

# Enhancement of Mammary Fibroadenomas in the Female Rat by a High Fat Diet\*

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There is considerable experimental and statistical evidence for the relationship of nutrition to tumor genesis. In man, the state of nutrition has been correlated with the development of various tumor types (16). Obesity in man has been associated with a higher incidence of cancer than that noted in normal and underweight persons (11). In the mouse, diets high in fat or caloric content have enhanced the formation of spontaneously occurring and experimentally induced neoplasms (1, 2, 8, 12-14, 17). In the white rat, prolongation of life span, retardation of spontaneous tumor development, and a delay in the onset of pathological aging processes were observed in association with prolonged undernutrition (9). (The types of tumors and the strain of rat were not stated by the authors.) A high fat diet accelerates the formation of estrogen-induced mammary carcinoma in the rat (7).

If increased rates of tumor genesis are to be evaluated in a group of given animals, one must take into account the actual spontaneous occurrence of tumors in large laboratory colonies of animals not necessarily bred as tumor-bearers. This occurrence has been well documented in colonies of various strains of rats and has been shown to be generally low (4, 6, 10). To our knowledge, there is no statistical survey of spontaneously occurring mammary tumors in the Sprague-Dawley strain. It is of interest to note that in one study of hypothalamic hyperphagia with the production of obesity in the Sprague-Dawley strain, there was no mention of spontaneous or increased formation of tumors (3).

The opportunity to observe the relation of a high fat diet to tumor genesis was afforded us during the course of an experiment involving the study of aging and other factors related to the

production of arteriosclerosis in the Sprague-Dawley albino rat of various ages and of both sexes.

## MATERIALS AND METHODS

Of a total of 380 penbred Sprague-Dawley rats, raised over a 2-year period in our laboratory for the experiment on atherosclerosis, 234 were placed on various diet and treatment schedules after being divided into various age groups (4-24 months) and according to sex. The sexes were separated just after weaning. The diets were aimed at raising or lowering serum lipid levels, with some animals existing in a moderately hypertensive state and others in hypo- and hyperthyroid states, as outlined in Table 1. The control group of 146 animals was similarly divided into various age groups, with the sexes kept separately, except for a few animals used for breeding purposes at specific times. The control animals had been given an ad libitum diet of Purina Laboratory Chow pellets, supplemented by lettuce and carrots given twice weekly. This diet has an available caloric content of 60 Calories/day. The experimental group was given 15 gm. (daily) of ground Purina Laboratory Chow containing 20 per cent olive oil as a basic diet. This diet has an available caloric content of 75 Calories/day. The olive oil, aside from enhancing the general palatability of the diet, was employed as a vehicle for various substances added to the diets. The drinking water of Groups VII, VIII, and IX contained 1 per cent NaCl and 0.2 per cent KCl. Serum and fecal cholesterol<sup>1</sup> and serum lipid<sup>2</sup> studies were made on experimental and control animals. The complete results of the histologic, metabolic, and chemical studies in this experiment on atherosclerosis will be reported later.

In both groups, control and experimental, similar tumors developed. Since they occurred almost exclusively in the female (there were two

\* Aided by a grant from the Leon Lowenstein Foundation, New York, New York.

† Mr. Grand performed the tissue culture work at The Cancer Institute at Miami.

Received for publication August 11, 1955.

<sup>1</sup> Kingsley-Schaffert method.

<sup>2</sup> Bloor method on 2 cc. of serum.

instances of tumors in males of the experimental group), the present report concerns itself with the relative incidence of tumors in the female rats in the experimental and control groups. Thus, 129 animals in the experimental group and 43 animals in the control group form the basis of the present study.

These tumors were removed surgically and studied histologically by paraffin section and hematoxylin-eosin stain. One mm. fragments of various parts of six of the mammary tumors (four fibroadenomas, two fibrosarcomas) were planted in a tissue culture medium consisting of: rat serum, 60 per cent; 50 per cent chick embryo

control group. Evaluation of the comparative incidence of tumors was therefore made from animals over 18 months of age (Table 1). In the control group, there was a tumor incidence of 12 per cent of 25 females of this age group, as compared with a tumor incidence of 41.9 per cent in 81 female experimental animals of this age group. The serum lipid values were distinctly higher in the experimental animals (Table 1). The serum cholesterol level was higher in all experimental groups, with the exception of Group I (Basic diet and 20 per cent olive oil). Since the incidence of tumors was high in Group I, there was no correlation between the height of the serum cholesterol level and

TABLE 1  
INCIDENCE OF FIBROADENOMAS OF THE BREAST IN FEMALE RATS 18 MONTHS OF AGE OR OLDER  
WITH TOTAL SERUM LIPID AND SERUM CHOLESTEROL LEVELS

GROUP	DIET AND TREATMENT	No. RATS	RATS WITH TUMORS			TOTAL SERUM LIPIDS† (mg. per cent)	SERUM CHOLESTEROL† (mg. per cent)
			No.	Per cent	Age* (months)		
I	80 per cent chow, 20 per cent olive oil	13	5	38.4	18-31	722	117
II	I plus 2 per cent cholesterol	11	6	54.5	21-27	1350	509
III	I plus 2 per cent desiccated thyroid	9	1	11.1	27-28		152
IV	II plus 2 per cent desiccated thyroid	6	1	16.7	26		347
V	II plus injection of I <sup>131</sup>	6	2	33.3	21-23		631
Vx	V plus 2 per cent cholic acid	6	5	83.3	23-28	3269	1594
VI	I plus injection of I <sup>131</sup>	3	2	66.7	24-25	926	200
VIx	VI plus 2 per cent cholic acid	3	1	33.3	28	741	129
VII	IX plus 0.25 mg. DOCA	6	4	66.7	21-24		129
VIII	VII plus 2 per cent cholesterol and unilateral nephrectomy	8	3	37.5	21-28	1210	254
IX	I plus unilateral nephrectomy	5	2	40.0	21-27	1362	189
XIII	I plus 2 per cent cholic acid and 2 per cent propylthiouracil	5	2	40.0	26	2263	460
Experimental, Totals:		81	34	41.9	21-27	1480	392
Control: Chow		25	3	12.0	18-28	550	115

\* Range of ages at time of appearance of tumors.

† Nonfasting.

extract, 20 per cent; and chicken plasma, 20 per cent. The Maximow double cover-slip method as outlined by Cameron (5) was employed. Transplantation of the benign mammary tumors was attempted in six cases in stock rats not pre-irradiated or treated with cortisone.

### RESULTS

After 10 months, 28 per cent of 129 female experimental animals had developed fibroadenomas, as compared with 7 per cent of 43 female control animals during the same period of time.

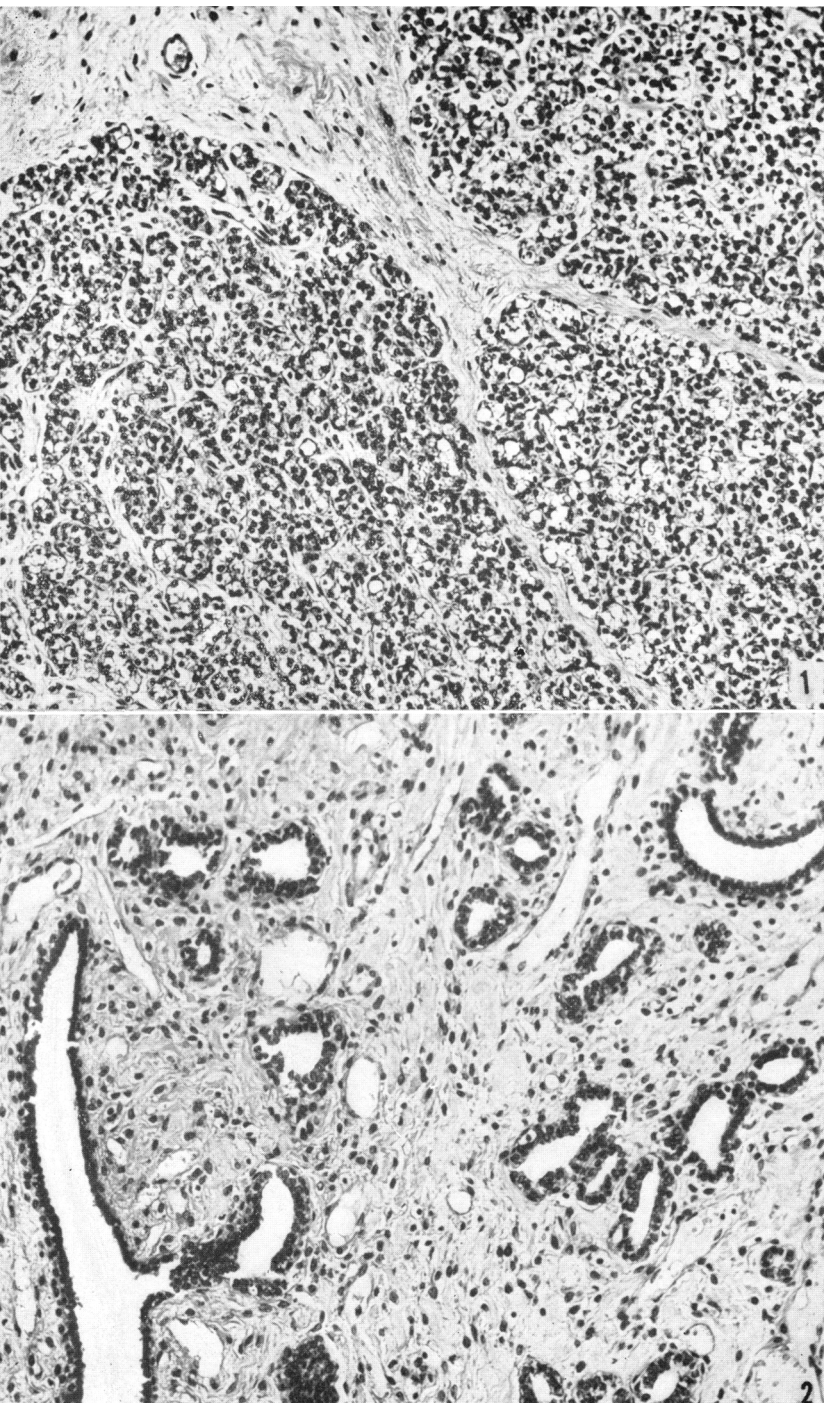
No tumors were found in animals under 18 months of age, either in the experimental or in the

tumor incidence. The lipid and cholesterol values were not significantly higher in tumor-bearing and nontumor-bearing animals within the same experimental group. There was little or no apparent difference in the age of the tumor-bearing animals among the control and experimental groups at the time these tumors developed. As to the period of time elapsing before the onset of the tumor, the longest time for tumor development occurred in Group III, 9 months, and the shortest in Groups Vx and XIII, 2 months.

Microscopically, the tumors contained mammary ducts, glands, and fibrous tissue in varying proportions within the same tumor, with some

FIG. 1.—More highly cellular type of mammary fibroadenoma. Hematoxylin-eosin.  $\times 150$ .

FIG. 2.—Usual microscopic pattern of mammary fibroadenoma. Hematoxylin-eosin.  $\times 150$ .



areas being predominantly fibrous with a slight increase in the number of glands, other areas showing about an equal proportion of glands and fibrous tissue, and still other areas showing a predominance of glandular tissue (Figs. 1 and 2). In two animals, fibrosarcomas developed at the site of previously resected fibroadenomas. No malignant tumors were found in the control group.

Tissue cultures of fibroadenoma did not show outgrowth, possibly because of partial necrosis of tumor tissue employed. Tissue cultures of the fibrosarcomas showed a rich outgrowth of fibroblasts within 24 hours, many showing mitoses. There was also a moderate number of macrophages and fat cells. Smaller numbers of lymphocytes and polymorphonuclear leukocytes were seen. No epithelial elements were noted. These cultures presented a fibrosarcomatous appearance. Transplantation attempts were not successful.

### DISCUSSION

This study demonstrates that there is an increased incidence of fibroadenomas of the breast in the Sprague-Dawley rat fed a high fat diet. Experiments with high fat diets in mice likewise indicate a definite enhancement of tumor formation. This has been shown by Tannenbaum, in the use of isocaloric diets, to be due to the fat per se, probably acting as a cocarcinogen and as a solvent for estrogen especially concentrated in breast tissues. The suggestion is also made that the influence of a fat-enriched diet on tumor formation in a given tissue may depend upon the extent to which the amount of fat in that tissue is modified by the diet (15). If the findings in the mouse can be compared with our findings in the rat, it would seem that a high incidence of fibroadenomas of the breast in a rat fed a high fat diet is related to both the high caloric intake and the specific effect of fat on tumor genesis.

The high incidence in the experimental groups was correlated with increased total serum lipid levels as contrasted to those of the controls, but not with any specific increase in serum cholesterol levels, as indicated by the low cholesterol level of Group I. This might imply that high serum lipids are related to tumor genesis.

### SUMMARY

During the course of an experiment related to the production of arteriosclerosis in the Sprague-Dawley rat, penbred animals of various ages were subjected to diets aimed at lowering or raising the

serum lipid levels. Fibroadenomas of the breast developed in the female and form the basis of the present study. These tumors occurred only in animals 18 months of age or over. There was an increased occurrence of tumors in animals fed a high fat diet. This is considered to be due to hypernutrition and the specific action of fat on tumor genesis. Serum lipid levels were higher in the experimental animals, suggesting that hyperlipemia is related to tumor genesis.

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