The Dependence of Tumor Formation on the Composition of the Calorie-Restricted Diet as Well as on the Degree of Restriction

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The inhibitory effect of caloric restriction on the formation of tumors in the mouse has been shown (11) to be dependent on the actual degree of caloric restriction. Previously it had been demonstrated (8) that restriction of caloric intake to about 60 per cent of the ad libitum intake causes an inhibition in tumor formation regardless of whether only the carbohydrate component is restricted (so-called caloric restriction per se) or an aliquot of the ad libitum ration is fed (restriction of all components). The question arose whether these two types of restricted diets affect tumor formation to the same extent. Also, since it is known that a fat-enriched diet enhances the formation of spontaneous mammary and induced skin tumors (1, 9) it was of interest to determine the effect of high-fat but restricted diets on tumor formation.

The present experiments, in which spontaneous mammary and induced skin tumors of the mouse were utilized, were performed in order to gather more data regarding the effect on tumor formation of the degree of caloric restriction, and at the same time to ascertain whether or not there is a differential effect due to the composition of the diet (at various caloric levels). Three types of restricted diets were used: (a) diets in which caloric restriction was achieved by decreasing the carbohydrate component only; (b) diets in which caloric restriction was achieved by reducing all components of the control ration (not necessarily in proportion to the caloric restriction); and (c) diets equivalent to the latter except that the fat content was increased by equicaloric substitution of fat for carbohydrate.

METHODS

The general methods, conditions, and criteria have been described (11). The special characteristics will be given under the separate investigations.

The constituents of the diets used in these experiments were Purina fox chow meal, skimmed milk powder, cornstarch,1 and hydrogenated cottonseed oil.2

1 Anheuser-Busch cornstarch 712-87.
2 Kremit (partially hydrogenated cottonseed oil) generously furnished by Armour & Co.

The fox chow meal and skimmed milk powder contained the protein, vitamins, and minerals, and (as far as growth is concerned) adequate essential fatty acids. Consequently, variation or equivalence of these two materials in the diets is referred to as variation or equivalence of “essential components.” The cornstarch and hydrogenated cottonseed oil utilized are practically free of vitamins, ash, and proteins; they were added to vary the caloric level and fat content of the diets. The compositions and caloric values of the diets were calculated from data supplied by the manufacturers of the foodstuffs.

It must be recognized that when caloric restriction per se is achieved by reducing the amount of cornstarch, there is concomitantly an increase in the percentage (not in the amount) of the essential components of the diet. Moreover, when cornstarch is replaced by an equicaloric amount of fat, the other constituents remaining constant, there is a decrease in the weight of the diet and a consequent increase in the proportion of the essential components. These latter points can be seen in the tables in which the diets are described.

EXPERIMENTAL

Experiment 1.—The 3 groups of this experiment were composed of either 50 or 54 dba male mice, all born between April 14 and May 23, 1941. They were placed on the respective experimental diets when they were 5 to 11 weeks old. Two weeks later they were given the first application of a 0.3 per cent solution of 3,4-benzpyrene in benzene. The solution was applied in the interscapular area by means of a dropping pipette.

The factors and conditions of the experiment were alike for all three groups, except for the differences in diet (Table 1a) and the number of semi-weekly applications of the carcinogen. The mice of the restricted groups, x5 and x25, received 25 applications (in 12 weeks), whereas those of the ad libitum group, x12 were given only 17 (in 8 weeks). There were special reasons for this procedure: the x12 group was also being used as a control (for other experi-
mental groups carried on at the same time) in which only a moderate tumor response was desired. Seventeen applications of the carcinogen solution would have produced only a few tumors in the restricted groups; therefore, these two groups were given 25 applications in order that the tumor response would be in a range where differences between the restricted groups, if any, would be more readily recognized.

The composition of the diet, mean caloric intake, and mean weight of the mice are given in Table IA. x12 are 13, 27, and 46, respectively. The curves of tumor formation are given in Fig. 1.

Although caloric restriction inhibits tumor formation, the method by which the restriction is achieved apparently modifies the degree of the effect. It appears that the mice receiving the diet restricted in carbohydrate only developed fewer tumors, and at a later mean time of appearance, than did the mice receiving the diet restricted in all components.

Experiment 2.—Four groups of 50 virgin dba female

<table>
<thead>
<tr>
<th>Diet constituents (gm. per mouse per day)</th>
<th>Mean daily caloric intake</th>
<th>Table IA: Diet, Mean Caloric Intake, and Mean Weight of Mice of Experiment 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fox chow meal</td>
<td>Skimmed milk powder</td>
<td>Corn starch</td>
</tr>
<tr>
<td>x12</td>
<td>1.4</td>
<td>0.9</td>
</tr>
<tr>
<td>x5</td>
<td>1.4</td>
<td>0.9</td>
</tr>
<tr>
<td>x25</td>
<td>0.8</td>
<td>0.5</td>
</tr>
</tbody>
</table>

* Calculated from food consumption, which varied during course of experiment.
† Approximate mean weight during course of experiment following last application of carcinogen.

<table>
<thead>
<tr>
<th>Number of mice developing skin tumors of mice</th>
<th>Mean time of appearance (weeks)</th>
<th>Per cent computing carcinomas</th>
<th>Number of mice tumor-free and alive at end of experiment (54th week)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Number</td>
<td>Per cent</td>
<td>Mean time of appearance (weeks)</td>
</tr>
<tr>
<td>x12</td>
<td>54</td>
<td>17</td>
<td>35 65 34 ± 2.6</td>
</tr>
<tr>
<td>x5</td>
<td>48</td>
<td>25</td>
<td>12 25 38 ± 6.1</td>
</tr>
<tr>
<td>x25</td>
<td>49</td>
<td>25</td>
<td>19 39 34 ± 7.1</td>
</tr>
</tbody>
</table>

* Number of mice alive at 13th week of experiment.

It is to be noted that, in comparison with x12, x5 was restricted only in carbohydrate. On the other hand, group x25 was fed a ration prepared by restricting all components (essentials as well as calories) of the x12 diet. The two restricted diets were approximately equicaloric.

The control mice grew normally, while those of the two restricted groups maintained a fairly constant weight; the mean weights of the latter groups were approximately the same. The experiment ran for 54 weeks. Table Ia contains the data on tumor incidence and average time of appearance.

At the termination of the experiment the carbohydrate-restricted group (x5) had 25 per cent skin tumors in comparison with 39 per cent for the all-component-restricted group (x25). These compare with 65 per cent for the “control” group (x12). While a direct comparison can be made only between x5 and x25, the x12 group may be considered a qualitative control, particularly since more tumors were formed here than in the restricted groups in spite of fewer applications of the carcinogen. With regard to the formation of carcinomas the percentages for x5, x25, and...
mice, all born between April 14 and 23, 1941, were utilized in this experiment. The groups were constructed by litter-mate distribution. After weaning, the mice were fed Purina fox chow checkers until they were 9 to 10 weeks of age, when the experimental diets were instituted; these were respectively, ad libitum (T2), calorie (carbohydrate) restricted (T5), all components restricted (T25), and the latter with fat substituted for some of the carbohydrate (T15). The 3 restricted diets were approximately equicaloric. The composition of the diet, mean caloric intake, and mean weight of the mice are shown in Table IIA.

The experiment was terminated at a time when only 3 mice were alive (136 weeks of age). The experiment ran smoothly and the mice were in good condition throughout. The data with regard to mammary tumor incidence and the time of appearance of these tumors are shown in Table IIB and Fig. 2.

The following results, in comparison with tumor production in the ad libitum control (T2), were obtained in the 3 restricted groups: The high-fat group (T15) developed more tumors and, on the average, at a significantly earlier time; the group restricted in essential components (T25) developed fewer tumors, but at about the same time; the group restricted in carbohydrate only (T5) developed significantly fewer tumors, and at a later time. The actual percentages of mammary tumors, on an effectual total basis, in the T2, T15, T25, and T5 groups were 75, 87, 66, and 47 respectively.

This experiment clearly indicates that although caloric restriction may inhibit tumor formation, the...
components of the restricted diet are of great importance, for they may be of such a nature (i.e., high-fat) that under proper conditions tumor production is enhanced over and above the control level. It is also obvious that the augmenting effect of the high-fat diet is not due to a caloric effect (since the 3 restricted groups were approximately equicaloric) but to some unexplained essential action of fat.

In both Experiments 1 and 2, diets restricted in carbohydrate only (caloric restriction per se) inhibited tumor formation to a greater extent than did an equicaloric diet restricted in all components. In Experiment 1, performed with induced skin tumors, and with diets restricted to approximately 60 per cent of the ad libitum intake, the differences were not statistically significant. On the other hand, in Experiment 2, performed with spontaneous mammary tumors and diets restricted to 80 per cent of the ad libitum intake, the differences were statistically significant. The agreement in results between the experiments supports the viewpoint that the nature of the restricted diet modifies the inhibitory effect on the formation of these two types of tumors in the mouse.

**Graded caloric restriction using diets of different composition.**—In Experiments 1 and 2 it was concluded that the composition of the restricted diet at a particular caloric level is a modifying factor for both the number of tumors and the average time at which they appear. In Experiments 3 and 4, which follow, the 3 types of restricted diets were studied at more than one caloric level.

The diets stemmed from a low-fat basic diet, 40, composed of Purina fox chow meal, skimmed milk powder, and cornstarch; this diet was sufficient in both essentials and calories to support good growth and health in the C3H male mice used in Experiment 3.

The types of diets were: (a) those constructed from basic diet 40 by restriction of cornstarch (calories) only; (b) those constructed from diet 40 but by restricting essentials (fox chow meal and skimmed milk powder) as well as cornstarch; (c) those comparable to the latter except that fat was substituted equicalorically for some of the cornstarch. The series may be briefly indicated as: (a) restricted in calories (carbohydrate) only; (b) restricted in calories and essentials (low in fat); (c) restricted in calories and essentials, but high in fat.

The diet and group numbers indicate the type of

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**Table IIIa: Composition of Diets Used in Experiments 3 and 4**

<table>
<thead>
<tr>
<th>Diet number</th>
<th>Fed to groups</th>
<th>Constituents (grams per mouse per day)</th>
<th>Percentage composition of diet</th>
<th>Calories per mouse per day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In experiment 3</td>
<td>In experiment 4</td>
<td>Fox chow meal</td>
<td>Skimmed milk powder</td>
</tr>
<tr>
<td>40</td>
<td>i40</td>
<td>i-series</td>
<td>1.4</td>
<td>0.8</td>
</tr>
<tr>
<td>33</td>
<td>i33</td>
<td>h33a &amp; b</td>
<td>1.4</td>
<td>0.8</td>
</tr>
<tr>
<td>28</td>
<td>i28</td>
<td>h28</td>
<td>1.4</td>
<td>0.8</td>
</tr>
<tr>
<td>26</td>
<td>i26</td>
<td>h26</td>
<td>1.4</td>
<td>0.8</td>
</tr>
<tr>
<td>24</td>
<td>i24</td>
<td>h24</td>
<td>1.4</td>
<td>0.8</td>
</tr>
<tr>
<td>22</td>
<td>h22</td>
<td></td>
<td>1.4</td>
<td>0.8</td>
</tr>
<tr>
<td>228</td>
<td>i228</td>
<td>h228</td>
<td>1.2</td>
<td>0.7</td>
</tr>
<tr>
<td>226</td>
<td>h226</td>
<td></td>
<td>1.1</td>
<td>0.6</td>
</tr>
<tr>
<td>224</td>
<td>i224</td>
<td>h224</td>
<td>1.0</td>
<td>0.6</td>
</tr>
<tr>
<td>222</td>
<td>h222</td>
<td></td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>140</td>
<td>i140</td>
<td>h133</td>
<td>1.4</td>
<td>0.8</td>
</tr>
<tr>
<td>133</td>
<td>h133</td>
<td>i-series</td>
<td>1.4</td>
<td>0.8</td>
</tr>
<tr>
<td>128</td>
<td>i128</td>
<td>h128</td>
<td>1.2</td>
<td>0.7</td>
</tr>
<tr>
<td>126</td>
<td>h126</td>
<td>i-series</td>
<td>1.1</td>
<td>0.6</td>
</tr>
<tr>
<td>124</td>
<td>i124</td>
<td>h124</td>
<td>1.0</td>
<td>0.6</td>
</tr>
<tr>
<td>122</td>
<td>h122</td>
<td>i-series</td>
<td>1.0</td>
<td>0.5</td>
</tr>
</tbody>
</table>

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On the other hand, in Experiment 2, performed with spontaneous mammary tumors and diets restricted to 80 per cent of the ad libitum intake, the differences were statistically significant. The agreement in results between the experiments supports the viewpoint that the nature of the restricted diet modifies the inhibitory effect on the formation of these two types of tumors in the mouse.

The last 2 figures of the group or diet number indicate the caloric level of the diet. For example, diets 40 and 140 were equicaloric, as were diets 28, 128, and 228.

The diets utilized in Experiments 3 and 4 are presented in Table IIIa.

**Experiment 3.**—This experiment was designed to test the effect of both graded caloric restriction and the composition of restricted diets on the formation of induced skin tumors. The nine groups were each composed of 50 C3H male mice (born between April 19 and July 17, 1943), and were equivalent as to age and weight of the mice. The diets were instituted when the mice were 5½ to 16 weeks of age. Two
The experimental diets have been discussed, and are listed in Table IIIA. The control diets, low-fat (i40) and high-fat (i140), contained equal amounts of essential components, and were approximately equicaloric. During the major part of the experiment most of the mice fed these 2 diets ate all the daily ration. The restricted mice consumed all their daily ration throughout the experiment.

At the beginning of the experiment the mean weights of the 9 groups ranged from 23.7 to 24.4 gm. The mean weight of the mice at various times of the experiment is shown in Table IIIB.

The experiment was terminated 55 weeks after the first application of the carcinogen. The data regarding the incidence and mean time of appearance of skin tumors are given in Table IIIc.

In each of the 3 series, tumor incidence is progressively decreased with progressive caloric restriction of the diet. There is also a tendency for the tumors to appear later, on the average, as restriction is increased. In addition, at a particular level of caloric restriction, the high-fat diet (i140, 128, or 124) permits the formation of a higher percentage of tumors than its low-fat counterpart (i40, 228, or 224). While the difference at each level is not statistically significant, the augmentation is consistent and in agreement with the results of other experiments. The lack of difference in this experiment between the effect of diets restricted in carbohydrate only (i24 and 28) and the corresponding equicaloric diets restricted in all components (i224 and 228) will be discussed later. These points are more readily seen in Fig. 3, in which the incidence of tumors as a function of both caloric intake and composition of diet is graphically presented.

An incidental finding is the data on hepatomas, which develop spontaneously in the males of strain C3H. The first one was seen in a 45 week old mouse of the i140 group. Of 34 mice of i140 group (high-fat control) sacrificed between 45 and 73 weeks of age, 12 (35 per cent) were found to have hepatomas. In contrast, hepatomas were found in only 3 of 32 mice (9 per cent) in the i40 group (low-fat control) sacrificed during the same interval; the first hepatoma observed in this group was in a mouse 65 weeks old.

In the 7 restricted groups, 158 mice were sacrificed between 62 and 73 weeks of age; only 2 had hepatomas. Apparently, as in the case of the induced skin tumor and spontaneous mammary tumors, the formation of a spontaneous hepatoma in C3H male mice is inhibited by caloric restriction, and augmented by a high-fat diet.

Experiment 4.—This experiment was designed as a companion investigation to Experiment 3, but in this experiment weeks later the first of 10 weekly applications of carcinogen was given: one drop of a 0.3 per cent solution of methylcholanthrene in acetone applied with a dropping pipette to the interscapular area.

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Experiment 4.—This experiment was designed as a companion investigation to Experiment 3, but in this
instance spontaneous mammary tumors were studied. The 15 groups were each composed of 30 virgin C3H females (born between April 19 and July 17, 1943) and were equivalent as to age and weight. The experimental diets were instituted when the mice were from 5 to 15 weeks of age. As in Experiment 3, the 3 diet types were studied at several caloric levels (Table IIIA). In this experiment diet 33 served as control, since it is at the ad libitum level for C3H female mice; throughout the major portion of the experiment most of the mice being fed this diet consumed all the daily ration. The mice receiving the restricted diets consumed all their daily ration throughout the experiment.

Because of inherent difficulties in constructing 15 uniform groups, two separate groups (h33a and h33b) were used as controls and were fed diet 33. This was done in order to obtain an impression, at least, of the variation to be expected between any 2 groups when such variation arises because of the manner of selecting mice for the individual groups of the experiment and other random factors.

Again, as in the previous experiments, graded caloric restriction produced graded weight levels. The mean weight of the mice of the various groups is shown in Table IVa.

Laboratory conditions prompted the termination of the experiment when the youngest mice were 64 weeks of age. The percentages of tumors formed by the 64th week, on an effectual total basis, are given in Table IVb. Although further extension of the experiment would have increased these percentages in some groups, previous experience (including the results of Experiment 2) suggests that the main differential results would be maintained.

The difference in tumor formation between the two control groups, h33a and h33b, is well within the expected limits, suggesting that with respect to the potentiality of tumor formation the 15 groups were relatively uniform at the beginning of the experiment.

As in Experiment 3, it can be noted that the greater the degree of caloric restriction in each of the 3 series, the greater the inhibition of tumor formation. Again, at the levels of caloric intake studied, the high-fat diets (100 series) caused the formation of more tumors than did the corresponding equicaloric low-fat diets (200 series).

The questionable existence of a difference in tumor formation between groups restricted only in carbohydrate and the corresponding groups restricted in all components will be taken up in the discussion.

In Fig. 4 the incidence of mammary tumors as a function of both caloric intake and composition (type of restriction) is graphically represented.

**DISCUSSION**

The results of Experiments 3 and 4 confirm the finding (11) that the inhibitory effect of caloric restriction per se (carbohydrate only) on the formation of spontaneous mammary and induced skin tumors of the mouse is dependent on the actual degree of restriction.
TABLE IVB: FORMATION OF SPONTANEOUS MA.XI:,t.,RY TUMORS (C3H VIRGIN FEMALE MICE) AS AFFECTED BY COMPOSITION OF DIET AND CALORIC INTAKE

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean caloric intake</th>
<th>Relative * amount of &quot;essentials&quot;</th>
<th>Per cent fat</th>
<th>Number of mice (effectual total)</th>
<th>Age at appearance of tumors (weeks)</th>
<th>Mice tumor-free and alive at 64 weeks of age</th>
</tr>
</thead>
<tbody>
<tr>
<td>h33a</td>
<td>11.7</td>
<td>1</td>
<td>2</td>
<td>30</td>
<td>16, 53</td>
<td>35-59, 48 ± 1.5</td>
</tr>
<tr>
<td>h33b</td>
<td>30</td>
<td>19</td>
<td>63</td>
<td>16</td>
<td>19</td>
<td>55-64, 45 ± 1.6</td>
</tr>
<tr>
<td>h28</td>
<td>10.0</td>
<td>8</td>
<td>27</td>
<td>30</td>
<td>2</td>
<td>41-63, 52 ± 2.6</td>
</tr>
<tr>
<td>h26</td>
<td>9.2</td>
<td>2</td>
<td>7</td>
<td>30</td>
<td>0</td>
<td>45-67, 46 ± 23</td>
</tr>
<tr>
<td>h24</td>
<td>8.5</td>
<td>0</td>
<td>0</td>
<td>30</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>h22</td>
<td>7.8</td>
<td>2</td>
<td>29</td>
<td>0</td>
<td>0</td>
<td>*</td>
</tr>
<tr>
<td>h228</td>
<td>10.0</td>
<td>12</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>*</td>
</tr>
<tr>
<td>h226</td>
<td>9.3</td>
<td>12</td>
<td>28</td>
<td>0</td>
<td>4</td>
<td>29-63, 50 ± 23</td>
</tr>
<tr>
<td>h224</td>
<td>8.5</td>
<td>29</td>
<td>28</td>
<td>0</td>
<td>0</td>
<td>*</td>
</tr>
<tr>
<td>h228</td>
<td>7.8</td>
<td>28</td>
<td>28</td>
<td>0</td>
<td>0</td>
<td>*</td>
</tr>
<tr>
<td>h133</td>
<td>11.9</td>
<td>30</td>
<td>18</td>
<td>0</td>
<td>27</td>
<td>22-54, 42 ± 1.6</td>
</tr>
<tr>
<td>h128</td>
<td>10.1</td>
<td>30</td>
<td>18</td>
<td>0</td>
<td>20</td>
<td>27-64, 50 ± 2.3</td>
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<td>h126</td>
<td>9.2</td>
<td>30</td>
<td>18</td>
<td>0</td>
<td>18</td>
<td>33-64, 50 ± 2.5</td>
</tr>
<tr>
<td>h124</td>
<td>8.5</td>
<td>29</td>
<td>28</td>
<td>5</td>
<td>17</td>
<td>41-63, 53 ± 3.2</td>
</tr>
<tr>
<td>h122</td>
<td>7.8</td>
<td>28</td>
<td>28</td>
<td>1</td>
<td>4</td>
<td>37</td>
</tr>
</tbody>
</table>

* Relative amounts of protein, vitamins, and minerals: h33 = 1.
† Number of mice alive in group when first tumor appeared in experiment; mice 22 weeks old.
‡ Little significance can be attached to these means because of the small number of tumors.

This relationship obtains whether the calorie-restricted rations are: (a) restricted only in the amount of carbohydrate (caloric restriction per se); (b) restricted in essentials (protein, vitamins, and minerals) as well as in carbohydrate (low-fat content); or (c) restricted in essentials and carbohydrate, but with high fat content. In addition, the average time of appearance of the tumors is also dependent, in general, on the degree of restriction: The greater the caloric restriction the later the tumors appear.

In Experiment 4 the curves (Fig. 4) showing the relationship between tumor formation and caloric intake for the 3 different types of diets appear to have a modified J shape. This is in general agreement with the previous conclusions regarding caloric restriction per se (11). On the other hand, the comparable curves of Experiment 3 (Fig. 3) appear to be linear, but this is probably due in part to the fact that fewer levels of caloric intake were studied.

Increasing the fat content of an ad libitum ration enhances the production of spontaneous mammary and induced skin tumors of the mouse. In this communication (Experiments 2, 3, and 4) it is further shown that there continues to exist an augmenting effect of a high-fat diet even at restricted levels of caloric intake.

Lavik and Baumann (1) investigated the differential effects of low-fat and high-fat diets at 2 levels of caloric intake, normal and restricted, on the production of skin tumors by methylcholanthrene. The incidence at each level of caloric intake was somewhat higher on the high-fat diet than on the corresponding low-fat diet. They interpreted this increase as possibly due to local contact of the fatty rations with the skin. Furthermore, they suggested that "much of the systemic or cocarcinogenic activity of dietary fat, if not all, is exerted through the medium of a voluntarily increased
intake of calories on diets high in fat." Our experiments do not support this view.

In earlier publications (9, 10) we have stated that the ingestion of a high-fat diet augments the formation of certain types of tumors mainly through a cocarcinogenic action on the developing tumor cell. Mice fed high-fat diets consumed more calories, possibly because of the greater compactness of the high-fat diet, and the augmentation in tumor formation may have been partly dependent on the increase in caloric intake. However, in the present experiments, such situations were obviated by the use of equicaloric diets which were fed at restricted levels where the mice consumed all food given them. The equicaloric consumption of the diets is evidenced by the rough equality in mean weight of corresponding groups. In every case where equicaloric diets were fed, the animals receiving the high-fat ration developed a higher incidence of tumors. Moreover, in the case of spontaneous mammary tumors, where mechanical contact with the fatty ration can not logically be assumed to be a factor, the augmenting effect of increased dietary fat is most pronounced; so great, in fact, that the groups in which the mice were fed diets approximately 85 per cent of the ad libitum caloric intake, but with a high-fat content, developed as many tumors as, or more than, the control groups on the ad libitum low-fat diet (T15 and T2; h128 and h33a or b).

Thus the evidence confirms our previous concept, that the enhancing effect of a high-fat diet on the formation of spontaneous mammary and chemically induced skin tumors is due mainly to some direct property of fat. What is important is that there exists an action of fat independent of a general caloric effect that might be produced by the added consumption of the whole ration, carbohydrate, or fat.

In contrast with the definite increase in tumor formation produced by increasing the dietary fat at various caloric levels, there is only a questionable difference between the inhibitory effect of diets prepared by limiting essential components (as well as calories) and that produced by diets limited only in the carbohydrate component (caloric restriction per se). In Experiments 1, 2, and 4 it appears that caloric restriction per se (restriction in carbohydrate only) is more effective in inhibiting tumor formation than is the restriction of essentials as well as calories. Even though Experiment 3 does not support this viewpoint and, in general, the differences are not of great magnitude, it is probable that a difference does exist. This is seen more definitely in the experiments on spontaneous mammary tumors than in those utilizing induced skin tumors. In all our studies on the consequence of caloric restriction or high-fat diets on the formation of tumors we have observed that the effects were of greater magnitude with spontaneous mammary tumors than with induced skin tumors.

There are probably many modifying factors in experiments designed to test whether diets restricted in carbohydrate only or in essentials as well as calories have differential effects on tumor formation. Possible factors are: the type of tumor studied; the carcinogeticity of the inciting agent, and dosage; the composition of the control ad libitum diet; and the extent of the differences in essential components. In Experiments 1 and 2, for example, where larger differences in tumor formation were observed, the diets restricted in essential components contained 40 per cent less essentials than did the carbohydrate-restricted diets; on the other hand, in Experiments 3 and 4 the differences in essential components were smaller: 14 per cent less at the 10 caloric level, and 27 per cent less at the 8.5 caloric level.

Thus the composition of the restricted diet as well as the degree of restriction is of importance in determining the extent of inhibition of tumor formation produced through caloric restriction. A restricted diet with a high-fat content definitely results in the least inhibition of tumor formation in comparison with either (a) an equicaloric diet that differs only in that it is low in fat, or (b) an equicaloric diet containing more protein, vitamins, and minerals than (a). Until more evidence is presented, one may accept the view that between the latter 2 types of restricted diets, there is a difference in the effects, often very slight.

The results reported previously (11) combined with those recorded in this communication suggest the tentative curves given in Fig. 5, indicating the relationship of tumor formation to both the degree of restriction and the composition of the restricted diet.

These curves have been drawn for spontaneous mammary and chemically induced skin tumors of the mouse. They may be valid for other types of tumors also, but certainly not for all. For example, it is known (9) that a high-fat diet has little or no effect on the formation of spontaneous lung tumors of the mouse. Therefore the 3 curves (Fig. 5) for that particular type of tumor may, in reality, be reduced to only 2 separate curves or even one. It has been suggested also (9) that the induction of sarcomas by hydrocarbons may actually be inhibited by a high-fat diet; in this case the 3 curves may have entirely different relationships.

It is realized that both the experiments and interpretations regarding the effects of the degree of caloric restriction and the composition of the restricted diet refer to the effects produced by the ingestion of the various diets. The consequent differences in absorption, interconversion of fats and carbohydrate, sparing mecha-
anisms, and intermediary metabolism in general, are not sufficiently defined at present to afford an explanation for the observed effects on tumor formation.

At this time it seems advisable to summarize some of the evidence regarding a possible role of nutrition in the prevention of human cancer. Included are: (a) the inhibiting effect of caloric restriction on the formation of induced skin tumors (1, 5, 8), induced sarcoma (5, 8), spontaneous mammary tumors (5, 8, 12, 13), spontaneous lung tumors (5), and induced and spontaneous leukemia (4, 13) of the mouse, and spontaneous tumors of the rat (3); (b) the fact brought out in this communication, that some inhibition of the formation of induced skin and spontaneous mammary tumors is obtained even with reasonable degrees of caloric restriction; (c) the inhibiting effect of a low-fat diet on the formation of induced skin tumors (1, 9), spontaneous mammary tumors (9), and spontaneous hepatoma (this paper); (d) the existence of a direct relation between the frequency of mammary carcinoma of the mouse and the average weight, the latter being greater in tumor-bearing mice (2); and (e) the data obtained from a study (6) of insurance statistics, indicating that overweight persons have a greater tendency to develop cancer.

These observations support our previous conclusions (6, 7, 8, 10) that the avoidance of overweight through calorie-restricted and low-fat diets may aid in preventing many types of cancer in man, or at least delay the onset.

SUMMARY

The present experiments on spontaneous mammary and induced skin tumors in mice were performed to gather more data regarding the effect on tumor formation of the degree of caloric restriction, and at the same time to ascertain whether or not there is a differential effect due to the composition of the diet (at various caloric levels).

Further evidence was obtained that caloric restriction inhibits the formation of tumors, and that a high-fat diet enhances the formation of the types of tumors studied. This finding refers not only to the incidence of tumors but also to the average time at which they appear. As an incidental finding it appears that the spontaneous hepatoma of C3H male mice is affected by both caloric restriction and a high-fat diet, in a manner similar to the other tumors studied.

It is confirmed that the formation of tumors (incidence and average time of appearance) is dependent on the degree of caloric restriction per se. Evidence is presented that this is true also for diets restricted in essentials as well as calories (high or low in fat).

At a particular restricted caloric intake, a high-fat diet is less inhibitory than a diet with a low-fat content. It is also possible that a diet restricted in carbohydrate only inhibits slightly more than an equicaloric diet containing less essential components. Thus tumor formation is dependent on the composition of the diet, as well as on the degree of caloric restriction.

Evidence is presented that the enhancing effect on tumor formation of a high-fat diet, ad libitum or restricted, is due mainly to some specific action of fat. This action is independent of a general caloric effect that might be produced by the added consumption of the whole ration, carbohydrate, or fat.

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The Dependence of Tumor Formation on the Composition of the Calorie-Restricted Diet as Well as on the Degree of Restriction

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