

# Dietary Fat and Growth Promotion of Rat Mammary Tumors<sup>1</sup>

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## Summary

A hypothesis is presented that explains the mammary tumor-promoting effects of high fat diets on the basis of alteration in the hormonal milieu, namely the relative concentrations of circulating prolactin to estrogen. Evidence from *in vivo* and *in vitro* studies drawn from work in our laboratory and others is reviewed in light of this hypothesis. It is postulated that mammary tumor cell proliferation is stimulated when the prolactin:estrogen ratio is high and is inhibited when the ratio is low. Chronic high fat intake elevates serum prolactin levels, thus raises the prolactin:estrogen ratio, and thereby promotes mammary tumor cell growth.

## Introduction

The dietary factors that influence cancer development in rodents have been reviewed by Carroll (5) in the present symposium. Evidence suggests that, among the dietary factors, fats in particular tend to promote mammary tumor growth. Exactly how dietary fat influences mammary tumorigenesis is not clear. Since growth and maintenance of normal and neoplastic mammary glands are chiefly regulated by prolactin and estrogen (although other hormones such as progesterone, glucocorticoids, and insulin may also play a role) (21), we have examined the role played by the endocrine system. Data obtained in our laboratory indicate that the mammary tumor-enhancing effect of high fat intake may be mediated by the pituitary via increased levels of circulating prolactin, which is ultimately expressed in a high prolactin:estrogen ratio.

## Dietary Fat, Prolactin, and Mammary Tumorigenesis

DMBA<sup>3</sup>-treated rats fed a high-fat diet developed more mammary tumors with a shorter latent period than did rats fed a low-fat diet (6). Since estrogen has been implicated in mammary tumor development, experiments were carried out to determine whether the effect of dietary fat was mediated through ovarian steroids. Administration of an antiestrogen drug, nafoxidine hydrochloride (Ull, 100A),

inhibited mammary tumor growth, but it did not eliminate the differential effects of high- and low-fat diets (6). Hence, it is unlikely that the tumor-enhancing effect of high fat intake is mediated via estrogen.

A 2nd hormone, prolactin, has been well documented to act as a promoter in mammary tumorigenesis (16). Boyns *et al.* (2) demonstrated that susceptibility to mammary tumor induction by DMBA in 3 strains of rats was correlated with the basal serum prolactin levels. Other studies demonstrated that, in mammary tumorigenesis in a given strain of rat, the tumor incidence corresponds directly to serum prolactin titers (22, 26, 29). Therefore, we studied whether the dietary fat effect is mediated by prolactin. When DMBA-treated rats were fed a high- or a low-fat diet and given an antiprolactin drug, 2-bromo- $\alpha$ -ergocryptine (CB154), the incidence in the 2 groups was similar, although at a lower level (6). In other words, the differential effect of high-fat and low-fat diets was eliminated. These experiments suggest that, when serum prolactin levels were depressed by ergocryptine (15, 22), the effects of different dietary fat levels on mammary tumor development were no longer expressed. Thus, the tumor-enhancing effect of high fat intake appears to be mediated by prolactin. On the basis of such studies, it appeared that the high fat effect might be mediated via a hypothalamopituitary function.

To validate the view that diet influenced the endocrine pattern, serum prolactin levels in rats fed a high-fat or low-fat diet were determined by radioimmunoassay. During the normal estrous cycle, prolactin levels peak in the late afternoon of proestrus and estrus (4). Blood samples collected in late afternoon showed that serum prolactin levels at proestrus-estrus were significantly higher in rats on a high-fat diet than those in rats on a low-fat diet; no differences were found in serum prolactin levels at metestrus-diestrus (7). Thus in some unknown manner, dietary fat levels influence cyclic surges of prolactin from the pituitary into the circulation. Whether the mechanism involves the hypothalamic prolactin-inhibitory factor, that is, a catecholaminergic pathway or an serotonergic pathway remains to be clarified.

## Hormonal Control of Mammary Tumor Growth

***In Vivo* Evidence.** Prolactin alone, in the absence of estrogen, cannot sustain growth of rat mammary tumors (10, 11). A certain basal level of estrogen is required for growth maintenance (19). Paradoxically, however, whereas estrogen in low doses stimulates mammary tumor growth, in

<sup>1</sup> Presented at the Conference on Nutrition in the Causation of Cancer, May 19 to 22, 1975, Key Biscayne, Fla. Supported in part by USPHS Research Grant CA 012376 from National Cancer Institute.

<sup>2</sup> Presenter.

<sup>3</sup> The abbreviation used is: DMBA, 7,12-dimethylbenz(a)anthracene.

high doses it inhibits mammary tumor growth (17, 23). Meites (22) reported that, in DMBA-treated ovariectomized rats given 2  $\mu\text{g}$  of estradiol benzoate every other day, mammary tumor development was enhanced, while the circulating prolactin level was elevated from about 14 to about 169 ng/ml serum. In similarly treated rats given 20  $\mu\text{g}$  of estradiol benzoate, tumor development was inhibited but prolactin titer remained at about 177 ng/ml serum. In these experiments, it seems that mammary tumor development was stimulated as a result of increased prolactin levels induced by low doses of estrogen. However, high doses of estrogen could not stimulate further increase of serum prolactin, and as the prolactin:estrogen ratio decreased, inhibition of tumor growth resulted.

**In Vitro Evidence.** *In vitro* systems offer better control for quantitative studies of hormone action than do intact animals. Accordingly, we used an epithelial cell line derived from a DMBA-induced rat mammary adenocarcinoma (8) to investigate the effects of estradiol and prolactin on growth. Since the cells grow well in medium containing 10% fetal calf serum, it is assumed that the hormonal make-up in the serum is optimal for growth. When the cells were grown in medium containing charcoal-extracted fetal calf serum (1) (estrogen level in the serum determined by radioimmunoassay was 228 pg/ml before and 12 pg/ml after charcoal absorption), growth was slightly but not significantly decreased compared to that of cells grown in untreated fetal calf serum<sup>4</sup>. At levels higher than 1  $\mu\text{g}/\text{ml}$ , estradiol was inhibitory to growth. These experiments indicated that estrogen at a range of 12 pg to 1  $\mu\text{g}/\text{ml}$ , in relation to the prolactin level in fetal calf serum is sufficient to support tumor cell growth. Further increase in estradiol titer depressed growth.<sup>4</sup>

However, when high doses of prolactin (> 50  $\mu\text{g}/\text{ml}$ ) were added to the cultures together with estradiol, the inhibitory effect of estradiol was counteracted and growth was stimulated.<sup>4</sup> These studies demonstrated that when the prolactin:estrogen balance in the growth medium is altered by increased estradiol concentration, cell growth is inhibited. The depressed state of cell growth can be reversed by the addition of a certain amount of prolactin in proportion to estradiol. Thus, our data indicate that prolactin and estradiol play a combined regulatory role in the growth of rat mammary tumor cells and suggest that normal growth depends on a specific ratio of prolactin to estrogen in the surrounding medium.

### Hypothesis

Based on the data discussed above, we propose that, under the influence of chronic high fat intake, the prolactin:estrogen ratio may be increased, leading to enhancement of growth and development of normal and neoplastic mammary gland tissue. Since the initiating agents in mammary tumorigenesis may vary and are by and large

unknown, the hypothesis is necessarily restricted to the promotional phase of mammary carcinogenesis. However, it may also be applied to explain the control of rhythmic growth and involution of the mammary gland during the estrous cycle (28).

### Ovariectomy Studies

We have designed an experiment to test the validity of our hypothesis. When 10 of 40 DMBA-treated rats developed palpable mammary tumors, all 40 were ovariectomized and divided into 2 groups: one was fed a high-fat diet, and the other was fed a low-fat diet. Fourteen weeks afterward, we observed that 5% of the ovariectomized rats on the high-fat diet had palpable mammary tumors, whereas only 1 rat in the low-fat group had palpable tumors. In general, about 90% of the DMBA-induced mammary tumors regress following ovariectomy (9, 10, 18). Meites (22) showed that serum prolactin levels in ovariectomized rats decreased to less than one-half of the normal basal level. These results conform with the notion that, in ovariectomized rats on high-fat diet, prolactin synthesis and secretion are stimulated, leading to a high prolactin:estrogen ratio and, as a result, tumor growth was enhanced. This experiment also showed that the high fat effect on prolactin is independent of the estrogen-prolactin feedback system. Again, direct confirmation will have to wait until we have completed assays of serum prolactin and estrogen levels in these rats.

### Implications

An experimental model may not accurately simulate the human conditions for which it was devised. However, the value of an experimental model is that it provides a simpler and more accessible system for the study and understanding of human diseases. One attractive aspect of our hypothesis derived from model studies is that it provides some new insights into a number of epidemiological observations.

De Waard (12) reported that in England and Holland mammary cancer incidence is higher than in Japan during premenopausal years. After menopause, the incidence drops in Japan, whereas in the Western countries it continues to increase. Comparison between Japanese and the United States population also shows a similar trend (3, 14, 20). Migrant studies of Japanese moving to the United States show a gradual increase in breast cancer incidence in the 2nd generation of migrant descendants. The 3rd generation descendants have an incidence rate similar to that of United States whites (3, 14). Wynder *et al.* (30) proposed that dietary fat intake may be responsible for these epidemiological observations.

Our hypothesis may provide an explanation of the mechanism(s) underlying these epidemiological data. It is probable that in high-risk populations, due to high intake of fat in the diet, serum prolactin levels may be periodically elevated as compared to those in the low-risk populations. In postmenopausal women in the West, the decrease in estrogen coupled with high fat intake may lead to an increase in prolactin:estrogen ratio. As a result mammary tumor

<sup>4</sup> P.-C. Chan, J. Tsuang, J. Head, and L. A. Cohen. Effects of Estradiol and Prolactin on Growth of Rat Mammary Adenocarcinoma Cells in Monolayer Culture. *Proc. Soc. Exptl. Biol. Med.*, in press.

growth is stimulated, as is observed in DMBA-treated ovariectomized rats fed a high-fat diet. In Japanese, possibly after menopause, estrogen-stimulated prolactin synthesis and secretion cease. In the absence of a high dietary fat stimulation, serum prolactin remains low, and therefore mammary tumor growth is not favored, as is observed in our ovariectomized rats fed a low-fat diet.

The role of prolactin in breast cancer in humans is now controversial (27). This is due to 2 main factors. As indicated in our animal model studies, elevation of serum prolactin in high-fat-fed rats is cyclical and is therefore detected only during the peak secretion period. Up to the present, in human studies, blood samples for prolactin assay have been collected in the morning when the serum prolactin level is lowest (24). Therefore, it is not surprising that the difference in serum prolactin levels between high-risk and low-risk population has not been conclusively demonstrated. Human serum prolactin determination in blood collected during peak secretion period may be more revealing. Another reason may be that, in analyzing serum prolactin levels in humans, a wide range of values has been observed. However, in reports, the data are usually combined and the average value is presented. In high-risk countries such as the United States, the standardized breast cancer incidence is 72.6/100,000 females (30) [77.4/100,000 in Connecticut, 1963 to 1965 (13)]. In low-risk populations such as Japan, it is 13.3/100,000 females (25) [10.8/100,000 in Miyagi, Japan, 1962 to 1964 (13)]. Thus, the probability of revealing elevated prolactin level in the few high-risk people in studies with small samples is low. To account for this, it is proposed that the percentage of cases with high titers (above a base line level, 20 ng/ml) be compared between high-risk and low-risk populations.

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*Cancer Res* 1975;35:3384-3386.

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