The Effect of the Proportion of Dietary Fat on the Rate of Formation of Mammary Carcinoma in Mice*

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Fat-enriched diets enhance the rate of formation of certain types of tumors in the mouse (1, 11, 16, 20); the subject has already been adequately reviewed (14, 18). In various experiments, performed at different times in our laboratory with the spontaneous mammary carcinoma and the induced skin tumor, diets containing approximately 12 per cent fat appeared to augment tumor formation very nearly as much as those containing about 30 per cent. It therefore seemed worthwhile to investigate the quantitative relation between the degree of fat-enrichment of the diet and the formation of tumors.

For the present experiments, the formation of the spontaneous mammary carcinoma of the mouse was investigated, inasmuch as it is more responsive to the action of dietary fat than is the formation of induced skin tumors (16). Two experiments were performed: in one, the diets were compounded of commercial components; in the other, of semi-purified components.

The results of the two experiments were in excellent agreement, and it was found that the rate of tumor formation (as measured both by incidence and by the average time of appearance of the tumors) tends to increase with increasing proportion of dietary fat. The effect was related to the proportion of dietary fat and was not the result of differences in caloric intakes or body weights of the mice.

GENERAL PROCEDURES

The following conditions and procedures were common to both experiments. The mice, virgin females, were of inbred strains born in our laboratory. From weaning until transfer to the experimental rations they were fed Purina laboratory chow checkers ad libitum. The several groups of each experiment were composed, so far as possible, by distribution of litter mates, and the mice were housed in groups of five in cages with solid bottoms. The diets were prepared by mixing a 1-week supply of the weighed components with sufficient water to make easily molded mashes which were spread in pans, cut into blocks of appropriate sizes, and stored in a refrigerator at 38° F. The mice were fed daily. Equicaloric amounts of the ration were fed at a level slightly below the known voluntary intake; this insured average equicaloric intakes1 among the several groups. The mice were weighed and inspected for tumors biweekly. Each animal was examined at post-mortem. Many tumors were examined histologically, including all those that were doubtful and all those not located in the mammae or liver. Records of the individual mice were kept, which yielded, in addition to the principal data, incidental information on longevity, metastases, and multiple mammary tumors.

EXPERIMENTS

Experiment 1.—Five groups were employed, each consisting of 52 virgin C3H females. They were 9–13 weeks old when transferred to their respective experimental rations. The basal portion of the daily diets for each mouse contained 1.0 gm. Purina Fox Chow meal, 0.5 gm. skimmed milk powder, 0.2 gm. casein, and 0.1 gm. brewers yeast; the remainder of the diets consisted of cornstarch and fat in such proportions that the diets of the five groups ranged from 1.6 per cent to 26.0 per cent fat. All five diets provided 11.4 Calories daily. The amounts of protein, salts, and vitamins were the same among the several rations, whereas the proportions of these constituents increased with increasing proportions of dietary fat. The diets fed groups A121, A122, A123, and A124 contained 1.6, 5.7, 12, and 26 per cent fat, respectively, and the

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The caloric values of the rations, and the protein, mineral, fat, and carbohydrate contents were calculated on the basis of data supplied by the manufacturers of the dietary constituents.
diet fed group A125 contained 24 per cent fat and 2 per cent cholesterol. The diets are detailed in Table 1.

Table 2 illustrates the mean body weights of the mice not bearing mammary tumors at several periods up to 1 year of age; after this the number of tumor-free mice decreased rapidly, and the averages were less reliable. From about the 24th week on, the mice of the two groups ingesting the rations containing 26 per cent fat (A124 and A125) weighed consistently less, on the average, than those on the diets containing 2, 6, or 12 per cent fat. At 7 months of age, the mean body lengths (nose to base of tail) were 10.8, 10.0, 10.8, 10.7, and 10.7 cm., respectively, with a mean standard error of 0.12 cm.

The experiment was terminated when the mice were 2 years old. The results are given in Table 3. CSH strain mice fed calorically and nutritionally adequate diets characterized by high proportion of dietary fat, enhance the formation of mammary carcinoma; therefore, no considerable difference in the final tumor incidence was observed among the several groups. That increasing the proportion of fat in the diet does enhance the formation of mammary carcinoma is shown, however, in the increasing tumor incidence (when compared at times up to 80 weeks) and in the earlier average appearance of the tumors. The effects were not arithmetically proportional to the fat intake; the augmentation resulting from an increase in dietary fat from 1.6 to 5.7 per cent (A121 compared with A122) was as great as that of an increase from 5.7 to 26 per cent (A122 compared with A124). The two diets containing 26 per cent fat, either without (A124) or with (A125) added cholesterol, enhanced tumor formation to about the same degree.

Among the tumor-bearing mice in groups A121, A122, A123, A124, and A125, multiple mammary carcinomas occurred in 27, 53, 51, 60, and 53 per cent, respectively. Thus, fewer of the mice fed the ration containing 1.6 per cent fat developed more than one mammary tumor than did those receiving higher proportions of dietary fat.

The survival times of the individual mice, subsequent to the development of mammary carcinoma, ranged from 2 to 25 weeks; the average survival time among the several groups varied from 11.1 to 12.7 weeks and was not related to the proportion of dietary fat. The incidences of grossly visible metastases to the lungs varied from 54 to 88 per cent among the several groups, also showing no regular relation to fat intake.

Experiment 2.—Five groups, each consisting of 60 virgin dba females 10–14 weeks of age, were employed. The diet for each mouse included a basal portion consisting of casein, gelatin, salts, yeast extract, synthetic B vitamins, and a supplement containing vitamins A, D, and E. This basal portion was complemented by fat and cornstarch in amounts which provided different proportions of dietary fat for the five experimental groups: 2.0, 4.1, 8.0, 16.0, and 23.7 per cent. The daily ration for each mouse provided 10.9 Calories. The diets are detailed in Table 4. The experiment was terminated when the few surviving mice were 2 years old.

The proportion of dietary fat had little effect on the rate of weight gain or on the final body weight (Table 5). The results on tumor formation (Table 6) are in agreement with those of the previous experiment: Increasing proportions of dietary fat tended to increase the incidence of mammary carcinoma and to shorten the mean age of tumor appearance. The effects of increasing the fat content from 2 to 8 per cent (AP1 compared with AP3) were as great as those of an increase from 8 to 24 per cent (AP3 compared with AP5). There was a negligible effect, if any, attributable to an increase from 10 to 24 per cent (AP4 and AP5). In Table 6, the incidence of mammary tumors is computed on the basis of an adjusted total of mice, which cor-
rects for deaths of nontumor mice during the course of the experiment (6); this was done because 11–16 of the mice in each group died without tumors. These deaths were not associated with the dietary regimen and were due principally to lymphomata and leukemia (5–7 deaths in each group) and a small number of other primary tumors. A few deaths were associated with uterine infections and granulomas.

The interval from the appearance of a mammary tumor to the death of the animal ranged from 1 to 25 weeks among the individual mice, and averaged from 9.6 to 10.4 weeks among the several groups, there being no association with the level of dietary fat. Of the mammary cancer mice of groups AP1, AP2, AP3, AP4, and AP5, respectively, 17, 33, 26, 34 per cent had more than one mammary tumor. The incidence of grossly visible metastases to the lungs was not associated with the levels of dietary fat. The growth rates of all tumors in groups AP1 (2 per cent fat) and AP5 (24 per cent fat) were determined (16); the growth indices (average daily increment in the sum of major and minor axes in units of 0.1 mm.) ranged from 1.0 to 13.3 and averaged 5.61 ± 0.45 time of tumor appearance) tends to increase with increasing proportion of dietary fat. The rate of tumor formation was not arithmetically proportional to the fat intake; rather, the stimulatory effect was as pronounced when the proportion of dietary fat was increased from approximately 2 per cent to 6 or 8 per cent as when it was increased from the latter levels to 24 or 26 per cent. In fact, there was little effect, if any, produced by an increase from 16 to 24 per cent. In agreement with this plateauing of the effect of dietary fat, Boutwell et al. (4) found that a diet containing 61 per cent fat stimulated the formation of induced skin tumors to about the same extent as one containing 27 per cent fat.

This type of relation is not uncommon for many

### TABLE 3

**FORMATION OF MAMMARY CARCINOMA IN C3H MICE INGESTING DIETS CONTAINING DIFFERENT PROPORTIONS OF FAT**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>DIETARY NUMBER*</th>
<th>AGE AT APPEARANCE OF TUMORS (weeks)</th>
<th>50 PER CENT OF MICE ALIVE AND TUMOR-FREE AT 104 WEEKS†</th>
</tr>
</thead>
<tbody>
<tr>
<td>A121</td>
<td>0.85 52</td>
<td>17 29 50 62 73 81 27–103</td>
<td>58.4 ± 3.1</td>
</tr>
<tr>
<td>A122</td>
<td>0.85 52</td>
<td>17 29 63 73 83 87 20–101</td>
<td>58.3 ± 2.7</td>
</tr>
<tr>
<td>A123</td>
<td>0.85 52</td>
<td>23 50 68 75 81 90 94 24–100</td>
<td>53.1 ± 2.8</td>
</tr>
<tr>
<td>A124</td>
<td>0.85 52</td>
<td>31 54 65 79 86 90 94 27–97</td>
<td>51.7 ± 2.3</td>
</tr>
<tr>
<td>A125</td>
<td>0.85 47</td>
<td>30 57 74 78 85 87 89 31–99</td>
<td>48.4 ± 2.3</td>
</tr>
</tbody>
</table>

*Number of mice alive when first mammary carcinoma was observed in experiment (effective totals).
†End of experiment.
‡Including ±1 per cent added cholesterol.
§Age at which 50 per cent of the mice had tumors.

### TABLE 4

**DIETS EMPLOYED IN EXPERIMENT 2**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>CORN-STARCH</th>
<th>KREMAX</th>
<th>TOTAL</th>
<th>FAT</th>
<th>PROTEIN</th>
<th>MINERALS</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP1</td>
<td>0.855</td>
<td>0.09</td>
<td>0.055</td>
<td>3.0</td>
<td>2.0</td>
<td>23.0</td>
</tr>
<tr>
<td>AP2</td>
<td>0.855</td>
<td>1.04</td>
<td>0.115</td>
<td>2.91</td>
<td>1.1</td>
<td>24.0</td>
</tr>
<tr>
<td>AP3</td>
<td>0.855</td>
<td>1.09</td>
<td>0.115</td>
<td>2.76</td>
<td>1.0</td>
<td>25.0</td>
</tr>
<tr>
<td>AP4</td>
<td>0.855</td>
<td>1.24</td>
<td>0.395</td>
<td>2.49</td>
<td>1.6</td>
<td>23.0</td>
</tr>
<tr>
<td>AP5</td>
<td>0.855</td>
<td>0.59</td>
<td>0.555</td>
<td>2.28</td>
<td>23.7</td>
<td>30.0</td>
</tr>
</tbody>
</table>

*0.7 gm. vitamin-free casein (Borden Labco); 0.09 gm. gelatin (United Chemical and Organic Products Co., No. 14X); 0.09 gm. Osborne-Mendel salt mixture, Wesson Modification (11); 0.006 gm. cottonseed oil containing 20 U.S.P. units of Vitamin A, 2 U.S.P. units of D, and 0.8 mg. of E (Distillation Products, Inc.). The B vitamins were supplied in 0.03 gm. yeast extract (Anheuser-Busch, No. 3) and the following synthetic vitamins: thiamin chloride, 60 mg.; pyridoxine HCl, 60 mg.; riboflavin, 50 mg.; folie acid, 10 mg.; biotin 0.03 mg.; niacin, 300 mg.; Ca pantothenate, 180 mg.; p-aminobenzoic acid, 600 mg.; inositol, 3 mg.; choline chloride, 15 mg.

†All diets supplied 10.8 Calories daily. Calorie value of cornstarch, 3.6 Calories per gram; of Kremax, 9.0 Calories per gram.

The results of the two experiments are in agreement and indicate that the rate of formation of spontaneous mammary carcinoma of the mouse (as measured both by tumor incidence and average
biologically active substances, such as vitamins and hormones. These produce successively greater effects as the dose is increased from a low to an optimal level; increases beyond this optimal level have no further effect.

There were no significant differences in caloric intake or body weight among the groups of an experiment, and, therefore, the enhancement of tumor formation was not mediated through either of these two factors.

In contrast to the stimulatory effect of high dietary fat on the formation of spontaneous mammary carcinoma was the lack of effect on the growth of the tumors, survival time of the mice following the appearance of the tumors, and the incidence of metastases to the lungs; all these indicate that fat enrichment of the diet has no significant effect upon the growth of spontaneous mammary carcinomas.

The numerous experiments performed by us and others on the effect of high-fat diets on tumor formation have resulted in probing of the mode of action of this experimental procedure. As yet, no single incontrovertible explanation has been offered.

We have suggested (16, 18) that substituting fat for isocaloric amounts of carbohydrate in the diet may affect the genesis of tumors by at least two means: (a) a local action (solvent effect), brought about by an increased fat content of the tissue involved, under which conditions the rate of transfer or amount of carcinogen is altered; and (b) an independent effect of fat on the developing tumor cell. This hypothesis was offered to explain the augmenting effect of a fat-enriched diet on the formation of spontaneous mammary and induced skin tumors, and the slight retardation of the genesis of carcino-gen-induced sarcoma. The lack of any effect of a high-fat diet on the incidence of spontaneous lung adenoma may be due to the fact that the lung is not a fat depot (13) and is therefore unaffected by varying percentages of fat in the diet. The effect 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.

Recently, Boutwell, Brush, and Rusch (4) have suggested that the increasing efficiency of utilization of diets due to increasing proportions of fat may be the factor responsible for the action of fat-enriched diets in accelerating the formation of skin tumors induced by 3,4-benzpyrene. Although they discussed certain qualifications, they concluded that “the difference in the net energy value of low and high fat diets is sufficient to explain the stimulating effect of fat on the induction of skin tumors with carcinogenic hydrocarbons.” This interpretation was based principally on the work of Forbes, Swift, and co-workers (2, 3, 7, 8, 9, 10) who have shown that, in rats, high-fat diets are metabolized with lower net energy expenditure (higher net body energy gain) than corresponding equicaloric low-fat diets. The question arises as to whether the latter’s findings may be interpreted as indicating that the enhancing effect of high-fat diets on the formation of tumors occurs through a decrease in energy expense of utilization. The higher net body energy might be considered equiv-
alent to an increase in caloric intake, which is known to augment the rate of formation of tumors (15, 19).

The data of Forbes, Swift, and co-workers indicate that increasing the proportion of fat, in equicaloric diets, from 2 to 30 per cent causes a decreasing energy expense of utilization and a corresponding gain in body energy. For mature rats, with cage activity excluded, this difference might amount to as much as 9 per cent of the total gross energy of the diet (8, 10). However, for both young and mature rats not restricted in activity, the difference in net body energy gain between animals on 30 per cent and those on 2 per cent fat diets appears no greater than 2 to 3 per cent of the total gross energy of the diet (2, 3, 7, 9). This is equivalent to 0.2 to 0.3 Calories per day for a mouse consuming 10 Calories and allowed normal activity. These latter conditions are comparable to those employed by Boutwell, Brush, and Rusch and those in the experiments reported in this paper.

It is our opinion that the results of Forbes, Swift, and co-workers were inadvertently misapplied by Boutwell, Brush, and Rusch (4). First, they employed the data (8, 10) concerned with resting animals (restricted activity) rather than the data dealing with animals permitted unrestricted cage activity (2, 3). Second, they did not discriminate between the gross energy of the total diet and the gross energy of the supplement (the supplement being that part of the diet supplied above the maintenance level). In Figure 2, page 745, of their paper, they used the data of Forbes, Swift, and co-workers (8, 10) that refer to the energy expense of utilization of the supplement. However, Boutwell, Brush, and Rusch, in their calculations, treat the curve as if it applied to the gross energy of the total diet. Thus, they calculated a value of 1.4 Calories as the difference between the dynamic effects of the 10-Calorie rations containing 2 or 27 per cent fat, instead of the value of approximately 0.3 Calories applicable to their experimental conditions and ours (see preceding paragraph).

This increase, approximately 0.3 Calories, in net body energy gain is definitely not large enough to produce the enhancement of tumor formation reported by us in this paper or by Boutwell, Brush, and Rusch. It would actually require an increase in net body energy, or an increase in caloric intake, of approximately 1.0--1.5 Calories per day to produce the observed effects on tumor formation.

The application of data on specific dynamic action obtained with rats to the interpretation of results on tumor formation in mice appears valid in this instance, as was indicated by Boutwell et al. Probably, many mammals expend less energy on utilization of high-fat diets (5), and it is unlikely that the mouse differs greatly in this respect from the rat. However, there are no data to support the view that for the mouse there is a daily net energy "sparing" of 1.4 Calories resulting from ingestion of 10 Calories of a high-fat diet as compared with one containing only 2 per cent fat. If the sparing effect were this large, one might expect the mice fed high-fat diets to weigh at least 1.5 to 2.0 gm. more than those fed low-fat diets, and this does not consistently occur, as can be seen from our data and those of Boutwell, Brush, and Rusch.

More significantly, if the augmenting effect of high-fat diets on tumor formation were mediated mainly through the increased net body energy gain, one would expect that high-fat diets would enhance the formation of all types of tumors affected by the level of caloric intake. However, of all the tumors of the mouse which respond to varying the level of caloric intake, only the formation of the spontaneous mammary carcinoma and tumors of the skin induced by ultraviolet radiation or carcinogenic chemicals are modified by fat-enrichment of the diet (16), whereas the incidence of induced sarcoma, spontaneous lung adenoma, induced leukemia, and spontaneous leukemia is not affected (12, 16).

Considering it as an effective increase in available calories, the small increase in net body energy that accompanies the consumption of a fat-enriched diet may be a minor factor in the enhancement of tumor formation. However, attempts to relate the mechanism through which fat-enriched diets enhance tumor formation to the mechanism through which caloric intake exerts its influence seem premature, inasmuch as so little is known about either mechanism.

**SUMMARY**

The primary objective of the study was to determine the relationship between the proportion of fat in the diet and the resultant enhancement of the formation of spontaneous mammary carcinoma. One experiment utilized C3H strain mice, 

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1 In long-term experiments, with growing animals, it is difficult to accurately ascribe the exact value of the dietary "maintenance" level, and consequently the "supplement"—in fact, these change with increasing weight of the mice. According to Boutwell et al. (4), 9 Calories maintained mice at 25-36 gm. throughout the experiment, whereas 10 Calories maintained mice at 31--32 gm. during the major part of the experiment. Thus, for their group on the 10-Calories ration, the "supplement" may be considered to have decreased from 2 to 0 Calories during the course of the experiment.

2 This estimate is based on data from experiments employing graded caloric restriction (4, 17).
with diets based on commercial components and four levels of dietary fat: 1.6, 5.7, 12, and 26 per cent; the second experiment utilized dba strain mice, with diets composed of semi-purified components and five levels of dietary fat: 2, 4, 8, 16, and 24 per cent. The diets were isocaloric, and the mean body weights were similar.

In both experiments, the rate of formation of mammary carcinoma, as measured both by incidence of tumors and by the average time of tumor appearance, tended to increase with increasing proportions of dietary fat. However, the effect was not arithmetically proportional to the level of fat: the enhancement of tumor formation resulting from increasing the dietary fat from 2 to 6 or 8 per cent was as great as that resulting from increasing the dietary fat from 6 or 8 per cent to 24 or 26 per cent. In contrast to the effect on tumor formation, phenomena related to the growth of the mammary carcinomas—increase in tumor size, survival time of the animal after appearance of the tumor, or the incidence of grossly visible metastases to the lungs—were not affected by the proportion of dietary fat in the range studied.

The decrease in energy expenditure of utilization of high-fat rations is too small to account entirely for the observed enhancement of tumor formation. Other suggestions are made, but it must be concluded that, at present, the mechanism through which fat-enriched diets accelerate the formation of spontaneous mammary carcinoma and carcinogen-induced skin tumors of the mouse is not known.

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