Overweight in man has been correlated with a higher incidence of cancer than that observed in normal or in underweight persons (12). Considerable experimental evidence indicates that diets high in fat or in caloric content increase the probability of the occurrence of many types of cancer in the mouse (1, 2, 5, 15, 18). Conversely, restricted caloric intake (13, 14, 17) prevents or delays the onset of both experimentally induced and spontaneously occurring neoplasms in the mouse. In the rat, dietary fat (6) was found to be much less effective in enhancing chemically induced skin cancer than in the mouse. In the absence of added fat in a semi-synthetic diet (11), no liver tumors were induced by p-dimethylaminoazobenzene, but the rats grew poorly and few survived the minimum latent period, while the addition of varying quantities of fat proportionately accelerated the formation of these liver tumors. An exception appears to be hydrogenated coconut oil (8). When 5 per cent hydrogenated coconut oil was the only source of fat in a synthetic diet, the formation of dimethylaminoazobenzene-induced liver tumors was retarded. Chronic undernourishment (7) effectively prolonged the life span of rats and delayed the onset of pathological ageing processes and also gave limited evidence of a retardation in the development of spontaneous tumors.

In the mouse, the amount of estradiol benzoate (16) required to produce a minimum duct response of the mammary gland is considerably increased as the level of food intake is decreased. Chronic underfeeding also results in pseudo-hypophysectomy (9) and lack of adrenotropic hormone (10). Because caloric intake in mice has proved to be such an important factor in the initiation of spontaneously occurring mammary cancers, and because rats on high-fat diets tend to ingest more calories per day than on diets of low fat content, it seemed important to assay the effects of dietary fat under conditions in which the caloric intake was rigidly controlled, even though it necessitated limiting considerably the number of rats that could be used. Accordingly the following experiments were undertaken.

MATERIAL AND METHODS

Pedigreed female rats of A X C Line 9935 between 4 and 5 months of age were used for the experiments. Cholesterol pellets containing 4 to 6 mg. of diethylstilbestrol1 were implanted subcutaneously in the scapular region to maintain a continuous state of hyperestrinism. A previous report (3) showed that on the laboratory stock diet of Friskie Dog Pellets supplemented by a green vegetable once a week, 85 per cent of the rats of this line, when similarly treated, developed multiple mammary cancers between the eleventh and twentysix-second months.

In the present experiments each rat was housed in an individual cage with free access to water. The daily portion of food was weighed out and presented in a food cup to which was added the daily supplement of crystalline vitamins. An attempt was made to recover and weigh all food that was spilled, but there was probably some unaccounted for waste of food by the rats on the ad libitum diets. The first 24 rats were pair fed. That is, the mates of the rats on the ad libitum low-fat diet received an isocaloric ration of high-fat diet equivalent to that consumed by the mate on the low-fat diet.

The base diet consisted of the following ingredients:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Per Cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellu flour</td>
<td>2.0</td>
</tr>
<tr>
<td>Halibut liver oil</td>
<td>0.4</td>
</tr>
<tr>
<td>Salt mixture</td>
<td>0.4</td>
</tr>
<tr>
<td>Crisco</td>
<td>5.0</td>
</tr>
<tr>
<td>Casin</td>
<td>30.0</td>
</tr>
<tr>
<td>Dextrin</td>
<td>60.0</td>
</tr>
</tbody>
</table>

plus the following crystalline vitamin2 supplement per kilo:

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Supplement per kilo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiamin</td>
<td>4 mg.</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>8 mg.</td>
</tr>
<tr>
<td>Pyridoxine HCl</td>
<td>4 mg.</td>
</tr>
<tr>
<td>Niacin</td>
<td>4 mg.</td>
</tr>
<tr>
<td>Calcium pantothenate</td>
<td>20 mg.</td>
</tr>
<tr>
<td>Choline HCl</td>
<td>2,000 mg.</td>
</tr>
<tr>
<td>Alpha-tocopherol</td>
<td>150 mg.</td>
</tr>
</tbody>
</table>

1 Supplied through the courtesy of Dr. D. F. Robertson of Merck and Co., Rahway, N.J.
2 Supplied through the courtesy of Dr. R. C. Pogge, Merck and Co., Rahway, N.J.
The base diet was combined with Crisco and dextrin to make up the three following rations:

<table>
<thead>
<tr>
<th>Group</th>
<th>1. Low Fat</th>
<th>II. Modified Low Fat</th>
<th>III. High Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base</td>
<td>30 gm.</td>
<td>30 gm.</td>
<td>30 gm.</td>
</tr>
<tr>
<td>Crisco</td>
<td>5 gm.</td>
<td>10 gm.</td>
<td>30 gm.</td>
</tr>
<tr>
<td>Dextrin</td>
<td>65 gm.</td>
<td>60 gm.</td>
<td>8.3 gm.</td>
</tr>
</tbody>
</table>

The low-fat diet contained 4.3 calories per gram, the modified low-fat diet, 4.6 calories per gram, and the high-fat diet, 6.3 calories per gram. The three diets contained respectively, 6.5, 11, and 46 per cent Crisco and 83, 78, and 38 per cent dextrin. Each rat on ad libitum rations was offered daily a weighed portion equal to a little more than she had consumed the previous day. The rats on restricted diets always consumed all that was offered them. An attempt to restrict the calorie intake to 22 calories per day (65 per cent of the amount consumed by those on ad libitum low-fat rations) resulted in failure, due to the death of a majority of the rats within 60 days. A restriction to 25 calories per day permitted the rats to live beyond the average survival period of the rats on ad libitum rations, but they lost considerable weight, were irritable, and constantly in search of food.

A restriction to 25 calories was made further.

The experiment included 84 rats in six groups as shown in Table 1. The first group of 12 rats consumed daily an average of 7.8 grams of the low-fat diet or 84 calories. Their paired mates consumed an iso-caloric portion equal to a little more than she had consumed the previous day. The rats on restricted diets always consumed all that was offered them. An attempt to restrict the calorie intake to 22 calories per day (65 per cent of the amount consumed by those on ad libitum low-fat rations) resulted in failure, due to the death of a majority of the rats within 60 days. A restriction to 25 calories per day permitted the rats to live beyond the average survival period of the rats on ad libitum rations, but they lost considerable weight, were irritable, and constantly in search of food.

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Each rat was weighed and inspected for mammary cancers once a week. At death, a thorough postmortem examination included a detailed description of every visible tumor, gross sectioning of all mammary glands and the inspection and weight of the liver, kidneys, adrenals, pituitary, and sex glands. Representative sections of each of these tissues and organs were preserved for microscopic examination. All cancer foci that could be identified were tabulated.

RESULTS

The results are tabulated briefly in Tables 2 and 3 and shown graphically in Charts 1, 2, and 3. All of the rats on ad libitum rations and the majority of the restricted rats survived for at least 6 months, the approximate minimum latent period for gross mammary cancers. The heaviest losses were among the restricted rats on the low-fat diet. These deaths occurred between the twenty-sixth and fifty-fifth days after being placed on the restricted diet and their records will not be considered further.

The rats fed ad libitum were heavier at death than the rats on the restricted diets but even those on the high-fat diets were not overweight. The livers were proportionately heavier in the rats on the high-fat diets than in the rats on the low-fat diets, but showed no evidence of fatty infiltration. The pituitaries were largest in two of the groups on the high-fat diet, i.e., those on the restricted high-fat diet and the high-fat mates of the rats on the ad libitum low-fat diet. The mean pituitary weight for the rats on the ad libitum low-fat diet was 69 ± 15 mg. compared with 152 ± 22 mg. for their paired mates on the high-fat diet and 96 ± 20 mg. for the rats on the ad libitum high-fat diet. On the restricted high-fat diet, the mean pituitary weight was 148 ± 24 mg. compared with 70 ± 14 mg. for rats on the restricted low-fat diet. Otherwise, there were no significant differences in the organs which were weighed as shown in Table 2. Although the rats on the restricted low-fat diet showed considerable inanition and, judging by our experience in attempting to reduce their consumption by an additional 3 calories daily, were fairly close to the starvation level, no changes were noted in their adrenals, which would indicate a depression in the physiological function of the pituitary.

The mammary cancer history is given in Table 3. Of the 67 rats which survived for 180 days, 58 or 87 per cent developed one or more mammary cancers. This is about the same proportion previously observed for rats of this line on the stock diet. The different groups varied somewhat. Comparing the paired mates on iso-caloric high-fat and...
low-fat diets, 9 of the 12 on the low-fat diet developed 34 gross tumors, while 12 mates had 52. Consideration of the cancer foci found on microscopic examination of the mammary tissue increased the number of cancer foci in the rats on the high-fat diet to twice that of their mates on the low-fat diet or 157 and 78, respectively. The gross tumors in the rats on the high-fat diet averaged 6.5 grams in weight compared with 5.0 grams for the tumors of their mates. The minimum and average latent periods were shorter for the tumors in the rats on the high-fat diet, being 153 and 288 days, respectively, compared with 175 and 304 days for the tumors in the rats on the low-fat diet. These observations appear to support the concept that fat is a mammary cancer accelerator. Inspection of Chart 2, however, reveals that four of the rats on the low-fat diet developed their first tumors somewhat earlier than their mates on the isocaloric high-fat diet and in one instance the rat on the low-fat diet had eighteen probably independent tumors, while its mate had only four. In another in-

TABLE 2
THE NUMBER OF RATS WHICH SURVIVED FOR AT LEAST 180 DAYS, THEIR AVERAGE SURVIVAL IN DAYS, POSTMORTEM BODY WEIGHTS IN GRAMS, AND THE AVERAGE PERCENTAGE WEIGHS OF SOME OF THE ORGANS

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NO. OF RATS</th>
<th>AVERAGE SURVIVAL IN DAYS</th>
<th>POSTMORTEM BODY WT.</th>
<th>LIVER WEIGHT</th>
<th>KIDNEY WEIGHT</th>
<th>ADRENAL WEIGHT</th>
<th>PITUITARY WEIGHT</th>
<th>OVARY WEIGHT</th>
<th>UTERUS WEIGHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad lib low-fat</td>
<td>12</td>
<td>392</td>
<td>139</td>
<td>4.9</td>
<td>0.9</td>
<td>0.4</td>
<td>0.04</td>
<td>0.04</td>
<td>0.06</td>
</tr>
<tr>
<td>High-fat paired mates</td>
<td>12</td>
<td>392</td>
<td>144</td>
<td>6.0</td>
<td>1.2</td>
<td>0.4</td>
<td>0.10</td>
<td>0.06</td>
<td>0.50</td>
</tr>
<tr>
<td>Ad lib high-fat</td>
<td>12</td>
<td>383</td>
<td>145</td>
<td>6.1</td>
<td>1.1</td>
<td>0.5</td>
<td>0.07</td>
<td>0.06</td>
<td>0.40</td>
</tr>
<tr>
<td>Restricted high-fat</td>
<td>10</td>
<td>488</td>
<td>110</td>
<td>5.4</td>
<td>1.2</td>
<td>0.5</td>
<td>0.13</td>
<td>0.06</td>
<td>0.58</td>
</tr>
<tr>
<td>Restricted 11% fat</td>
<td>9</td>
<td>508</td>
<td>116</td>
<td>5.7</td>
<td>1.0</td>
<td>0.4</td>
<td>0.08</td>
<td>0.03</td>
<td>0.55</td>
</tr>
<tr>
<td>Restricted low-fat</td>
<td>12</td>
<td>431</td>
<td>96</td>
<td>4.8</td>
<td>1.4</td>
<td>0.5</td>
<td>0.07</td>
<td>0.05</td>
<td>0.66</td>
</tr>
</tbody>
</table>

The rats on the ad libitum high-fat diet consumed more fat and an average of six more calories daily, but one of the twelve died without any evidence of mammary cancer and another had only two small growths. The minimum and average latent periods as shown in Chart 3 were essentially the same as for the rats on the ad libitum low-fat ration. One real difference seemed to be in the size of the tumors (Table 2). The average weight of the gross tumors at death was 9.3 grams or 80 per cent larger than the mean weight of 5.0 grams for the tumors in the rats on the ad libitum low-fat diet and more than 40 per cent higher than the average weight of 6.5 grams for the tumors in the latter's pair-fed mates on the high-fat diet. Another factor seemed to be the prevalence of tumors of the uterus in rats of this group as shown in Chart 2. Four of the 12 rats had malignant tumors involving the uterus: an adenocarcinoma, a squamous cell cancer, a mixed tumor of the ovary and uterus, and another tumor of the uterus which was probably an adenocarcinoma. No tumors of this organ were observed in the rats on the other dietary regimens, although fifty-one of them survived for more than 250 days (the number of days before the observation of the first of these tumors). Spontaneous tumors of the uterus have been observed in untreated rats of this line in...
about the frequency of 1 in 200. Two additional
tumors, which may be unrelated to the treatment,
were a benign adenoma of the mammary gland in
a rat on the restricted high-fat diet and a car-
cinoma of the adrenal in a rat on the ad libitum
low-fat diet. However, adrenal tumors (4) are a
fairly common sequel to estrogenic treatment in
rats of another inbred line.

In general physical appearance, the 12 rats on
the ad libitum high-fat diet compared unfavorably
with the 12 rats on the same diet, limited in quan-
tity to equal the caloric value of an ad libitum low-
fat mate. It seemed that above a certain level ad-
ditional fat consumption was detrimental to the
physical well being of the rat and interfered with
the initiation of the mammary cancer. These rats
did not live quite as long or develop as many tu-
mors per rat, but once a tumor could be identified,
it appeared to grow much faster than the tumors
of the rats in any of the other groups.

The average survival period of the rats on re-
stricted diets was considerably prolonged and the
minimum and average latent periods for the tu-
mors were increased as shown by Chart 3. The per-
centage of rats which eventually developed tu-
mors was not significantly lessened as shown in
Table 3 and Chart 2. The growth of the tumors
was greatly retarded as shown (Table 3) by their
average weights. The number of cancer foci identi-
fied by microscopic examination of the mammary
tissue was not less in the rats on the restricted low-
fat diets than had been observed in the rats on the
ad libitum low-fat diet. The percentage of rats that
developed neoplasms was lowest (50 per cent)
among the rats on the restricted high-fat diet, but
the average number of gross and microscopic tu-
mors per tumor-bearing rat was 12 or higher than
in any other group. Probably the employment of a
larger number of rats would reduce these devia-
tions, but not alter the general observations that
the state of inanition caused by a restriction in the
caloric intake of the rat delays considerably the
onset of the malignant process in the mammary
gland, even when there is no evidence of hormonal
insufficiency, and that increased fat concentration
in the diet stimulates mammary growth and secre-
tion and accelerates the growth of the once formed
cancer cells.

Histologically, the mammary glands from rats
fed diets of high fat content showed more hyper-
trophy and secretory activity than did the breast
tissues of the rats on diets of lower fat content. In
general, more gross tumors per rat were observed
and a greater number of microscopic foci were
identified as neoplastic in the rats on the high-fat
diets than in those of other groups. The multiple
minute tumors were readily found in the breast tis-
sue that showed the greatest hyperplasia and se-
cretory activity.
The 386 gross tumors and 337 additional neoplastic foci found by microscopic study of the preserved mammary tissues were all classified as adenocarcinomas. The larger growths were mostly papillary and cystic. The histologic features of all of these tumors were comparable, but the development of the neoplasm could more easily be traced in the rats fed diets low in fat content because the developmental changes were less abrupt. Early alterations were generalized hyperplasia (Fig. 1) followed by an enlargement of the lobules with a coalescence of the contained follicles (Figs. 2 and 3) to form a somewhat homogenous eosin stained material. In the expanded follicles, the limits of each acinus were indistinct and the lining epithelium was partly shed. Alterations in the follicular epithelial cells were observed in the areas showing the grosser architectural changes. Cell boundaries of rigidly controlled calorific intake, 84 A × C Line 9938 female rats, with cholesterol pellets containing 4 to 6 mg. of diethylstilbestrol implanted in their scapular region, were distributed into 6 groups and placed on iso-caloric synthetic rations of varying fat and carbohydrate composition.

2. Diets adequate in protein, minerals, and vitamins, but varying in fat content from 6.5 per cent to 46 per cent Crisco with sufficient dextrin to equalize the calorific content were fed ad libitum and restricted to rats in individual cages.

3. The calorific intake varied from 40 calories daily for rats on the ad libitum high-fat diet to 34 calories daily for those on the ad libitum low-fat diet and their paired mates on the high-fat diet, and was restricted to 25 calories daily in iso-caloric portions of the high-fat, modified low-fat, and low-fat diet in three additional groups.

### TABLE 3

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Number of rats</th>
<th>Percent with cancer</th>
<th>Number of carcinomas</th>
<th>Average weight</th>
<th>Minimum latent period</th>
<th>Average latent period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad lib low-fat</td>
<td>12</td>
<td>9</td>
<td>75</td>
<td>54</td>
<td>78</td>
<td>175</td>
</tr>
<tr>
<td>High-fat paired mates</td>
<td>12</td>
<td>12</td>
<td>100</td>
<td>52</td>
<td>157</td>
<td>6.3</td>
</tr>
<tr>
<td>Ad lib high-fat</td>
<td>12</td>
<td>11</td>
<td>92</td>
<td>47</td>
<td>109</td>
<td>9.3</td>
</tr>
<tr>
<td>Restricted high-fat</td>
<td>10</td>
<td>5</td>
<td>50</td>
<td>33</td>
<td>62</td>
<td>4.1</td>
</tr>
<tr>
<td>Restricted 11% fat</td>
<td>9</td>
<td>9</td>
<td>100</td>
<td>30</td>
<td>81</td>
<td>3.5</td>
</tr>
<tr>
<td>Restricted low-fat</td>
<td>12</td>
<td>12</td>
<td>100</td>
<td>40</td>
<td>86</td>
<td>4.0</td>
</tr>
<tr>
<td>Total</td>
<td>67</td>
<td>58</td>
<td>87</td>
<td>254</td>
<td>73</td>
<td>53</td>
</tr>
</tbody>
</table>

of the very early neoplasms were sharply defined, the nuclei were larger and the nucleoli more conspicuous than in the surrounding epithelial cells. Under low power observation, the follicles of these early tumors appeared hyperchromatic (Figs. 4 and 5).

In somewhat larger tumors a sharp separation from the adjacent non-neoplastic breast stroma was evident. The central portions of the follicles had fused to form small cystic spaces (Fig. 6) and fingerlike projections from the walls of ruptured acini hung freely into these cystic spaces. The large tumors seen in the gross specimens were composed of solid masses (Fig. 7) and rounded cords of epithelial cells interspersed with cysts containing projecting masses of loose, extremely hyperchromatic cells (Fig. 8). These nodular areas showed little evidence of secretory activity and the solid cords of tumor cells simulated the appearance of early intra ductal carcinoma of the human breast.

### SUMMARY

1. In order to assay the effects of dietary fat on mammary cancer development under conditions...
Fig. 1.—Generalized hyperplasia of the mammary gland of a rat on the high-fat diet after 327 days of treatment with a pellet containing 5 mg. of diethylstilbestrol. X150.

Fig. 2.—Increased secretory activity and hyperchromatism in the mammary gland of a rat on the low-fat diet after 399 days of treatment with a pellet containing 5 mg. of diethylstilbestrol. X150.

Fig. 3.—Same as Fig. 2. X250.

Fig. 4.—Coalescent lobules and hyperchromatism in the mammary gland of a rat on the low-fat diet after 394 days of treatment with a pellet containing 5 mg. of diethylstilbestrol. X150.
Fig. 5.—Tumor nodule in the mammary gland of a rat on the low-fat diet after 288 days of treatment with a pellet containing 5.0 mg. of diethylstilbestrol. ×150.

Fig. 6.—Multiple tumor nodules separated by normal stroma in the mammary gland of a rat on the high-fat diet after 399 days of treatment with a pellet containing 5.5 mg. of diethylstilbestrol. ×125.

Fig. 7.—Solid nodule of carcinoma in the mammary gland of a rat on the low-fat diet after 327 days of treatment with a pellet containing 5.0 mg. of diethylstilbestrol. ×150.

Fig. 8.—Malignant papillary area in the mammary gland of a rat on the low-fat diet after 399 days of treatment with a pellet containing 5.0 mg. of diethylstilbestrol. ×150.
9. When the high-fat diet was restricted to 25 calories daily, or fed ad libitum at an average consumption of 40 calories daily, neither the number of tumors induced nor the latent periods differed significantly from those on ad libitum low-fat diets.

10. The only consistent effect of the high-fat diet appeared to be an acceleration of the growth rate of the induced tumors.

11. Extensive hypertrophy and evidence of an increased secretory activity were consistent histologic features of the mammary gland of the rats on the high-fat diet, although no evidences of hormone insufficiency were observed in the mammary glands of the rats on low-fat or restricted diets.

12. All 236 gross tumors of the mammary glands and 337 additional microscopic foci were characterized as adenocarcinomas. All of the tumors were generally similar in structure, but the developmental changes appeared more abrupt in the tumors of the rats on the high-fat diet.

REFERENCES


The Effect of Dietary Fat and Carbohydrate on Diethylstilbestrol-induced Mammary Cancer in Rats

W. F. Dunning, M. R. Curtis and M. E. Maun