

The Effect of Fat-Enriched Diet on the Incidence of Spontaneous Mammary Tumors in Obese Mice (40683)¹

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Summary. C3H/HeJ mice show a high incidence of spontaneously occurring mammary tumors. Mice made experimentally obese were observed to have an earlier appearance and a greater incidence of tumors than were the normal-weight mice. A fat-enriched diet increased the observed tumor incidence in normal-weight mice to about that of obese mice given a regular diet, while a fat-enriched diet further enhanced the already augmented observed yield of mammary tumors in the obese mice given a regular diet. Statistical analysis supported the dependence of tumor incidence and appearance time on the type of diet. The calories consumed by normal mice on a fat-enriched diet was considerably below that of obese mice on a regular diet, yet produced about the same tumor incidence. A specific effect of the fat-enriched diet is suggested.

High caloric diets, particularly those modified to include a high portion of fat, have been reported to promote or enhance the occurrence of several types of tumors in various species of animals (1-3). Mice, force-fed to enhance weight gains, show an increase in tumors. In contrast, dietary restriction delayed or inhibited the formation of spontaneously occurring or experimentally induced tumors (4-6). In the present study virgin female mice were made obese by a single injection of goldthioglucose (7-16). These mice reached weights of 50-60 g compared to controls of 25-30 g and showed an increased tendency to tumor formation. This was evidenced by an earlier appearance and a greater incidence of spontaneously occurring mammary tumors than found in control mice (8-10). The excess weight gain has been shown to be due to increased food intake and to primarily represent adipose tissue (11). These

mice can mobilize the excess fat during starvation and regain it on *ad libitum* refeeding (11).

Whether dietary fat has a tumorigenic effect apart from its caloric value has been a matter for speculation for at least three decades (1-17-19). Recently, Chan and Cohen reported that rats on a high fat diet had a significantly greater incidence of mammary tumors than those on a low fat diet, although the diets were isocaloric and weight gains comparable (12). The present study was undertaken to reexamine the possible effect of a fat-enriched diet as distinct from its caloric value on mammary tumor incidence in mice.

Materials and methods. C3H female mice with a spontaneous incidence of mammary tumors were obtained from the Department of Genetics, University of California, at 4 weeks of age and kept virginal throughout the experiment. The animals were housed seven to a cage and allowed free access to Purina Chow during the initial stages of the experiments. At 3 months of age, the mice were randomized into two groups, one of which was left untreated and the other injected with goldthioglucose and allowed to become obese. Mice from both groups were fed either commercial Purina Chow or Purina with added 10% Wesson oil by weight. The obese mice on the fat-enriched diet were pair-fed against the obese groups on regular feed. This was done by averaging the weight in grams of diet consumed by mice on the regular diet twice weekly and feeding fat-enriched diet in the same amounts to the companion group. The same procedure was followed in pair-feeding nonobese animals on the fat-enriched diet against the nonobese on the regular diet. The mice were weighed twice weekly and then inspected by palpation for the presence of tumors and the time of onset noted. All animals were autopsied at the time of natural death or termination of the exper-

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iment. The experiment was terminated when 50% of the mice of each of the four groups had developed tumors. All palpable tumors were verified histologically to be mammary carcinomas.

Results. Table I lists the groups of animals, the number of mice in each group, the number of mice in each group developing tumors within 12 months, and their average age and weight at tumor onset. At the end of 12 months the tumor incidence was 71% for obese mice on a fat-enriched diet; 48% for obese mice on a regular diet; 44% for non-obese mice on a fat-enriched diet; and 29% for nonobese mice on a regular diet. The average times of tumor onset for the four groups were 269, 298, 299 and 338 days, respectively. The average weights at tumor onset were 30.0 and 29.7 for nonobese animals on the regular and fat-enriched diets. For obese animals, the average weight was 53.3 g on the regular diet and 55.6 g on the fat-enriched diet.

Figure 1 presents the observations differently from Table I. It plots the ages of individual mice in the four groups at tumor onset against the cumulative percentage of animals developing tumors. (For example, each animal in a group of 34 was plotted as contributing 3.0% to the cumulative percentage, and the experiment was terminated when 17 mice had developed tumors.) The time at which 50% of the mice in each group had developed tumors was 298 days for obese mice on a fat-enriched diet; 369 days for obese mice on a regular diet; 397 days for normal-weight mice on a fat-enriched diet; and 484 days for normal-weight mice on a regular diet. At 298 days of age, when 50% of the obese mice on a fat-enriched diet had tumors, only 11% of the controls on the regular diet had tumors.

To statistically evaluate the influence of the fat-enriched diet on tumor incidence at 12 months, first the interaction between (i) the treatment effect, i.e., fat enrichment versus no fat enrichment; and (ii) the stratification effect, i.e., presence or absence of obesity, was assessed. According to the exact method for contrasting odds ratios (21), there is essentially no evidence of any interaction ($P = 0.47$). Consequently, a model may be entertained in which the ratios of the odds for tumor appearance at 12 months, of the fat-enriched to non-fat-enriched populations, are assumed to be identical for both obese and nonobese animals. The Mantel-Haenszel test (20) is then appropriate for assessing the null hypothesis that the common value of the odds ratio is unity, i.e., that there is no treatment effect (22). This null hypothesis was rejected ($P = 0.05$). Within the obese group alone or in the nonobese group alone, tumor incidence

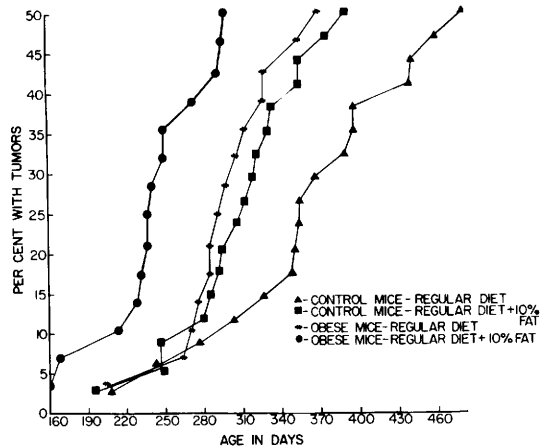


FIG. 1. Plot of ages of individual mice in the four groups at tumor onset against the cumulative percentage of animals developing tumors.

TABLE I. INCIDENCE OF SPONTANEOUS MAMMARY TUMORS IN OBESE AND NORMAL WEIGHT MICE ON REGULAR (PURINA) AND FAT-ENRICHED (PURINA + 10% FAT) DIETS WITHIN 12 MONTHS OF AGE

Group of mice and diet	Number of animals in each group at onset of experiment	Number of mice with tumors	Percentage of mice with tumors	Average weight at tumor onset (g)	Average age at tumor onset (days)
Normal weight, Purina	34	10	29.4	30.0	338
Normal weight, Purina + 10% fat	34	15	44.1	29.7	299
Obese, -Purina	29	14	48.3	53.3	298
Obese, -Purina + 10% fat	28	20	71.4	55.6	269

between animals given the fat-enriched and control diets was not statistically significant. The effect of the fat-enriched diet on time of tumor appearance (see Fig. 1) was also evaluated, using Gehan's test. In the nonobese animals the effect was not significant ($P = 0.1$), but in the obese animals the effect was significant ($P = 0.01$). Combining the two sets of animals and adjusting for the influence of obesity (in the same way that the Mantel-Haenszel statistic adjusts for a stratifying variable), the difference was significant ($P = 0.004$).

In addition, the correlation between the time of tumor appearance and the weight of animals at tumor appearance time in obese animals was assessed in the control and fat-enrichment groups separately, using Spearman's correlation coefficient (24). There is more evidence of correlation in the control group than there is in the fat-enrichment group ($P = 0.04$ vs $P = 0.15$). However, these two separate one-sided inferences may be combined, using Fisher's method for combining P values (23) and the summary assessment is significant ($P = 0.05$). The correlation is negative, i.e., the later the time of appearance of a tumor, the lighter the animal at the time. There was no significant correlation in the nonobese animals.

Discussion. Tumor incidence appears to be influenced by at least two experimental factors in the present series. (i) The addition to the diet of 10% by weight of fat in the form of Wesson oil significantly increased tumor incidence in a sample composed of both goldthioglucose-injected, obese and noninjected, nonobese mice. (ii) Goldthioglucose injection itself resulted in a large observed increase in incidence of tumors compared with noninjected animals. This occurred whether injected mice were given a regular or a fat-enriched diet.

Much of the diet effect seemed to derive from the injected animals and the question must be asked whether an increased incidence of tumors after goldthioglucose injection would be due to the action of goldthioglucose itself rather than the resultant obesity. Enhanced tumorigenesis due to goldthioglucose per se appears unlikely since mice made obese by goldthioglucose and later reduced and maintained at the weight level of control

mice do not show the increased incidence and earlier appearance of tumors noted in the obese groups (10). Also, mice given multiple small doses of gold far in excess of a single toxic dose used to induce obesity do not become obese and do not show an increase in tumor incidence. Hence, it appears likely that hyperphagia resulting from the hypothalamic lesion produced by a single toxic dose of goldthioglucose (13) or the obesity per se would be responsible for an increased tumor incidence in the injected animals.

Purely descriptively, the influence of obesity and of the fat-enriched diet may be related as follows: The fat-enriched diet increased the incidence and shortened the times of appearance of mammary tumors in a combination of normal and obese mice. Goldthioglucose injection in the doses given also resulted in an increased and earlier incidence of tumors. The magnitude of that effect was quite similar to the effect achieved by the fat enrichment of the diet, as may be seen from the two middle curves in Fig. 1 which are almost superimposable. It should be recalled that the food intake of the normal animals on the fat-enriched diet was kept at the level of the controls given normal diet. Hence their caloric intake was only 5% above that of the controls and their average weight at onset of tumors was not different from the 30.0-g average of normal mice given a regular diet. Goldthioglucose-injected animals have a 50% higher food consumption than controls (11) and the average weight at onset of tumors of the injected group on a regular diet was 53.3 gms. Thus both increased food intake (or weight) and the fat-enriched diet are implicated in tumorigenesis.

At this time, the pathogenesis of increased tumor incidence resulting from either obesity, or fat-enriched diet, or both is not known. Recently, Chan and Cohen (12) have reported that ovariectomized rats on a high-fat isocaloric diet had palpable mammary tumors whereas there were almost none in animals on a low-fat diet. They attributed this yield of tumors to the high prolactin to estrogen ratio induced in ovariectomized rats by a high-fat intake. However, caution is indicated in ascribing the effect of fat-enriched diet to specific estrogenic disturbances known to be involved in the incidence of mammary

tumors, since fat-enriched diet has also increased the yield of other tumors such as hepatomas (14-15) in which a hormonal influence has not been well demonstrated.

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