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High Dietary Fat, Elevation of Rat Serum Prolactin and Mammary Cancer[1] (38758)

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(Introduced by J. H. Weisburger)

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In a previous communication (1) we reported that 7,12-dimethylbenz (a) anthracene (DMBA)-treated rats fed a 20% fat semisynthetic diet exhibited a higher mean mammary tumor incidence (56%) than rats fed a 0.5% fat diet (34%), thus confirming the earlier studies of Carroll and coworkers (2, 3). Also, on the basis of experiments using hormone antagonists we tentatively concluded that the high fat effect was mediated through elevated serum prolactin and that changes in the estrogen-receptor system were probably not involved.

We now submit direct evidence, based on the radioimmunoassay of serum prolactin, that animals maintained on high-fat diets (HF) exhibit significantly higher serum prolactin levels during the proestrus–estrus period than animals fed low fat diets (LF).

Materials and Methods. Forty virgin female Sprague–Dawley rats (ARS/Sprague–Dawley, Madison, WI) were housed five in a cage in a temperature (24° ± 1°), humidity (50%) and light controlled (12 hr/day) room and fed Purina Lab Chow and water *ad libitum*. When 50 days old, the rats were separated into two groups of 20 each and fed a high (20%) lard semisynthetic diet, as described previously (1), or a low (0.5%) lard diet. After 2 and 5 mo on the diets, tail blood was taken once a week for 3 wk and the serum separated and stored at −20°. Bleeding was carried out under ether anesthesia at 4:00 PM and completed within 2 min. Also, during these 3 wk, vaginal smears were taken daily at 4:30 PM to determine the estrous stage. Radioimmunoassay of serum prolactin from cycling rats was carried out using reagents and methods supplied by the Hormone Distribution Program, National Institutes of Arthritis, Metabolic and Digestive Diseases, Bethesda, MD. Rat prolactin was iodinated with ^{125}I obtained from Cambridge Nuclear Corp., Billerica, MA. Sheep anti-rabbit gamma-globulin was obtained from Grand Island Biologicals, Grand Island, N.Y. Each prolactin determination was performed using two dilutions of serum. Purified rat prolactin served as a reference standard.

Students *t* test and analysis of variance were used for statistical evaluation of the data.

Results. During the proestrus–estrus (P–E) period the serum prolactin titer in HF rats was significantly higher than that in LF rats at 2 and 5 mo respectively (Fig. 1). There was no difference in prolactin levels between the two groups of rats during the metestrus–diestrus (M–D) phase. The paired P–E and M–D prolactin values for individual rats were plotted as a scatter diagram and regression lines calculated (Fig. 2). Statistical evaluation revealed that (a) the slopes were not significantly different and (b) the HF lines exhibited a significant elevation at the Y-axis intercept.

Discussion. It is generally agreed that at least 3, and possibly more prolactin regulatory mechanisms exist (4, 5). These include (a) hypothalamic inhibition by a prolactin-inhibiting factor (PIF) which may in fact be a catecholamine, (b) an estrogen-regulated positive feedback system (6–8), (c) a prolactin-releasing system which probably involves thyrotropin releasing hormone.

Using antiestrogen, we demonstrated indirectly that the tumor enhancing effect of HF diet might not be mediated via the estrogen pathway (1). Therefore, the elevated levels of serum prolactin observed in rats on HF diet could relate to the catecholaminergic pathway in the hypothalamus. Experiments in rats have shown that after ingestion of a high carbohydrate diet there is an increase in the rate of synthesis of the neurotransmitter serotonin in the brain (9). Conversely, high

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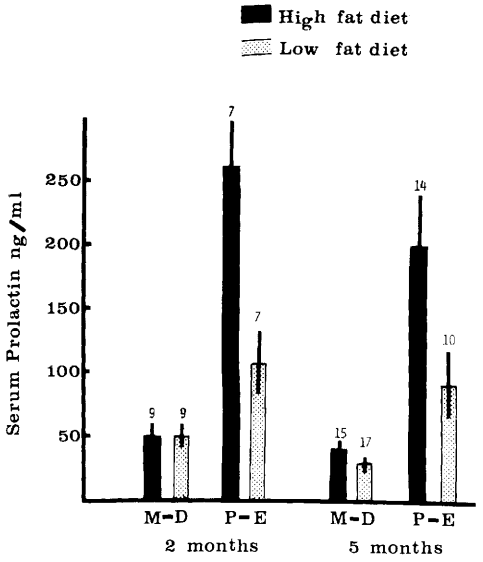

FIG. 1. Serum Prolactin Titer. The vertical line on top of each bar represents ± SEM; numbers above the bar designate the number of individual rats assayed at metestrus–diestrus (M–D) and proestrus–estrus (P–E). The difference between the mean prolactin values of the HF group and the LF group at P–E was significant ($P < 0.05$).

fat intake may cause a decrease in hypothamic catecholamine (PIF) synthesis, which in turn could increase prolactin synthesis and secretion. Clearly, further studies on the effect of dietary fat on hypothalamic function are required.

There is a circadian rhythm governing rat prolactin secretion superimposed on an underlying estrous rhythm. Serum prolactin is higher during late afternoon than during the morning (10). In the afternoon at proestrus and estrus during the regular estrous cycle specifically, serum prolactin level rises at 1300 hr, peaks at 1500–1900 hr, and returns to the basal level before midnight (11–13). Unless the HF or LF diet causes a shift in the time of the peaking of prolactin level at proestrus and estrus, it is highly unlikely that the differences between HF and LF groups were the result of biased selection of "peak" samples.

Since the weight gain over the experimental period was not significantly different when the HF and LF groups were compared (1, 14) one can conclude (a) that the feeding habits (caloric intake) were similar in both groups and (b) that the difference in prolactin levels was actually due to the experimental variable being tested (i.e. the fat composition of the diet) and not to extraneous factors such as differences in total ingested calories, obesity or malnutrition.

We observed that some rats on a LF diet developed mammary tumors with a latent period similar to that of HF rats (1). The regression lines representing the prolactin profile of individual rats (Fig. 2) also show that certain rats on LF diet exhibited a prolactin profile similar to that of HF rats. If it could be proved that the rats with prolactin values high on the regression line correspond to the rats which are more sensitive to tumor induction, the regression line could be used to predict the risk of individual rats to carcinogenesis by DMBA. This hypothesis is currently under study in our laboratory.

If it is granted that high fat intake does result in elevated serum prolactin titers during the relatively short proestrus–estrus surge how could these intermittent increases influence the development of mammary carcinoma? At present one can only offer a conjecture. Prolactin is thought to act as a classical promoter substance in rat mammary carcinogenesis (15). Strains of rats with varying degrees of susceptibility to DMBA mammary carcinogenesis have been shown to exhibit different serum prolactin titers which correspond to differences in tumor incidence (16). Possibly, therefore, the periodic surges of elevated concentrations of prolactin promote tumor growth in a manner analogous to dose effects of croton oil in promoting mouse skin carcinogenesis (17).

Summary. We investigated the concept that the enhanced development of DMBA-induced mammary adenocarcinomas in rats fed a high fat diet might be mediated by prolactin via the hypothalamic-pituitary system rather than by a direct effect of fat on the mammary gland itself. Prolactin levels in serum from female Sprague-Dawley rats fed high (20%) and low (0.5%) lard diets for 2 and 5 mo were measured by radioimmunoassay. The levels in rats at proestrus–estrus on high-lard diets were significantly higher (250 ng/ml) than those on a low lard diet (100 ng/ml). At metestrus–diestrus prolactin levels were approximately 50 ng/ml regardless of diet. Thus, the tumor-enhancing effect

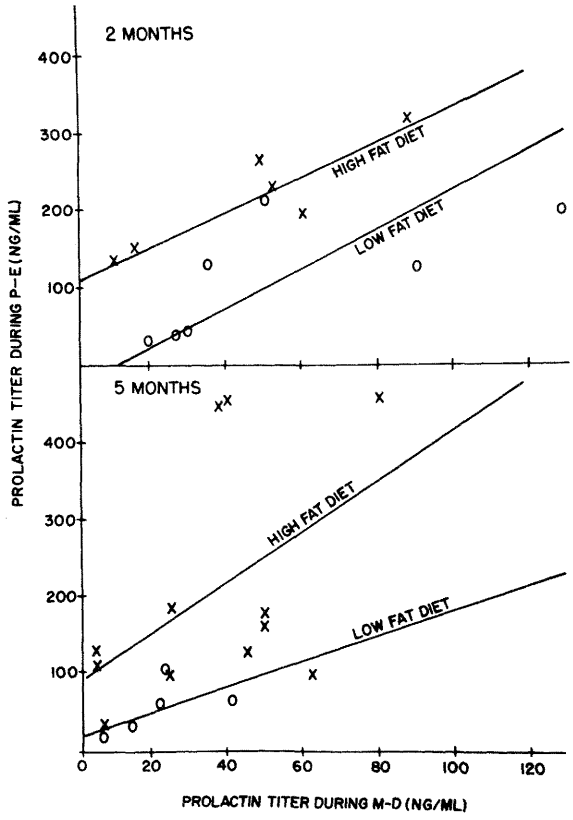

FIG. 2. Scatter diagram with regression lines for paired prolactin values from individual rats at P–E or M–D after 2 mo and 5 mo on HF (×) and LF (0) diets. Regression coefficients for HF and LF lines at 2 mo were b = 0.61 and 1.28; at 5 mo, b = 3.2 and 1.6, respectively. The differences in elevations for HF and LF at 2 mo were significant.

of a high fat diet may be indirect and mediated by neuro–hormonal changes in the hypothalamic centers controlling prolactin synthesis and secretion.

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