right atrial cannula. This allowed for frequent sampling of blood from unanesthetized, undisturbed rats. Silastic cannulas (0.025 inches I.D.; 0.047 inches O.D.) were inserted into the right external jugular vein, and threaded through the superior vena cava into the right atrium. The free end of the cannula was threaded subcutaneously across the back of the neck and exited 1-2 cm caudal from the base of the skull. After the cannula was secured in place, it was flushed with approximately 0.2 ml of sterile heparinized saline (100 IU/ml) and closed with a knot at the free end of the cannula. Cannulated rats were then injected with 0.2 ml Longicil to prevent infection and placed in individual wire cages.

At least 2 hours prior to blood collection, the free end of the cannula was cut immediately proximal to the closure knot and a silastic extension tube was attached to the cannula and extended outside the cage. Cannulated rats with attached extension tubes could move freely in their cages and were free to consume food and water.

Serum from collected blood was separated by centrifugation and stored at -20°C until assayed for prolactin, using the radioimmunoassay method developed by Niswender et al. (1969).

Dietary Treatments

The composition of the semi-synthetic diets used in the following experiments are shown on Table 1. Diets were prepared 1 to 2 times per week, or as required, with the proportion of ingredients added on a percentage-weight basis. Prepared diets were stored at 4°C until fed. Rats were fed ad libitum with fresh food provided at least every two days. The casein, cellulose, salt mixture and vitamin supplement were obtained from U.S. Biochemical Corp., Cleveland, OH. Sucrose and corn oil were purchased from local sources.

Drug and Endocrine Manipulations

In studies examining the effects of high fat diets on mammary tumor development and growth, serum estrogen and prolactin levels were controlled by using the following combinations of endocrine and drug treatments: bilateral ovariectomy without hormone replacement to reduce circulating levels of estrogen and prolactin; bilateral ovariectomy followed by administration of estradiol benzoate (EB) at a dose of 2 μg/rat/day to replace basal levels of estrogen and serum prolactin; bilateral ovariectomy followed by injections of EB and 2-bromo-α-ergocryptine (CB-154) at a dose of 0.2 mg/kg/day to replace physiological levels of estrogen while maintaining reduced serum prolactin levels; bilateral ovariectomy followed by administration of haloperidol (0.5 mg/kg/day) to elevate serum prolactin levels while

Table 1.--Composition of Variable Fat Diets (% of Total Diet).

Treatment	Casein	Sucrose	Corn Oil	Cel-lulose	Salt Mixture[a]
Control Fat (4.5%)	25	59.5	4.5	7	4
High Fat (20%)	25	42.0	20.0	8	5

[a]Twenty-one grams of total vitamin fortification was added to each kilogram of diet mixture.

maintaining reduced estrogen levels; bilateral ovariectomy followed by injections of haloperidol and EB to elevate serum prolactin levels in the presence of physiological levels of estrogen. Sham operated rats were used as controls in which normal cyclic levels of estrogen and prolactin were present.

The EB and CB-154 were suspended in a solution of 0.85% physiological saline containing 0.3% ethanol. Haloperidol was dissolved in 0.3% tartaric acid. All drugs were administered by subcutaneous injection into the back of the animal. Sham operated and ovariectomized control rats received injections of vehicle alone.

The effects of high fat diets on induced prolactin surges also were examined. In this study, proestrus-like surges were induced by administration of EB and progesterone into long-term ovariectomized rats. Six weeks after bilateral ovariectomy, animals were injected with EB at a dose of 20 µg/rat; 72 hours later, the animals were injected with progesterone at a dose of 5 mg/rat. The resulting surge of prolactin was observed 4-5 hours following progesterone administration.

Tissue Preparation and Prolactin Receptor Assay

Rats bearing multiple DMBA-induced mammary tumors were killed by decapitation at the end of the experiment. All identifiable mammary tumors were immediately excised, wrapped in aluminum foil, and frozen on dry ice. Mammary

tumor tissues were stored at -50°C until assayed for prolactin binding activity. All mammary tumors were homogenized in 0.3 M sucrose for 45 sec. in a Waring Blender with a special microcup attachment. The homogenate was centrifuged at 11,000 rpm for 20 min and the pellet was discarded. The supernatant was centrifuged at 40,000 rpm for 60 min to obtain the particulate membrane pellet. This pellet was resuspended in a Tris buffer (0.025M Tris, pH 7.6 and 10 mM $CaCl_2$). Protein concentrations for each membrane preparation was determined by the method of Lowry et al. (1951). All samples were then diluted to uniformity with the Tris buffer so that 500 µg of membrane protein were present in 100 µl of membrane preparation.

Ovine prolactin was iodinated by the lactoperoxidase-glucose oxidase method of Tower et al. (1977). ^{125}I-labeled prolactin was diluted in Tris buffer to give approximately 70,000 cpm/100 µl. Individual tumor samples were assayed in quadriplicate. Total binding of ovine prolactin was determined in tubes containing 100 µl of ^{125}I-labeled prolactin, 300 µl of membrane preparation containing 1.5 mg protein and 100 µl of Tris buffer. Parallel incubation to determine non-specific ovine prolactin binding were performed using the same reactants, except 100 µl of excess unlabeled ovine prolactin (1 µg/100 µl) replaced the 100 µl of Tris buffer diluent. In all tubes, the total incubation volume was 0.5 ml. All assay tubes were incubated for 2 days at 40°C after which 3 ml Tris buffer were added to

terminate the incubation, and the tubes were centrifuged at 16,000 rpm for 30 min. The resulting pellets were counted for 1 min in a Nuclear-Chicago gamma counter. Specific binding was determined for each sample by subtracting the cpm bound in the presence of excess unlabeled ovine prolactin (i.e., non-specific binding cpm) from the cpm bound in the absence of ovine prolactin (i.e., total binding cpm) and was expressed as a percentage of total radioactive label used in each incubation.

Statistical Analysis

Significant differences in hormone levels between treatment groups were determined by using either students' "t" test or by analysis of variance (ANOVA) and the Student-Newman-Keuls' (S-N-K) statistical tests, when appropriate. Differences in body weight, tumor diameters, tumor number, and latency period of mammary tumor development were tested for significance by ANOVA and S-N-K analyses. A difference of $p<0.05$ was considered to be significant.

EXPERIMENTAL

Estimate of Caloric Intake in Rats Fed High Fat and Control Fat Diets

Objectives

Nutritional effects have been shown to influence murine mammary tumorigenesis, particularly with respect to caloric intake (Tennenbaum, 1959). Since the semi-synthetic diets used in this and subsequent experiments differed in their caloric content, it is conceivable that the stimulation of mammary tumor development by high fat diets may be due to differences in caloric intake between rats fed high and control fat diets. The purpose of this experiment was to estimate the caloric intake of rats fed a 20% high fat diet and a 4.5% control fat diet, and to relate any observed differences in caloric intake to possible involvement in stimulation of mammary tumor development by high fat diets.

Procedure

Fifty female Sprague-Dawley rats approximately 250 grams in body weight were fed ad libitum either a 4.5% control fat or a 20% high fat diet. Over a 4 week treatment period, daily food consumption was measured by subtracting the amount

of food remaining each day from the amount of food given the previous day. The daily caloric intake for control fat fed rats was calculated by multiplying the grams of food consumed by each rat by the caloric content of each diet. The caloric contents for the control fat and the high fat diets were 3.795 Kcal/gram and 4.480 Kcal/gram, respectively. Body weights were measured and recorded at weekly intervals.

Results

Figure 1 shows the food consumption and caloric intake of rats fed control fat and high fat diets during the 4 week treatment period. As shown after 4 days on their respective diets, rats fed the 20% high fat diet consistently consumed less of their diet than similar rats fed the 4.5% control fat diet. After the fourth day of dietary treatment, rats fed the 20% high fat diet consumed an average of between 1 and 5 grams less of their diet per day than rats fed the 4.5% control fat diet. Caloric intake, calculated for rats fed either diet, demonstrated that beginning 4 days after initiation of dietary treatment, there were no significant differences in caloric intake between rats fed the high fat and control fat diets.

Also shown on Figure 1 are the average body weights measured during the 4 week treatment period. No differences in body weight were observed between rats fed the 4.5% control and the 20% high fat diets.

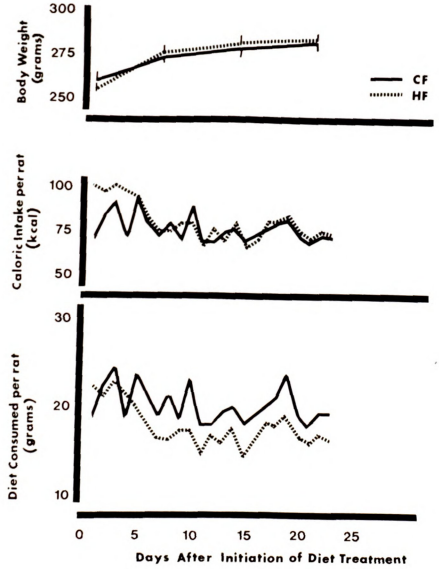

Figure 1. Food Consumption, Estimate of Caloric Intake and Body Weight Gains in Rats Fed 4.5% Control Fat and 20.0% High Fat Diets. Vertical bars represent S.E.M.

Conclusions

These results indicate that rats have the ability to regulate their caloric intake whether they are fed a high fat or a control fat diet. After a 4 day period of adjustment to their dietary regimen, rats fed the high fat diet consumed less of their diet and maintained a daily caloric intake similar to rats fed the control fat diet. The ability of rats to control their caloric intake is further indicated by the observation that over a 4 week dietary treatment period, no significant difference in body weight was observed in rats fed the high fat diet compared to rats fed the control fat diet.

Therefore, the enhanced development of mammary tumors by consumption of a high fat diet cannot be explained on the basis that the high fat diet had a higher caloric content than rat diets containing normal amounts of fat.

Effects of High Fat Diet on Serum Prolactin Levels During the Estrous Cycle in Female Rats

Objectives

Prolactin has been reported to have a major facilitatory role in murine mammary tumorigenesis. Therefore, elevation of serum prolactin levels by high dietary fat may be one mechanism by which high fat diets enhance mammary tumor development. The effects of high fat diets on serum prolactin levels during the estrous cycle have not been thoroughly investigated. Chan et al. (1975) claimed that

consumption of high fat diets elevated serum prolactin levels in rats during the proestrus-estrus stages of the estrous cycle, and suggested that such an effect may account for stimulation of mammary tumor development by high fat diets. However, other investigators have not been able to confirm such an increase in serum prolactin levels by high fat diets (Cave et al., 1979). Furthermore, since the rat is more active and consumes most of its daily food ration at night, any acute or short-term effects of high dietary fat on serum prolactin may not be observed from blood sampled only during the day. Also, by using indwelling right atrial cannulas, blood may be sampled from conscious, undisturbed rats, and this had not been done previously. This would allow for more accurate physiological measurements of serum prolactin levels in blood sampled from rats treated with different dietary fat treatments, and would not be influenced by drug-induced anesthesia or physiological stress. The purpose of this study was to observe, in detail, the effects of high fat diet consumption on serum prolactin levels during the entire estrous cycle of cycling female rats, and to attempt to relate these effects to the stimulation of mammary tumor development by high fat diets.

Procedure

Sixty mature female Sprague-Dawley rats were fed either a 20% high fat or a 4.5% control fat diet for 4 weeks, during which time estrous cycling patterns were monitored by daily collection of vaginal smears. After 4 weeks, 24

normally cycling rats (12 from each group) were implanted with indwelling right atrial cannulas and maintained on their respective dietary regimen. Beginning the day after cannulation, and continuing for the next 4 days serial blood samples were taken at 4 hour intervals during each stage of the estrous cycle. Additional blood samples were taken at hourly intervals between 1600 and 1900 hr during the afternoon of proestrus.

Serum prolactin was measured by radioimmunoassay according to the method of Niswender et al. (1969). Differences in serum prolactin levels between dietary treatment groups at each time sampled during the estrous cycle were tested for statistical significance by students' "t" test. Differences were considered to be statistically significant if $p<0.05$.

Results

The effects of dietary fat on serum prolactin levels are shown in Figure 2. Both control fat and high fat diet rats showed normal serum prolactin levels throughout the estrous cycle and a typical surge of prolectin during the afternoon of proestrus. Basal serum prolactin levels for both control fat and high fat fed rats ranged from 20 to 60 ng/ml throughout the cycle. Prolactin levels during the afternoon of proestrus were elevated to between 300 and 400 ng/ml in both treatment groups. Additionally, a smaller surge of prolactin was observed during the afternoon of

PRL
(ng/m

Fig

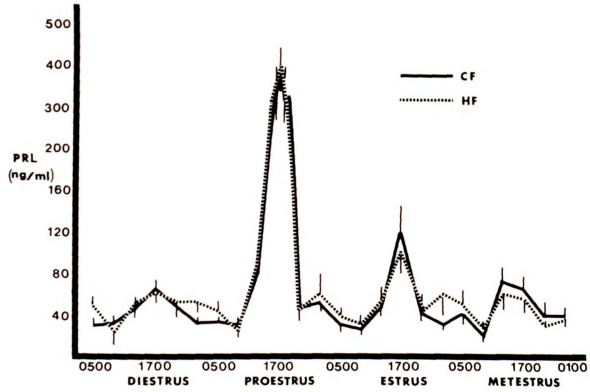

Figure 2. Effects of High Fat Diet on Serum Prolactin Levels During the Estrous Cycle of Female Sprague-Dawley Rats. CF represents 4.5% control fat diet. HF represents 20.0% high fat diet. Vertical bars represent S.E.M. n=10

estrus. No significant difference in serum prolactin at any time during any stage of the estrous cycle was detected between rats fed either the 4.5% control fat or the 20% high fat diet.

Also, the estrous cycling behavior was not altered by consumption of the high fat diet during the 4 week treatment period. A total of 20 out of 28 rats fed the 4.5% control fat diet and 19 out of 29 rats fed the 20% high fat diet showed consistent 4-5 day estrous cycles, as indicated by daily vaginal smears. Additionally, in both dietary treatment groups, 10 out of 12 rats implanted with indwelling cannulas maintained their normal cycling pattern during the following 4 days of blood sampling.

Conclusions

These results show that consumption of a high fat diet does not significantly influence serum prolactin at any time during the estrous cycle. In contrast to results reported by Chan et al. (1975), no elevation of serum prolactin levels during the afternoons of estrus or proestrus were observed in rats fed high fat diets when compared to rats fed control fat diets. Chan et al. (1975) claimed that high fat diets stimulate serum prolactin levels during the afternoons of proestrus and estrus. However, the data reported by these investigators combined serum prolactin values during proestrus and estrus, and showed a large amount of variability. The prolactin values obtained in the present experiment are believed to be more precise and to

represent a more meaningful assessment of the effects of dietary fat on serum prolactin levels. Therefore, stimulation of mammary tumor development by a high fat diet does not appear to be mediated through elevation of serum prolactin levels.

Effects of High Fat Diet on the Surge of Prolactin Induced by Estrogen and Progesterone in Ovariectomized Female Rats

Objectives

To clarify further the effects of high fat diets on serum prolactin levels, the effects of dietary fat on induced surges of prolactin were investigated. It has been shown that surges in prolactin and gonadotropins resembling those occurring just prior to ovulation, can be induced in long-term ovariectomized rats treated with estrogen and progesterone (Freeman et al., 1976; Huang et al., 1980). The purpose of this experiment was to compare the surges of prolactin induced by estrogen and progesterone in ovariectomized rats fed a 4.5% control fat and a 20% high fat diet.

Procedure

Twenty female Sprague-Dawley rats, 200-225 grams in body weight were bilaterally ovariectomized. Two weeks after ovariectomy, rats were placed on either a 4.5% control fat or a 20% high fat diet. Six weeks after ovariectomy, or 4 weeks after initiation of the dietary treatment, all rats were implanted with indwelling right atrial cannulas

and injected with 20 µg of estradiol benzoate (EB); 72 hours later, all rats were injected with progesterone at a dose of 5 mg per rat. Blood was sampled via the indwelling cannula immediately prior to (1100 hrs) and following progesterone administration, at 1400, 1600, 1700, 1800, 1900, and 2200 hrs.

Serum prolactin was measured by radioimmunoassay according to the method of Niswender et al. (1969). Statistical significance was tested between the two dietary treatment groups using the students' "t" test and a level of significance of $p<0.05$.

Results

The effects of dietary fat on serum prolactin levels in EB-progesterone treated ovariectomized rats are summarized on Figure 3. As shown, a surge in prolactin was induced. Prior to progesterone treatment, basal serum prolectin levels in both control fat and high fat fed rats were approximately 30 ng/ml. Five hours after injection of progesterone, serum prolactin levels were observed to peak at nearly 400 ng/ml in both the control fat and high fat treatment groups. Subsequently, serum/prolactin levels declined to nearly 100 ng/ml at 2200 hrs, 11 hours after progesterone treatment. At no time were serum prolactin levels significantly different between rats fed either the 20% high fat or the 4.5% control fat diet.

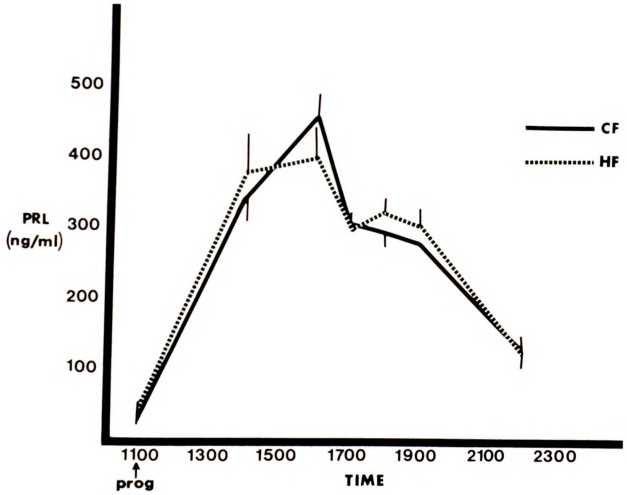

Figure 3. Effects of High Fat Diet on the Surge of Prolactin Induced by Estrogen and Progesterone in Ovariectomized Female Rats. CF represents 4.5% control fat diet. HF represents 20.0% high fat diet. Vertical bars represent S.E.M. PROG: indicates progesterone injection (5 mg per rat).

Conclusions

These results indicate that ovariectomized rats fed diets containing either control or high levels of fat show similar prolactin surges induced by EB and progesterone injections. They support previous observations that high fat diets do not stimulate serum prolactin during the pre-ovulatory surge on the afternoon of proestrus. Thus, they strengthen the conclusion that the stimulatory effects of high fat diets on mammary tumor development are not mediated via elevation of serum prolactin levels.

Role of Prolactin and Estrogen in Stimulation by High Fat Diet of Development of DMBA-Induced Mammary Tumors

Objectives

The stimulatory effects of high fat diets on mammary tumor development in rats are well established (Carroll, 1975; Aylsworth, 1979). However, the mechanism(s) involved are not clear. Some investigators believe that enhancement of the pre-ovulatory surge of prolactin on the afternoon of proestrus is the mechanism through which such stimulatory effects of high dietary fat are mediate (Chan et al., 1975). However, consideration of the observations previously presented in this thesis, as well as other published reports (Cave et al., 1978), makes it apparent that high fat diets do not influence serum prolactin levels significantly at any stage of the estrous cycle in the rat. Therefore, the

stimulatory effects of high fat diets on mammary tumor development are mediated by other mechanisms.

Chan and Cohen (1975) also suggested that prolactin may exercise a greater influence on high fat stimulation of mammary tumor development than estrogen, since administration of the anti-prolactin drug, (CB-154), could abolish the stimulatory effects of high fat diets on mammary tumorigenesis, whereas administration of an anti-estrogen drug, Nafoxidine, could not do so.

In the present study, the influence of estrogen and prolactin upon the stimulatory action of high dietary fat during mammary tumor development was examined. The objective of this study was to determine whether high fat diets could effectively stimulate mammary tumorigenesis in rats whose levels of estrogen and prolactin were maintained at controlled levels. Attempts were made to determine the optimal requirements for estrogen and prolactin to permit high fat diets to exert their stimulating effects on mammary tumorigenesis, and to evaluate the relative importance of estrogen as against prolactin in this process.

Procedure

Two hundred forty female Sprague-Dawley rats, 55 days of age, were injected with DMBA as described previously (see p. 67). Five days after DMBA administration, animals were either bilaterally or sham ovariectomized. Beginning 10 days after DMBA administration, and continuing for the

duration of the experiment, rats were fed either a 20% high fat, or a 4.5% control fat, semi-synthetic diet. Ten days after DMBA administration, reduced levels of estrogen and/or prolactin were selectively replaced in ovariectomized rats, as follows: reduced levels of both estrogen and prolactin were maintained in ovariectomized rats; physiological levels of estrogen were injected while reduced serum prolactin levels were maintained, by administering both estradiol benzoate (EB, 2 µg/rat/day) and bromoergocryptine (CB-154, 0.2 mg/kg/day); physiological levels of estrogen and basal levels of prolactin were maintained in ovariectomized rats, by injection of EB (2 µg/rat/day); serum prolactin levels were elevated and serum estrogen levels were reduced by injection of haloperidol (0.5 mg/kg/day) to ovariectomized rats; serum prolactin levels were elevated and physiological levels of estrogen were maintained by administration of haloperidol and EB. Sham operated rats were used as controls, in which normal cyclic levels of estrogen and prolactin were present.

Developing mammary tumors in each rat were palpated and measured at 7 to 10 day intervals, beginning 4 weeks after DMBA administration. Also measured were average tumor number, percent tumor induction, and average latency period for tumor development. Blood was collected 3, 9, and 16 weeks after DMBA injection, and was radioimmunoassayed for prolactin to insure that the effects of the administered drugs on serum prolactin levels were similar in rats fed high and low fat diets.

Results

The effects of high fat diets on DMBA-induced mammary tumor development, as influenced by normal and reduced circulating levels of estrogen and prolactin, are presented on Figure 4 and Table 2. It was observed that mammary tumor development in rats on a high fat diet was stimulated when compared to similarly treated rats fed a control fat diet. Beginning 7 weeks after DMBA administration and continuing throughout the experiment, sham-operated rats fed the high fat diet showed a significantly greater accumulation of mammary tumor mass than sham-operated control fat diet rats. Sham-operated rats fed high fat diets also showed an increase in average tumor number and percent tumor induction, and a decreased average latency period when compared to sham-operated rats fed a control fat diet (Table 2).

Reduction of both serum prolactin and estrogen levels by ovariectomy resulted in near complete suppression of mammary tumor development in both control fat and high fat diet rats (Figure 4). Average tumor number and percent tumor induction were similarly reduced in ovariectomized rats fed either a high fat or a control fat diet.

Table 3 shows the effects of sham and bilateral ovariectomy on serum prolactin levels. Serum prolactin levels were reduced in ovariectomized rats fed either a control or a high fat diet. Moreover, at no time did serum prolectin levels differ significantly between rats fed either a control or a high fat diet. Therefore, in rats with normal

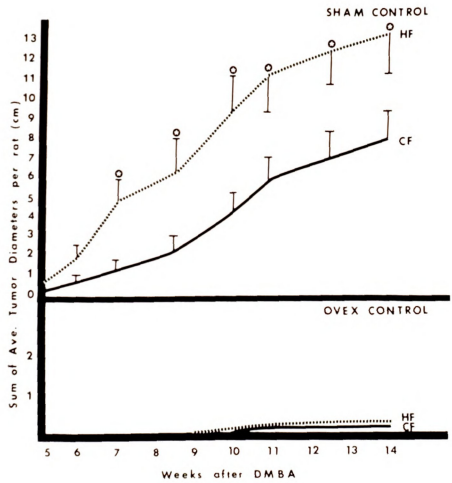

Figure 4. Effect of High Fat Diet on Mammary Tumor Development: Influence of Normal or Reduced Circulating Levels of Prolactin and Estrogen.

A. Animals were sham ovariectomized (SHAM) 5 days after DMBA administration.
B. Animals were bilaterally ovariectomized (OVEX) 5 days after DMBA administration.

CF represents 4.5% control fat diet; HF represents 20.0% high fat diet.

✪ indicates $p<0.05$ vs similarly treated rats fed control fat diets.

Table 2.--Effects of High Fat Diet on Mammary Tumor Development as Influenced by Altered Endocrine States.

Endocrine Treatment	Dietary Treatment	No. of Rats	Average Tumor No. Per Rat	% Tumor Induction	Total No. of Tumors	Mean Tumor Latency Period (wk)
Sham-Control	CF	17	5.35 ± 0.87[a]	88.2%	92	9.79 ± 0.27[a]
	HF	18	9.33 ± 1.26[b]	100.0%	173	8.85 ± 0.19[b]
OVEX-Control	CF	15	0.06 ± 0.06	6.7%	1	10.00 ± 0.00
	HF	17	0.18 ± 0.13	11.2%	3	12.17 ± 1.17[c]
OVEX + EB	CF	17	5.38 ± 1.00	70.6%	91	13.01 ± 0.25
	HF	14	5.57 ± 1.11	92.9%	76	12.13 ± 0.39[b]
OVEX+EB+CB154	CF	19	1.79 ± 0.42	68.4%	35	15.91 ± 0.54
	HF	15	4.00 ± 1.00[b]	66.7%	60	16.00 ± 0.47
OVEX + HALO	CF	19	0.32 ± 0.17	21.0%	7	10.29 ± 2.34
	HF	17	1.00 ± 0.58	52.9%	18	17.05 ± 1.02[c]
OVEX+EB+HALO	CF	16	3.81 ± 0.90	75.0%	64	14.81 ± 0.44
	HF	18	6.19 ± 0.76[b]	93.8%	98	12.41 ± 0.41[b]

[a] Mean ± S.E.M.

[b] $p<0.05$, when compared with similarly treated rats fed a control fat diet.

[c] Statistical comparison inappropriate due to small number of tumors.

Table 3.--Effects of Dietary and Endocrine Treatments on Serum Prolactin Levels.

Endocrine Treatment	Dietary Treatment	Serum Prolactin (ng/ml)		
		3[a]	9	16
Sham Control	CF	51.1 ± 7.6[b]	45.6 ± 10.4	39.7 ± 8.4
	HF	69.6 ± 11.1	50.9 ± 18.1	33.7 ± 7.3
OVEX Control	CF	22.9 ± 2.3	18.6 ± 3.8	20.8 ± 3.8
	HF	19.1 ± 3.0	22.1 ± 3.0	20.7 ± 5.5
OVEX + EB	CF	39.4 ± 3.9	34.5 ± 8.1	35.6 ± 6.2
	HF	38.4 ± 5.81	26.3 ± 3.8	44.9 ± 10.1
OVEX+EB+CB154	CH	8.4 ± 0.9	14.3 ± 4.3	6.2 ± 1.4
	HF	10.0 ± 0.6	9.9 ± 1.3	12.4 ± 6.1
OVEX + HALO	CF	283.3 ± 8.8	237.5 ± 11.4	236.8 ± 9.1
	HF	292.7 ± 9.1	223.1 ± 13.4	253.4 ± 16.5
OVEX+EB+HALO	CF	326.3 ± 13.9	438.5 ± 26.5	433.1 ± 42.9
	HF	356.5 ± 22.6	470.1 ± 21.8	448.3 ± 29.6

[a]Weeks after DMBA treatment.

[b]Mean ± S.E.M.

circulating levels of estrogen or prolactin, high fat diets stimulated mammary tumorigenesis, whereas reduction of circulating levels of both estrogen and prolactin by ovariectomy suppressed mammary tumorigenesis equally in rats fed low or high fat diets.

The effects of replacement with estrogen after ovariectomy on mammary tumor development in rats fed control or high fat diets are shown on Figure 5 and Table 2. Replacement of estrogen by daily injections of EB in ovariectomized rats resulted in mammary tumor development approximately equal to that of sham operated rats fed a control fat diet, with respect to accumulation of tumor mass (Figure 5), average tumor number, and percent tumor induction (Table 2). However, such treatment appeared to delay the appearance of palpable tumors as indicated by an increased average latency period in ovariectomized EB-treated rats as compared to sham operated control rats. Moreover, no significant difference in mammary tumor development was observed in ovariectomized EB-treated rats fed either a high fat or a control fat diet, as indicated by accumulation of tumor mass (Figure 5) or average tumor number (Table 2). However, the average latency period was reduced, and percent tumor induction was increased in high fat, ovariectomized, EB-treated rats when compared to similarly treated rats fed a control fat diet (92.9% vs 70.6%). Examination of Table 3 reveals that serum prolactin levels in ovariectomized rats treated with 2 μg of EB per day were approximately the same as sham operated

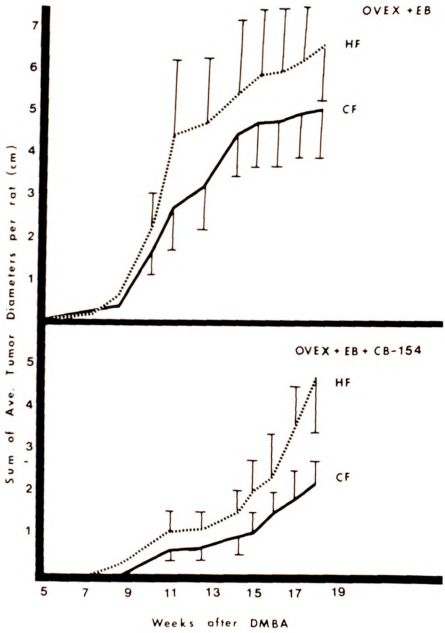

Figure 5. Effects of High Fat Diet on Mammary Tumor Development: Influence of Replacement of Circulating Levels of Estrogen and Prolactin or Only Estrogen.

A. Replacement of serum estrogen and prolactin levels in ovariectomized (OVEX) rats by daily administration of estradiol benzoate (EB).
B. Replacement of serum estrogen levels in ovariectomized (OVEX) rats while maintaining reduced prolactin levels by daily administration of estradiol benzoate (EB) and bromoergocryptine (CB-154).

CF represents 4.5% control fat diet; HF represents 20.0% high fat diet.

control rats, 90 minutes following injection of EB. No significant differences in serum prolactin levels were observed in rats fed high or control fat diets.

The effects of replacement of physiological levels of estrogen and reduced levels of prolactin on mammary tumor development in rats fed control and high fat diets are shown on Figure 5 and Table 2. Treatment of ovariectomzed rats with EB and CB-154, to replace physiological levels of estrogen while maintaining reduced serum prolactin levels, resulted in suppression of mammary tumor development when compared to sham-operated control fat diet rats. No significant differences in mammary tumorigenesis were observed in ovariectomized rats treated with EB and CB-154 and fed either control or high fat diets, as indicated by accumulation of tumor mass (Figure 5), percent tumor induction, or average latency period (Table 2). However, an increase in average tumor number was observed in ovariectomized EB and CB-154 treated rats fed a high fat diet, as compared to similarly treated rats fed a control fat diet.

Table 3 shows that serum prolactin levels were reduced in ovariectomized EB and CB-154 treated rats when compared with sham-operated control rats. Also, no significant differences in serum prolactin levels were observed in control and high fat diet fed ovariectomized rats treated with EB and CB-154.

Effects of elevated serum prolactin levels, without replacement of physiological levels of estrogen, on mammary

tumor development in ovariectomized rats fed high and control fat diets are shown on Figure 6 and Table 2. Elevation of serum prolactin levels through daily administration of haloperidol, in the absence of normal circulating levels of estrogen, resulted in markedly suppressed mammary tumor development in both control and high fat fed rats. Accumulation of tumor mass, average tumor number and percent tumor induction were reduced in ovariectomized-haloperidol treated rats when compared to sham-operated control rats. No differences in mammary tumor development were observed in ovariectomized rats treated with haloperidol and fed either a control or high fat diet.

Serum prolactin levels were substantially elevated in ovariectomized rats treated with haloperidol when compared to sham-operated control rats (Table 3). No difference in serum prolactin levels was observed in ovariectomized-haloperidol treated rats fed either a high or a control fat diet.

The effects of elevated serum prolactin levels and replacement with physiological levels of estrogen on mammary tumor development in ovariectomized rats fed control and high fat diets are shown on Figure 6 and Table 2. At 11 weeks following DMBA administration, and throughout the experiment, ovariectomized rats fed the high fat diet and treated with haloperidol and EB accumulated a significantly greater tumor mass when compared to similarly treated rats fed the control fat diet (Figure 6). Upon termination of

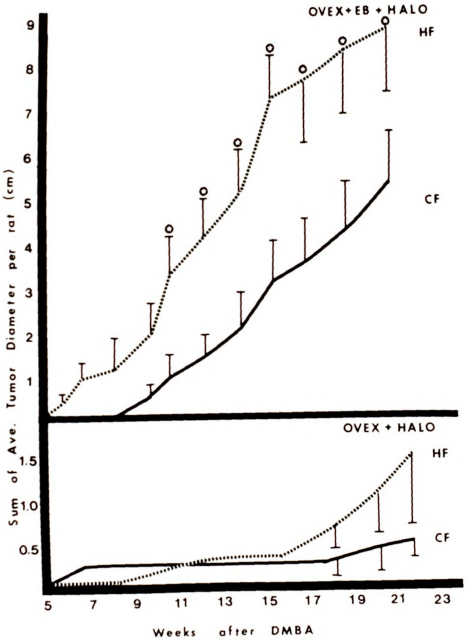

Figure 6. Effects of High Fat Diet on Mammary Tumor Development: Influence of Elevated Prolactin With or Without Estrogen Replacement.

A. Circulating prolactin levels were elevated in ovariectomized (OVEX) rats by daily administration of haloperidol (HALO).
B. Circulating levels of estrogen were replaced and prolactin levels were elevated in ovariectomized (OVEX) rats by daily administration of estradiol benzoate (EB) and haloperidol (HALO).

CF represents 4.5% control fat fiet; HF represents 20.0% high fat diet.

✪ indicates $p<0.05$ vs similarly treated rats fed control fat diets.

the experiment, high fat diet ovariectomized rats treated with haloperidol and EB showed an increase in average tumor number, and percent tumor induction, but a shorter latency period for tumor palpation when compared to similarly treated rats fed a control fat diet (Table 2).

Serum prolactin levels were elevated in ovariectomized rats treated with EB and haloperidol (Table 3). No significant differences in serum prolactin levels were observed in ovariectomized EB and haloperidol treated rats fed either a control or high fat diet.

Conclusions

These results show that high fat diets can stimulate the development of DMBA-induced mammary tumors in the presence of controlled blood concentrations of prolactin and estrogen. Although serum estrogen and prolactin levels were controlled in ovariectomized rats by daily injections of haloperidol and estrogen, high fat diets stimulated all aspects of mammary tumor development when compared to similarly treated rats fed a control fat diet. High fat diets also enhanced some aspects of mammary tumor development in ovariectomized rats with varying amounts of hormone replacement. Therefore, it appears that high fat diets can stimulate mammary tumor development by a mechanism independent of alterations of estrogen or prolactin secretion. These data suggest that high fat diets stimulate mammary tumor development by another mechanism, possibly by an increase

in the biological responsiveness of the incipient mammary tumor tissue to circulating levels of estrogen and prolactin. Even though mammary tumors in both high fat and control fat diet rats were exposed to similar circulating hormone levels, development of tumor tissue in rats fed high fat diets was enhanced.

These results demonstrate that stimulation of mammary tumor tissue by high fat diets required near normal circulating estrogen and prolactin levels. Only when mammary tumor development was maximally stimulated, as observed in sham-operated controls and in ovariectomized rats injected with haloperidol and EB, were all parameters of mammary tumor development significantly increased by high fat diets. This indicates that the presence of adequate circulating levels of both estrogen and prolactin are required for enhancement of mammary tumor development by high fat diets. This contradicts the report by Chan and Cohen (1975) that stimulation of mammary tumorigenesis by high fat diets occurred despite inhibition of the peripheral action of estrogen by the antiestrogen, nafoxidine. These results also contradict their view that the stimulating effects of high fat diets on mammary tumor development are mediated via an increase in prolactin secretion.

Relation of Length of Treatment With High Fat Diet and Carcinogen Administration to Mammary Tumor Development

Objectives

High fat diets appear to stimulate mammary tumor development during the promotional stage of tumorigenesis since their effects are observed only when administered after, but not before carcinogen treatment (Carroll & Khor, 1970; Hopkins et al., 1976). Other promotional characteristics of high fat diets on mammary tumorigenesis, however, have not been described. One of the properties of promoting agents is their dose-response influence on tumorigenesis. An increase in dose or duration of treatment with a promoting agent enhances the response of potential tumor tissue. The effects of promoting agents are largely reversible, since removal of promoting influences decrease their stimulatory effects. A delay in administration of a promoting agent after carcinogen induction does not decrease tumor development. Therefore, administrations of a similar dose of a promoting agent at different times during the developmental phase of tumorigenesis does not alter tumor responsiveness.

The objective of this study was to assess some of the properties of dietary fat treatment as a promoting agent on mammary tumor development. This investigation attempted to determine whether high fat dietary treatments of equal time duration influenced mammary tumor development similarly when administered at different periods of carcinogen administration. This study also determined whether high fat diets

could stimulate mammary tumor development in a dose-response fashion, where dose represents time duration of high fat diet treatment. In addition, the influence of consumption of a semi-synthetic diet on mammary tumor development was observed in rats fed either a semi-synthetic or a commercially prepared diet containing equalivalent amounts of fat.

Procedure

Female Sprague-Dawley rats, 55 days of age, were injected with DMBA as previously described (Huggins, 1965). Two days following DMBA administration, rats were placed on one of the following dietary regimens: a 4.5% control fat diet for the duration of the experiment (0-16 weeks); a 20% high fat diet for the duration of the experiment (0-16 weeks); a 20% high fat diet for one week after DMBA administration, followed by a 4.5% control fat diet (0-1 week); a 20% high fat diet for 3 weeks after DMBA administration, followed by a 4.5% control fat diet (0-3 weeks); a 20% high fat diet for 6 weeks after DMBA administration, followed by a 4.5% control fat diet (0-6 weeks); a 20% high fat diet for 3 weeks beginning 2 weeks following DMBA administration, preceeded by and followed by a 4.5% control fat diet (2-5 weeks); a 20% high fat diet for 3 weeks beginning 4 weeks after DMBA administration, preceeded by and followed by a 4.5% control fat diet (4-7 weeks). An additional control group was fed a commercially prepared diet in pelleted form (laboratory rat chow) containing approximately 4.5% fat for the entire

experiment. Developing mammary tumors were palpated and measured at weekly intervals beginning 4 weeks after DMBA administration. Mammary tumor development was assessed in each treatment group by examination of accumulation of tumor mass, expressed as the sum of the average tumor diameter per rat, the average tumor number, the percent tumor induction, and the average latency period for tumor development.

Results

The results of treatment with a semi-synthetic 4.5% control fat diet, a 20% high fat diet, and a commercially prepared pelleted diet containing approximately 4.5% fat, are shown on Figure 7 and Table 4. The high fat diet stimulated most aspects of mammary tumor development, confirming previously described results. No significant differences in mammary tumor development were observed between rats fed a 4.5% control fat diet or a commercially prepared diet containing approximately the same amount of fat. Accumulation of mammary tumor mass was not significantly different during the 16 week treatment in rats fed the control fat diet or the laboratory rat chow (Figure 7). Furthermore, no significant differences in average tumor number or mean tumor latency period were observed (Table 4).

The effects of different durations of treatment with a high fat diet on DMBA-induced mammary tumor development are shown on Figure 8 and Table 5. Treatment with the high fat diet for one week after DMBA treatment had no effect on

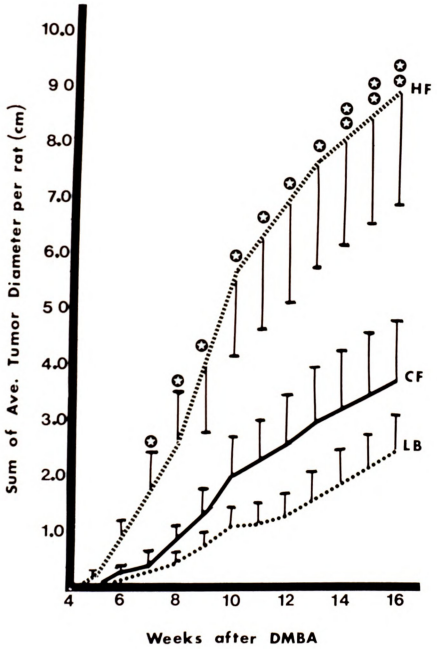

Figure 7. Effects of High Fat and Control Fat Semi-synthetic Diets on Mammary Tumor Development. CF represents 4.5% control fat semi-synthetic diet; HF represents 20.0% high fat semi-synthetic diet; LB represents commerically prepared laboratory rat chow in pelleted form. ✪indicates $p<0.05$ vs similarly treated rats fed control fat diets.

Table 4.--Effects of Semi-Synthetic Control Fat and High Fat Diet on Mammary Tumor Development.

Dietary Treatment	No. of Rats	Average Tumor No. Per Rat	% Tumor Induction	Total No. of Tumors	Mean Tumor Latency Period (wks)
LB (0 - 16)	24	2.00 ± 0.48[a]	62.5%	48	11.69 ± 0.53[a]
CF (0 - 16)	24	3.04 ± 0.45	75.0%	73	10.88 ± 0.28
HF (0 - 16)	23	6.52 ± 1.02[b]	78.3%	150	8.64 ± 0.40[b]

[a]Mean ± S.E.M.

[b]$p<0.05$ when compared to CF (0 - 16).

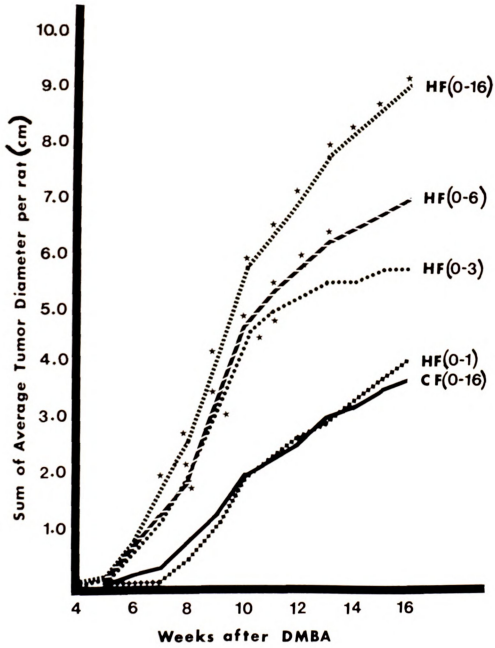

Figure 8. Effects of Duration of High Fat Diet Treatment on Mammary Tumor Development. HF represents 20.0% high diet; CF represents 4.5% control fat diet; ( ) denote period of dietary treatment expressed in weeks. ★ indicates $p<0.05$ vs similarly treated rats fed control fat diet.

Table 5.--Effects of Duration of High Fat Diet Treatment on Mammary Tumor Development: Evidence for a Time Dose-Response Relationship.

Dietary Treatment	No. of Rats	Average Tumor No. Per Rat	% Tumor Induction	Total No. of Tumors	Mean Tumor Latency Period (wks)
CF (0 - 16)[c]	24	3.04 ± 0.45[a]	75.0%	73	10.88 ± 0.28[a]
HF (0 - 1)	24	2.88 ± 0.40	75.0%	69	11.06 ± 0.35
HF (0 - 3)	22	4.23 ± 0.35[b]	81.8%	93	9.10 ± 0.57[b]
HF (0 - 6)	25	4.60 ± 0.51[b]	76.0%	115	9.52 ± 0.41[b]
HF (0 - 16)	23	6.52 ± 1.02[b]	78.3%	150	8.64 ± 0.40[b]

[a]Mean 1 S.E.M.

[b]$p<0.05$ when compared to CF (0 - 16).

[c]Numbers in parentheses denotes period of dietary treatment.

mammary tumorigenesis. No differences in accumulation of tumor mass (Figure 8), percent tumor induction, average tumor number, or average tumor latency period were observed between rats fed the high fat diet for one week after DMBA administration and rats fed the control fat diet for the entire treatment period. Examination of mammary tumor development in rats fed a high fat diet for a longer period of time suggests that a dose-response relationship exists for the stimulation of mammary tumorigenesis by high fat diets. With increasing duration of treatment with high dietary fat, from 0, 3, 6, to 16 weeks, enhanced mammary tumor development was observed as indicated by accumulation of tumor mass (Figure 8), average tumor number and average tumor latency period (Table 5). No differences in percent mammary tumor incidence were observed in rats fed a high fat diet for different lengths of time.

Data presented in Figure 8 also suggest that withdrawal of the high fat diet and subsequent control low fat diet treatment, resulted in a partial reversal of the stimulatory effects on mammary tumor development by the high fat diet. Mammary tumor development between 4 and 10 weeks following DMBA administration progressed at nearly identical rates in groups of rats treated with a high fat diet for the entire experiment (0-16 weeks), for only 6 weeks after DMBA administration (0-6 weeks), or for only 3 weeks after DMBA (0-3 weeks). When the high fat dietary treatment was withdrawn 3 or 6 weeks after DMBA administration, subsequent mammary

tumor development was diminished when compared to rats maintained on the high fat diet for a longer period. These results suggest that the early stimulatory effect of a high fat diet on mammary tumor development ceased after the removal of the high fat dietary treatment.

The effects of a high fat diet fed to rats for equal time durations but at different periods during early mammary tumorigenesis are shown in Figure 9 and Table 6. These data show that a delay in administration of high fat dietary treatment does not significantly alter development of mammary tumors. In general, rats fed the high fat diet from 0 to 3 weeks, from 2 to 5 weeks, or from 4 to 7 weeks after DMBA, showed similar accumulation of mammary tumor mass (Figure 9) and average tumor number (Table 6). However, mammary tumors developed more rapidly in rats fed the high fat diet from 0 to 3 weeks after DMBA treatment, as indicated by a significantly reduced mean tumor latency period observed in these rats as compared with rats fed from 2 to 5 weeks and from 4 to 7 weeks after DMBA treatment.

No significant differences in body weight were observed in any treatment group throughout the experiment.

Conclusions

These data suggest that a high fat diet acts as a promotor in the classical sense, as defined by Berenblum (1954), to enhance mammary tumorigenesis. A dose-response relationship is suggested in which increasing duration of

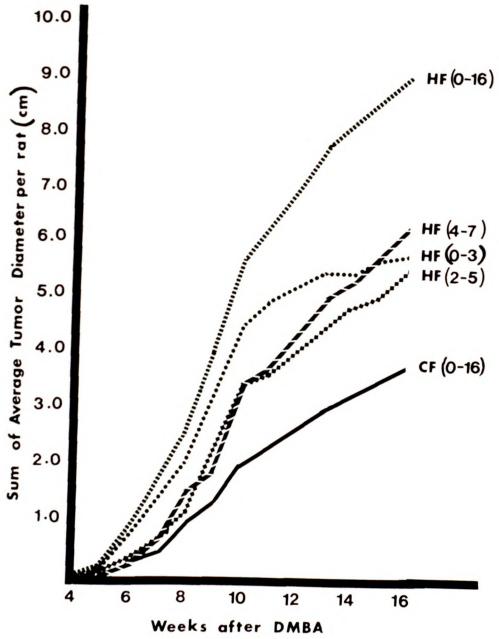

Figure 9. Effects of High Fat Dietary Treatments of Equal Duration Administered at Different Times During Early Mammary Tumorigenesis. HF represents 20.0% high fat diet; CF represents 4.5% control fat diet. ( ) denote period of dietary treatment.

Table 6.--Effects of High Fat Dietary Treatments of Equal Duration on Mammary Tumor Development When Applied at Different Times During Early Mammary Tumorigenesis.

Dietary Treatment	No. of Rats	Average Tumor No. Per Rat	% Tumor Induction	Total No. of Tumors	Mean Tumor Latency Period (wks)
CF (0 - 16)[c]	24	3.04 ± 0.45	75.0%	73	10.88 ± 0.28
HF (0 - 3)	22	4.23 ± 0.35[b]	81.8%	93	9.10 ± 0.57[b]
HF (2 - 5)	25	4.16 ± 0.21[b]	60.0%	104	10.56 ± 0.32
HF (4 - 7)	24	5.04 ± 0.81[b]	95.8%	121	10.90 ± 0.25
HF (0 - 16)	23	6.52 ± 1.02[b]	78.3%	150	8.64 ± 0.40[b]

[a]Mean ± S.E.M.

[b]$p<0.05$ when compared to CF (0 - 16).

[c]Numbers in parentheses denotes period of dietary treatment.

high fat dietary treatment (i.e., dose) resulted in increased mammary tumor development (response).

Administration of a high fat dietary treatment for equal durations of time, but at different periods during early tumorigenesis, did not significantly alter the subsequent development of mammary tumors. Thus, the actions of a high fat dietary treatment, like classical promotors, are not dependent upon some critical period during mammary tumor development, but are equally effective in promoting mammary tumor development regardless of when they are administered. These results support findings by Ip (1980) that when the administration of high fat dietary treatment was delayed for up to 20 weeks after DMBA administration, high fat diets were still capable of enhancing mammary tumor development. These observations conflict with those of Carroll and Khor (1975) who reported that a delay in the initiation of dietary treatment until 4 weeks after DMBA administration resulted in little or no stimulation of mammary tumor development.

The effects of high fat diets on mammary tumor development appear to be largely reversible, since cessation of high fat diet treatment resulted in less mammary tumor development when compared with rats continually maintained on the high fat diet. Therefore, these dietary effects are not permanently induced modifications, but rather are dependent upon continuous high fat administration.

This study also demonstrates that the consumption of a semi-synthetic diet does not alter mammary tumor development, since no significant differences in mammary tumorigenesis were observed between rats fed a commercially prepared laboratory chow and a semi-synthetic diet with similar composition. These results appear to contradict the report by Chan and Dao (1980) that commercially prepared "non-purified" diets reduced mammary tumor development when compared to similar semi-synthetic diets.

Effects of High Fat Diet on Mammary Tumor Growth in Rats With Established DMBA-Induced Mammary Tumors

Objectives

Much of the research concerned with the effects of high fat dietary treatment on mammary tumor development has been concerned primarily with the initial developmental stages of mammary tumorigenesis. These stimulatory effects of high fat diets on development of carcinogen-induced mammary tumors are well documented and are further confirmed by the data presented in this thesis. Wicha et al. (1978) noted that polyunsaturated fatty acids can stimulate the growth of normal mammary gland cells and mammary tumor cells *in vitro*, but this observation has not been extended to include *in vivo* conditions. The purpose of this study was to determine whether the stimulatory effects of a high fat dietary treatment shown during the developmental stages of mammary

tumorigenesis could also be observed in its established stages of growth.

Procedure

Virgin female Sprague-Dawley rats, 55 days of age, were injected with DMBA, as described previously (Huggins, 1965). Approximately 12 weeks after DMBA administration, rats bearing multiple mammary tumors greater than 1 cm in average tumor diameter were placed on either a 20% high fat or a 4.5% control fat diet. Pretreatment measurements of average tumor diameter were recorded and treatment groups were adjusted, so that each group was similar with respect to average tumor diameter, average tumor number, and average body weight.

Mammary tumor growth was monitored by weekly tumor measurements, and results were expressed as percent change in average tumor diameter. In addition, newly developing tumors were palpated and measured at weekly intervals. Blood was sampled by oribtal sinus puncture in the morning between 1000 and 1100 hr, 2 weeks after initiation of dietary treatment, and by decapitation upon termination of the experiment. Separated serum was measured for prolactin by radioimmunoassay (Niswender et al., 1969).

Results

Figure 10 shows the effects of a high fat dietary treatment on the growth of established mammary tumors,

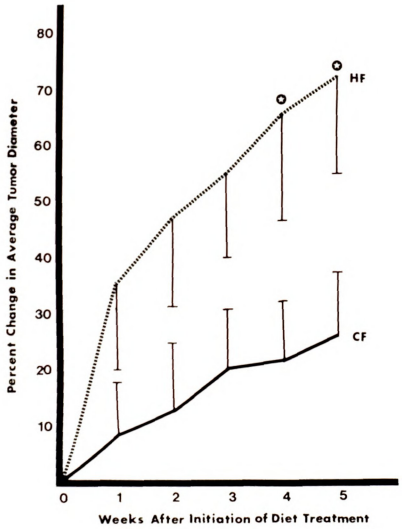

Figure 10. Effects of High Fat Diet on the Growth of Established DMBA-Induced Mammary Tumor Growth in Rats. CF represents 4.5% control fat diet; HF represents 20.0% high fat diet. ✪ indicates $p<0.05$ vs similarly treated rats fed control fat diet.

expressed as percent change in average tumor diameter. Mammary tumor growth in rats fed the high fat diet was markedly stimulated when compared to rats fed the control low fat diet. During the 4 and 5 week periods after initiation of dietary treatment, mammary tumor size was significantly greater in rats fed the high fat diet when compared with rats given the control low fat diet regimen. After 5 weeks of treatment, average tumor size was increased by approximately 70% in rats fed the high fat diet, whereas average tumor size was increased by approximately 25% in rats given the low fat dietary treatment.

The high fat diet also enhanced growth of newly palpable mammary tumors during the 5 week treatment period, as shown in Figure 11 and in Table 7. Following treatment, rats fed the high fat diet showed a significant increase in the number of newly developing mammary tumors when compared to rats fed the control fat diet.

No effects on body weight or in serum prolactin levels were observed during the 5 week treatment period (Table 7).

Conclusions

Results from this study show that a high fat diet can stimulate growth of previously established DMBA-induced mammary tumors, thereby lending support to the observations of Wicha et al. (1978) that polyunsaturated fatty acids stimulate mammary tumor growth *in vitro*. In the experiment presented here, consumption of a polyunsaturated high fat diet stimulated mammary tumor growth *in vivo*.

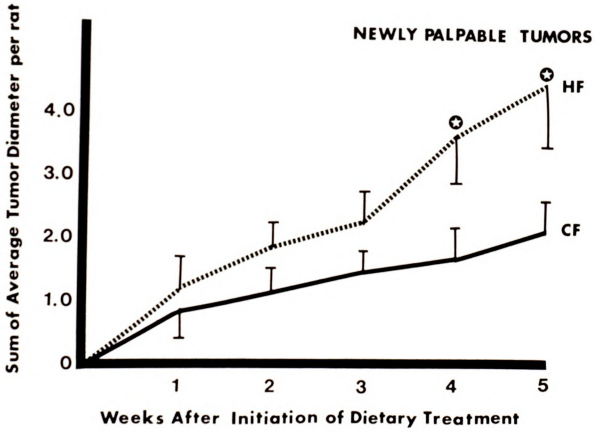

Figure 11. Effects of High Fat Diet on Mammary Tumor Development During Latter Stages of Mammary Tumorigenesis: Newly Palpable Tumors. CF represents 4.5% control fat diet; HF represents 20.0% high fat diet. ✪ indicates $p<0.05$ vs similarly treated rats fed control fat diet.

Table 7.--Effects of High Fat Diet on Growth of Established Mammary Tumors.

Dietary Treatment	No. of Rats	Serum Prolactin (ng/ml)		Final Average Tumor No. Per Rat	Final Body Weight (grams)
		14[a]	35		
CF	15	35.6 ± 4.0	21.6 ± 3.0	5.2 ± 0.51	296.1 ± 10.8
HF	16	40.1 ± 5.2	23.7 ± 3.5	6.9 ± 0.63[c]	294.1 ± 10.5

[a]Days after initiation of dietary treatment.

[b]Mean ± S.E.M.

[c]$p<0.05$ vs CF.

The high fat diet also stimulated development and growth of newly palpable mammary tumors when administered for 12 weeks after DMBA treatment. These observations confirm the study by Ip (1980), which determined that a delay of up to 20 weeks in administration of high fat dietary treatment following DMBA induction resulted in enhanced mammary tumor development. The concept that high fat diets act as promoting agents to enhance mammary tumorigenesis also is supported by these findings, since by definition, promotors stimulate tumor development even when treatment has been delayed after carcinogen administration.

The Effects of a High Fat Diet on Specific Prolactin Binding in DMBA-Induced Mammary Tumors

Objectives

It is apparent from studies previously discussed that a high fat diet does not stimulate mammary tumor development by an increase in serum prolactin levels. Furthermore, it has been determined that high fat diets are capable of stimulating mammary tumorigenesis when circulating estrogen and prolactin are maintained at constant levels, thus not excluding the possibility that high fat diets may sensitize the incipient mammary tumor tissue to hormone action.

High fat diets may exert a direct effect on mammary tumor development by increasing prolactin receptor activity within the mammary tumor. This would result in an increase in the activity of normal circulatory levels of prolactin,

thereby stimulating mammary tumor development and growth. The purpose of this study was to measure the prolactin binding capacity of mammary tumors obtained from rats fed high fat and low fat diets, and to correlate these results with any observed effects on mammary tumor development.

Procedure

Female Sprague-Dawley rats were injected with DMBA according to the previously defined procedure and placed on either a 4.5% control fat or a 20% high fat diet. At 10 to 12 weeks following DMBA administration, rats bearing multiple mammary tumors 1-2 cm in average tumor diameter were sacrificed and their tumors were excised and frozen. After a sufficient number of tumors had been collected from both groups, membranes from these tumors were prepared and assayed for prolactin binding capacity according to the radioreceptor assay procedure described previously.

Results

The effects of a high fat diet on specific prolactin binding to mammary tumor membranes are shown on Figure 12. No significant differences in specific prolactin binding were observed in mammary tumor membranes obtained from rats fed either the control fat dietary regimen or the high fat regimen.

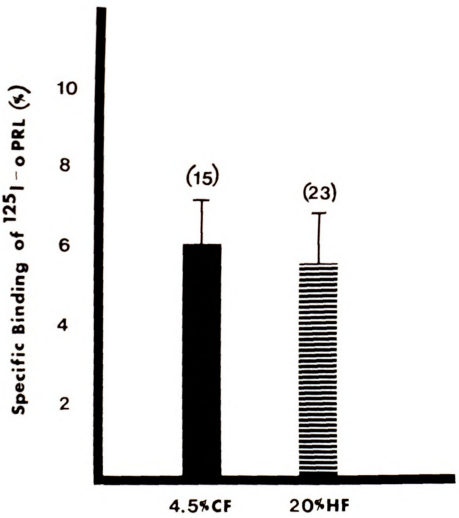

Figure 12. Effects of High Fat Diets on Specific Prolactin Binding to Mammary Tumor Membranes. ( ) denote number of samples in each group.

Conclusions

These results indicate that high fat diets do not influence prolactin binding to membranes of mammary tumors, and therefore cannot provide an explanation for the increase in development of carcinogen-induced mammary tumors observed in rats fed a high fat diet. This appears to contradict a recent report by Cave et al. (1981) observing that lactogenic hormone binding is increased in rats fed high fat diets. Differences in the method of assessing specific prolactin binding, however, may account for the discrepancy between these two studies.

DISCUSSION

Many mechanisms have been proposed in an attempt to explain how high fat diets stimulate mammary tumorigenesis. However, to date, no conclusive mechanism has been established to wholly account for the stimulatory effects of high fat diets on mammary tumor development. The research reviewed in this thesis has primarily focused upon investigation of the involvement of the endocrine system, specifically estrogen and prolactin, in the enhancement of mammary tumorigenesis by dietary fat.

The most extensively investigated and widely accepted premise describing the stimulatory role of dietary lipids in mammary tumor development has involved the endocrine system, particularly anterior pituitary prolactin secretion. The development of this premise by Chan et al. (1975) was based upon observations that suppression of serum prolactin levels could block the stimulatory effect of high fat diets on mammary tumorigenesis. Furthermore, these investigators reported that high fat diets elevated serum prolactin levels during selective periods of the estrous cycle. These observations led to the conclusion that the effects of high

fat diets on mammary tumor development are mediated indirectly through the hypothalamic-hypophyseal system, resulting in the elevation of serum prolactin levels.

Conclusions drawn from the data presented in this thesis, however, show that the stimulation of mammary tumor development by high fat diets does not involve an alteration in anterior pituitary prolactin secretion as has been proposed by Chan et al. (1975). Serum prolactin levels during the estrous cycle were not influenced by dietary fat. Furthermore, when circulating levels of prolactin and estrogen were controlled by endocrine and drug manipulations, high fat diets continued to stimulate mammary tumor development. These results suggest that high fat diets may stimulate mammary tumor development by a direct mechanism, possibly through involvement of a sensitization of the incipient mammary tumor tissue to circulating levels of estrogen and prolactin.

Support for the above suggestion was provided by Dunning et al. (1949) who noted that mammary glands from rats fed high fat diets showed an enhanced proliferative and secretory response to implanted diethylstilbesterol. Furthermore, Cave et al. (1981) showed that the binding of lactogenic hormone was increased in mammary tumors from rats fed high fat diets, thereby rendering the tumor tissue more responsive to circulating levels of prolactin and growth hormone. However, the observations made in this thesis could not confirm this report by Cave et al. (1981) since it was determined

that there was no change in specific prolactin binding to mammary tumor membranes from rats fed control fat and high fat diets.

Consumption of high fat diets alters whole body composition, resulting in an increase in the proportion of fat. An increase in the size of the mammary fat pad may increase the localization of lipid soluble substances, including estrogens, in the mammary gland. This effect also may enhance the aromatic conversion of steroids to estrogens in the fat pad surrounding the incipient mammary tumor. Both effects would tend to potentiate the estrogenic influence on developing mammary tumors to rats fed a high fat diet.

There also is other evidence that the stimulatory effects of high fat diets on mammary tumor development may not involve an endocrine mechanism. The growth of the R3230AC transplantable rat mammary tumor carcinoma is stimulated by high fat diets (Hillyard & Abraham, 1979) and inhibited when serum prolactin levels are elevated (Hilf et al., 1967). If the stimulation of mammary tumor growth by high fat diets involved either an elevation of serum prolactin levels (as proposed by Chan et al., 1975), or a sensitization of the tumor tissue to circulating levels of prolactin, then the growth of this tumor could expect to be inhibited rather than stimulated by high fat diets. Early work by Tannenbaum (1942) demonstrated that the development and growth of hormone independent mammary tumors in mice were stimulated by high fat diets, lending further support

to the view that hormones are not involved in this phenomenon.

The development and growth of many non-endocrine related tumors also have been shown to be enhanced by high fat diets. These observations would suggest that a common mechanism may be involved in the promotion of tumorigenesis that does not include the endocrine system. Also, the distribution and clearance of estrogen from the mammary gland does not differ in rats fed high fat and low fat diets (Carroll & Khor, 1975). Non-endocrine related mechanisms for the stimulation of mammary tumor development were not investigated in this thesis. The fatty acid composition of cell membrane phospholipids of mammary gland and tumor tissue is modified by the amount and type of fat consumed by the animal. Such modifications in the cell membrane, by incorporation of large amounts of polyunsaturated fatty acids, may cause alterations in membrane permeability, protein involvement in the transport of nutrients, the adenyl cyclase system, and hormone-receptor interactions which may ultimately be manifested in enhanced tumor development.

Additionally, the peroxidation of polyunsaturated fatty acids during lipid metabolism has been implicated in the stimulation of mammary tumor development by high fat diets. During normal lipid metabolism, polyunsaturated fatty acids are converted to various lipid peroxides, which have been demonstrated to have many deleterious effects on the structure and function of various cellular components when

present in high concentrations. It is possible that consumption of high fat diets containing large amounts of polyunsaturated acids increases the production of lipid peroxides which then may act to promote mammary carcinogenesis.

Therefore, it can be concluded that the effects of high fat diets on mammary tumorigenesis do not involve an elevation in serum prolactin levels, but are probably mediated by a direct mechanism on the mammary tumor tissue. This direct action of lipids on the incipient mammary tumor tissue may include sensitization of the tissue to hormones, or non-endocrine related phenomenon in which the biophysical nature or the metabolism of the neoplastic cell is changed, resulting in promotion of mammary tumorigenesis.

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