The Influence of the Degree of Caloric Restriction on the Formation of Skin Tumors and Hepatomas in Mice*

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Chronic caloric restriction inhibits the formation of various tumors of the mouse: spontaneous mammary carcinoma, spontaneous lung adenoma, induced skin tumor, induced sarcoma, and spontaneous and induced leukemia. Many workers have contributed to this knowledge, and the field has been recently reviewed (1). Studies have been conducted in an attempt to learn more about the conditions and factors that modify the caloric effect. Two of these (2, 3) have demonstrated that, for both the carcinogen-induced skin tumor and the spontaneous mammary carcinoma of the mouse, the extent of inhibition of tumor formation is directly dependent on the degree of caloric restriction.

The present paper is concerned with the extension of the studies on the effect of caloric restriction to the spontaneous hepatoma of the C3H male mouse. At the same time the relationship of graded caloric restriction to the formation of the carcinogen-induced skin tumor was re-investigated.

METHODS

Strain C3H male mice, bred in our laboratory, were utilized in these experiments. They were fed a commercial ration, Purina fox chow checkers, from weaning until the experimental diets were instituted. Litter mates were distributed among the groups of an experiment so far as possible. The mice fed ad libitum were housed 5 animals to a cage. In the groups fed calorie restricted rations, each set of 5 was kept in 2 cages; at biweekly weighings the lighter animals were placed in one cage, the heavier in the other. Throughout the experiment, the 5 calorie restricted mice of a set competed for the available food with others of nearly the same weight; thus they consumed approximately equal amounts of food.

The diets were composed of Purina fox chow meal, skimmed milk powder, and cornstarch. A week's supply of the weighed constituents was mixed with sufficient water to form an easily molded mash which was spread in pans, cut into blocks of appropriate size, and stored in a refrigerator (38° F). The diets were fed daily, usually at 1 P.M. All animals had free access to drinking water.

Each mouse was numbered and its course recorded separately. At 2-week intervals the animals were inspected for neoplasms and weighed. All mice were autopsied postmortem: when the tumors became large, at death, or at the termination of the experiment. Tumors were recognized grossly; microscopic sections were made of a considerable proportion of the lesions, as well as of the few that were questionable. Most of the carcinogen-induced skin tumors were papillomas when first observed and many of these progressed to carcinomas; a few were sarcomas. The hepatic tumors observed at autopsy were spontaneous benign hepatomas; they have been described previously by several investigators (4, 5, 6).

EXPERIMENTS

Experiment 1.—Six groups of 50 mice were employed. The daily rations for the mice of the several groups ranged from 2.3 to 4.0 gm. The ration for mice of group 1–23 was composed of 1.4 gm. Purina fox chow meal and 0.9 gm. skimmed milk powder. As indicated in Table 1, all diets contained

<table>
<thead>
<tr>
<th>Group</th>
<th>Purina fox chow meal (gm)</th>
<th>Skimmed milk powder (gm)</th>
<th>Cornstarch (gm)</th>
<th>TotalGrams</th>
<th>Calories</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–23</td>
<td>1.4</td>
<td>0.9</td>
<td>0</td>
<td>2.3</td>
<td>8.1</td>
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<td>1–26</td>
<td>1.4</td>
<td>0.9</td>
<td>0.3</td>
<td>2.6</td>
<td>9.2</td>
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<tr>
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<td>0.6</td>
<td>2.9</td>
<td>10.3</td>
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<tr>
<td>1–32</td>
<td>1.4</td>
<td>0.9</td>
<td>0.9</td>
<td>3.2</td>
<td>11.4</td>
</tr>
<tr>
<td>1–36</td>
<td>1.4</td>
<td>0.9</td>
<td>1.3</td>
<td>3.6</td>
<td>12.8</td>
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<tr>
<td>1–40</td>
<td>1.4</td>
<td>0.9</td>
<td>1.7</td>
<td>4.0</td>
<td>14.3</td>
</tr>
</tbody>
</table>

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sisted of this basic ration to which varying amounts of cornstarch, from 0 to 1.7 gm., were added. Inasmuch as the cornstarch is almost entirely carbohydrate (and about 10 per cent water) the diets for the several groups contained the same amounts of protein, fats, vitamins, and minerals (contributed by the fox chow meal and the milk powder), and differed only in the amounts of carbohydrate. The computed caloric values ranged from 8.1 calories for the diet of group 1–23 to 14.3 calories for that of group 1–40. Correspondingly, as the amount of cornstarch was increased the proportion of protein and other essentials decreased.

The experimental diets were instituted when the mice were 8 to 10 weeks old. Initially, the mice of group 1–40 ate only 3.8 gm. of food, but within a few months they consumed all their daily ration. The mice of the other groups consumed their entire allotment of food from the outset. Three weeks after institution of the diets, the mice were given the first application of a 0.3 per cent solution of methylcholanthrene in acetone; a single drop of the solution was applied to the interscapular area by means of a dropping pipet. Seven such applications were given, at 5 day intervals, over a period of 30 days.

Changes in body weight of the mice are shown in Figure 1. Within 10 to 20 weeks after institution of the rations, the several groups reached average weight levels which were maintained fairly well throughout the experiment. These average body weight levels were approximately in direct proportion to the daily caloric intake. The experiment was terminated 51 weeks after the first application of carcinogen. The pertinent data are given in Table 3. Groups 1–40, 36, 32, 29, 26, and 23 had, respectively, 64, 47, 1.5, 11, 0, and 0 per cent hepatomas. As with the induced skin tumor, the incidence of hepatomas among the several groups decreased with decreasing caloric intake. The decrease in mean size of the hepatomas with decrease in caloric intake suggests that the hepatomas may have originated later in the more restricted groups. However, the differences in size are not large and might be de-

At the termination of the experiment the remaining animals were examined for hepatomas. A few of these mice had skin tumors; inasmuch as there was no correlation between the presence of a skin tumor and that of a hepatoma, all the mice, whether or not they had skin lesions, were employed for evaluating the incidence of hepatomas. The pertinent data are given in Table 3. Groups 1–40, 36, 32, 29, 26, and 23 had, respectively, 64, 47, 13, 11, 0, and 0 per cent hepatomas. As with the induced skin tumor, the incidence of hepatomas among the several groups decreased with decreasing caloric intake. The decrease in mean size of the hepatomas with decrease in caloric intake suggests that the hepatomas may have originated later in the more restricted groups. However, the differences in size are not large and might be de-

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**TABLE 2**

<table>
<thead>
<tr>
<th>Daily ration (gm)</th>
<th>Number of mice</th>
<th>Week after first application of carcinogen</th>
<th>Mean time of appearance of skin tumors (weeks)</th>
<th>Number of mice alive and tumor-free at end of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>10</td>
<td>20</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>1–23</td>
<td>2.3</td>
<td>37</td>
<td>0</td>
<td>0</td>
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<td>1–26</td>
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<td>45</td>
<td>0</td>
<td>2</td>
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<tr>
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<td>50</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>1–32</td>
<td>3.2</td>
<td>48</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>1–36</td>
<td>3.6</td>
<td>50</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>1–40</td>
<td>4.0</td>
<td>49</td>
<td>12</td>
<td>19</td>
</tr>
</tbody>
</table>

*Initially there were 50 mice in each group. The "number of mice" is the total adjusted for deaths of mice without tumors (7).
†Experiment terminated 51 weeks after first application of carcinogen.
‡The 6 tumors in this group were observed at the 39th week.

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**FIG. 1.—Growth of strain C3H male mice on different levels of caloric intake (Experiment 1).**
pendent on modification of the growth rates by the different caloric consumptions.

Experiment 2.—In the preceding experiment caloric restriction was achieved by limiting only the amount of carbohydrate; this means of caloric restriction leads to a greater proportion of protein, fat, vitamins, and minerals in the rations of the restricted animals. The present experiment was undertaken to ascertain whether formation of the spontaneous hepatoma, like other mouse tumors, responds to simple underfeeding (all components of the ration reduced proportionately) in the same manner as to caloric restriction per se (restriction in carbohydrate only).

Two groups of mice, 13 to 14 weeks of age, were employed. The diet was composed of Purina fox chow meal, 40 per cent, skimmed milk powder, 20 per cent, and cornstarch, 40 per cent. The mice of group n40 (50 animals) were given 4.0 gm. daily, those of group n42 (29 animals) 2.5 gm. On the average, the mice of group n40 ate from 3.8 to 4.0 gm. and subsequent to 7 months of age weighed between 37 and 40 gm. The mice of group n42 ate the 2.5 gm. given them and, after 4 months of age, weighed between 22 and 24 gm., on the average.

When the mice were 58 to 59 weeks of age, they were sacrificed and examined for hepatomas. The data are given in Table 4. Forty-four per cent of the mice of group n40 had hepatomas; no hepatomas were observed in the 21 mice of group n42 surviving to the end of the experiment. In agreement with the results of Experiment 1, a 35 to 40 per cent reduction in food intake significantly inhibited the formation of hepatomas.

DISCUSSION

Inhibition of formation of spontaneous hepatomas in the C3H male mouse by chronic caloric restriction was achieved either by limiting only the carbohydrate (caloric restriction per se) or by proportionate reduction of all components of the diet. This finding is in accordance with similar observations for all previously studied tumors of the mouse. It was also shown that the incidence of hepatomas was dependent on the degree of caloric restriction. The results on the formation of skin tumors, obtained here with a moderate dose of carcinogen, agrees with previously published experiments in which higher doses of carcinogen were utilized (2): With decreasing caloric intake below the ad libitum level, there is a decreasing incidence of tumors, or a delay in their appearance, or both.

The data may be examined by methods analogous to those employed in biological assay. When the probit of the incidence of spontaneous hepatomas is plotted against the logarithm of the daily food intake, a very nearly linear relation is obtained (Figure 2). A similar linear dependence exists for skin tumors (3), as well as for other mouse tumors (4). The data for each tumor type are given in Table 4. The straight lines satisfactorily fit the data, the respective chi-square values being 5.1 and 0.85.
ists for the induced skin tumor (Figure 2). The greater slope of the regression line for hepatomas, as compared with that for skin tumors, quantitates the greater response of hepatoma formation to caloric restriction.

Previously published data on the formation of both spontaneous mammary carcinoma and benz-pyrene-induced skin tumors in mice (2, 3) were analyzed in the same way and, within the limits of statistical requirements, the probits of the incidence of tumors were also linear functions of the logarithms of the caloric intakes. Referred to units of per cent animals with tumors, plotted against caloric intake on an arithmetic scale, the data imply that when the tumor incidences range between 0 and 100 per cent, the graph of the relation between tumor incidence and caloric intake should be a \( f \) shaped curve with the upper arm longer than the lower arm—i.e., an integration of a positively skewed distribution curve.

It was previously suggested that there might be a critical level at which tumor inhibition is most readily affected by a unit change in caloric intake. Now it is evident that the point of maximum change in tumor incidence is not dependent on a particular level of caloric intake, but rather is determined by the range of tumor incidences between the highest and lowest levels of food intake. Inasmuch as the probit of the incidence of tumors is a linear function of the logarithm of the food intake, the greatest change in the percentage of tumors, for proportionate changes in food intake, occurs in the region of a 50 per cent incidence of tumors. This is supported by the present and previously reported (2, 3) graded caloric restriction experiments, in which the tumor incidences in the \( ad \) libitum group to the most restricted group ranged a) from 100 to 0 per cent; b) from 100 to near 50 per cent; and c) from near 50 to 0 per cent, as obtained in Experiment 1 with skin tumors and hepatomas.

**SUMMARY**

1. Chronic caloric restriction inhibits the formation of the spontaneous hepatoma of the CSH male mouse. This caloric effect is in accord with that obtained with all previously studied tumors.
2. In an experiment in which graded caloric intakes were given to several groups of mice, it was demonstrated that with decreasing caloric intake there was a decreasing incidence of both methylcholanthrene-induced skin tumors and spontaneous hepatomas.
3. The relationship between caloric intake and incidence of these two types of tumors may be mathematically stated: the probit of the incidence of tumors is a straight line function of the logarithm of the daily caloric intake. This implies, and the data agree, that the largest inhibition of tumor formation, for proportionate reduction in food intake, occurs near the level of 50 per cent incidence of tumors.

**REFERENCES**

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