Effect of Low Environmental Temperature, Dinitrophenol, or Sodium Fluoride on the Formation of Tumors in Mice*

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It has been demonstrated consistently that chronic caloric restriction inhibits the formation of various spontaneous and induced tumors of the mouse (1, 2). The mechanism of this effect is unknown. However some insight might derive if it were known whether the decreased caloric intake, the accompanying restriction in body weight, or the decreased total metabolism, was most directly related to the inhibition of tumor development.

The present investigation was undertaken to acquire information relevant to this question by examining the effects on tumor formation of experimental procedures which would result in retarded body growth, despite unchanged or augmented caloric intake. Two of the procedures were the feeding of dinitrophenol and housing of the mice at low environmental temperature. The third procedure was the feeding of sodium fluoride, inasmuch as under suitable conditions it effects a significant retardation in body weight without a parallel change in food consumption.

In these experiments four types of tumors were utilized—spontaneous mammary carcinoma, induced sarcoma, induced skin tumor, and primary lung adenoma. Only the spontaneous mammary carcinoma was investigated with all three experimental procedures; the results have been reported previously (3).

GENERAL METHODS

The following methods and conditions were common to all the experiments. The mice either were obtained from the Roscoe B. Jackson Memorial Laboratory (in which case the strain designation is preceded by JAX) or were bred in our laboratory by brother to sister mating. They were fed Purina fox chow or dog chow checkers from weaning (or when received in the laboratory) until the beginning of the experiment, generally when the mice were 7 to 12 weeks of age. Since the study was performed over a period of years, the foodstuffs employed in preparing the diets differed among the various series of experiments. In all instances the control diets supported relatively good growth and health. The rations were prepared by mixing the weighed foodstuffs with sufficient water to make easily molded mashes, which were spread in pans and cut into blocks of appropriate size. When sodium fluoride (C.P.) or sodium salt of 2,4 dinitrophenol (Eastman-Kodak) was fed it was dissolved in water and incorporated into the mash. In the earlier experiments the diets were made daily; it was later found expedient to prepare them weekly, and store them in a refrigerator. The diets were fed daily; by weighing the food remaining in the cages at the end of each week, the actual food consumptions were estimated. Drinking water was available at all times.

The mice were housed in groups of five in cages with solid bottoms. Each animal was numbered and a separate record of its progress was kept. They were weighed and inspected for tumors at 2 week intervals except during the period in which the tumors appeared rapidly, when they were inspected weekly. The animals were examined postmortem—at sacrifice when the tumors became very large, at death, or at the termination of the experiment. The lesions were recognized as tumors by their appearance and progressive growth; the tumor type was established by gross examination and sectioning. Histological examinations were made of many tumors selected at random and of the few doubtful lesions. In general, the experiments were terminated either when the rate of formation of tumors had passed its peak and very few new tumors were appearing, or when the surviving animals were too few to modify the nature of the results.

EXPERIMENTS

SPONTANEOUS MAMMARY CARCINOMA

Experiment 1.—Each of the four groups was composed of 50 dba female mice born in the laboratory; litter-mates were distributed among the groups. The experimental diets consisted of 35 per cent Purina fox chow meal, 24 per cent skimmed milk powder, and 41 per cent cornstarch; they were instituted when the mice were 7 to 10 weeks old. The mice of the control group, P40, and of group P30 were fed the described ration; the diet fed group P47 contained sodium fluoride at the
level of 0.09 per cent; and that fed group P48 contained 0.25 per cent of the sodium salt of 2,4 dinitrophenol. When the mice of group P50 were 19 to 22 weeks old they were transferred to a room kept between 45 and 55° F.; this contrasts with the average temperature of 80° F. in the laboratory in which the other three groups were housed.

The mice were fed 3.4 gm. daily and during the main course of the experiment the average daily food consumptions were: control group, 3.0 gm.; sodium fluoride group, 2.7 gm.; sodium dinitrophenol group, 3.3 gm.; and cold room group, 3.4 gm. At first the mice fed the ration containing dinitrophenol (P48) ate less food than the control mice and lost considerable weight. Within a few weeks, however, they increased their food consumption and returned to their original body weights.

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When the mice housed in the cold room (P50) were about 80 weeks of age, it was observed that a few were comatose when inspected in the morning; such mice survived only if removed to the general laboratory (88° F.). Because this phenomenon was increasing in severity, the whole group was transferred to the general laboratory when the mice were 85 weeks old. This change, late in the experiment, had no effect on the rate of tumor formation.

The experiment was terminated when the surviving mice were 97 to 100 weeks old. The results are given in Figure 2 and Table 1. All three experimental procedures significantly inhibited tumor formation. The incidences of mammary tumors were: control group (P40), 74 per cent; sodium fluoride group (P47), 42 per cent; dinitrophenol group (P48), 2 per cent; and cold room group (P50), 10 per cent. The decreased incidence of tumors in the experimental groups was not due to an increased death rate among these mice. Even at 75 weeks there were, in each group, only 4 to 9 mice dead without tumors, yet the tumor incidences were 23, 2, and 8 per cent for the experimental groups P47, P48, and P50 respectively, in comparison with 46 per cent for the control group.

In this long-term experiment there were certain observations not directly related to the main objectives of the study. In the group fed sodium fluoride, 9 mice had chronic nephritis, and 33 of the animals, at one time or another after 1 year of age, developed "overgrown" teeth. The first few mice with "overgrown" teeth died from starvation; the rest were maintained by the simple expedient of clipping their teeth whenever necessary. In the group fed dinitrophenol, and the group housed at a low environmental temperature, the principal pathological change at death was pneumonia; the next most common finding, associated only in a few cases with pneumonia, was edema (hydrothorax, ascites, or anasarca).

**INDUCED SARCOMA**

In the following experiments sarcomas were induced by subcutaneous injection in the interscapular area of 3:4 benzpyrene dissolved in 0.2 cc. of an oily fraction of lard.
Experiment 2.—Three groups of 40 JAX-Swiss female mice, about 10 weeks old, were employed. Each mouse was injected with 0.15 mg. of benzpyrene. The control group (L30) was placed on a diet of cracked spring wheat, 64 per cent; Purina dog chow meal, 18 per cent; skimmed milk powder, 7 per cent; and white milled flour, 11 per cent. Group L7 was given the same diet but containing 0.25 per cent sodium salt of dinitrophenol.

The ingestion of the sodium fluoride or dinitrophenol rations resulted in decreased body weight (Table 2). The experiment was terminated 52 weeks after the injection of carcinogen, more than 12 weeks after the last sarcoma had appeared in any group. There were no significant differences in tumor formation (Table 2).

### TABLE 1

<table>
<thead>
<tr>
<th>GROUP *</th>
<th>NUMBER OF MICE</th>
<th>MICE DEVELOPING TUMORS</th>
<th>MEAN AGE AT TUMOR APPEARANCE (WEEKS)</th>
<th>NUMBER OF MICE †</th>
</tr>
</thead>
<tbody>
<tr>
<td>P40: Control</td>
<td>40</td>
<td>50</td>
<td>74</td>
<td>79</td>
</tr>
<tr>
<td>P47: NaF</td>
<td>48</td>
<td>47</td>
<td>69</td>
<td>70</td>
</tr>
<tr>
<td>P48: DNP</td>
<td>48</td>
<td>50</td>
<td>74</td>
<td>50</td>
</tr>
<tr>
<td>P50: Cold room</td>
<td>48</td>
<td>50</td>
<td>74</td>
<td>50</td>
</tr>
</tbody>
</table>

* NaF indicates 0.09% sodium fluoride in diet; DNP, 0.08% sodium 2,4-dinitrophenol in diet; cold room, mice housed at 45° to 55° F.
† Number of mice alive when first tumor was observed in experiment.
‡ Number of mice obtained by adjustment for the deaths of animals without tumors (4).
§ Experiment terminated when mice were 97 to 100 weeks old.
¶ The single tumor of this group appeared at 65 weeks.

Experiment 3.—Two groups of 40 JAX-Swiss female mice about 10 weeks of age were employed. Each mouse was injected with 1.5 mg. of benzpyrene. The mice of group L50 were fed the same basic diet employed in experiment 2; those of group L67 were fed the same diet for 12 weeks, when sodium fluoride was added at the level of 0.09 per cent. As in the preceding experiment the mice fed sodium fluoride ate slightly more food (on the

### TABLE 2

<table>
<thead>
<tr>
<th>EXPERIMENT*</th>
<th>NUMBER OF MICE</th>
<th>MICE DEVELOPING SARCOMAS</th>
<th>TIME OF TUMOR APPEARANCE (WEEKS)</th>
<th>MEAN BODY WEIGHTS†</th>
</tr>
</thead>
<tbody>
<tr>
<td>2: JAX-Swiss female; 0.15 mg. benzpyrene</td>
<td>L30: Control</td>
<td>40</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>L50: DNP</td>
<td>40</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>3: JAX-Swiss female; 1.5 mg. benzpyrene</td>
<td>L407: NaF</td>
<td>40</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>4: JAX-ABC female; 0.1 mg. benzpyrene</td>
<td>L67: NaF</td>
<td>40</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>5: C57 Black male; 0.15 mg. benzpyrene</td>
<td>A88: Control</td>
<td>40</td>
<td>20</td>
<td>40</td>
</tr>
</tbody>
</table>

* Experiment number, strain and sex of mice, and dosage of carcinogen administered subcutaneously.
† See footnote †, Table 1.
‡ Number of mice alive when first tumor was observed in experiment.
§ Experiments 2, 3, and 4 ended 4-2 weeks after injection of carcinogen; experiment 5 ended 40 weeks after injection.
¶ The mean weights are those of the mice without tumors; time in weeks after injection of benzpyrene.

Group L207 was fed the basic diet for 12 weeks, after which sodium fluoride was incorporated at a level of 0.09 per cent.

Each mouse was given 4.5 gm. of its experimental diet daily. The control mice ate an average of 3.3 gm. daily, those fed sodium fluoride about 3.6 gm., and those fed dinitrophenol all the food given them, 4.5 gm. The sodium fluoride mice drank twice as much, and the dinitrophenol mice about 30 per cent more water than did the control mice. The ingestion of the sodium fluoride or dinitrophenol rations resulted in decreased body weight (Table 2).
groups.

The experiment was terminated 52 weeks after the injection of the carcinogen, 10 weeks after the last sarcoma had appeared. Fifty per cent of the control mice developed tumors compared with 64 per cent in the sodium fluoride group (Table 2).

Experiment 4.—Two groups of 40 JAX-ABC female mice about 9 weeks old were utilized. One-tenth mg. benzpyrene in 0.2 cc. lard was injected subcutaneously. The diets were the same as those of experiment 3; feeding of sodium fluoride was begun 7 weeks after injection of the carcinogen. The mice of the group given sodium fluoride, L67, ate somewhat less than those of the control group, L60 (3.2 gm. compared with 3.5 gm. daily); they drank about twice as much water. Their body weights were considerably less than those of the controls.

The experiment was terminated 52 weeks after injection of the carcinogen inasmuch as few sarcomas had formed after the forty-third week (2 in the fluoride group, none in the control group). As in the preceding two experiments there were few non-tumor deaths. Thirty-three per cent; fed at a level of 4.0 gm. daily to the mice of group A82. The mice of group A88 were given the same diet, while the mice of the control group ate an average 3.5 gm. daily compared to 3.2 gm.) yet their growth was retarded. They drank more than twice as much water as the control mice.

The dinitrophenol-treated mice ate about 20 per cent more food (average of 3.8 compared with 3.2 gm.) and drank about 30 per cent more water. The control mice grew to an average weight of 40 gm. while those fed dinitrophenol hardly maintained their initial weight of 25 gm. (Table 2). The experiment was terminated 40 weeks after the injection of carcinogen as the rate of appearance of tumors had definitely decreased. There was no difference in tumor formation between the two groups.

Summary of sarcoma experiments.—In experiment 1 the addition of sodium fluoride or dinitrophenol to the diet resulted in a striking reduction in the incidence of spontaneous mammary carcinomas. In contrast, diets containing the same levels of sodium fluoride or dinitrophenol had no inhibiting effect on the formation of benzpyrene-induced sarcomas; actually in the three experiments with sodium fluoride there is a consistent suggestion of a slight augmentation of sarcoma formation. There was no decrease in the incidence of sarcomas even though the body weights of the experimental mice ranged, on the average, from 10 to 40 per cent less than those of the control mice.

<table>
<thead>
<tr>
<th>TABLE 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>THE EFFECT OF DINITROPHENOL ON THE FORMATION OF BENZPYRENE-INDUCED SKIN TUMORS AND ON THE BODY WEIGHT OF THE MICE</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GROUP*</th>
<th>NUMBER OF MICE†</th>
<th>MEAN BODY WEIGHT§</th>
<th>TIME OF TUMOR APPEARANCE (WEEKS)</th>
<th>NUMBER OF MICE§</th>
<th>MEAN BODY WEIGHT§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>42</td>
<td>19</td>
<td>13-39</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td>DNP</td>
<td>42</td>
<td>18</td>
<td>13-39</td>
<td>21</td>
<td>20</td>
</tr>
</tbody>
</table>

* DNP indicates 0.27 per cent sodium salt of dinitrophenol in diet. † Number of mice alive when first tumor was observed. § The mean weights are those of mice without tumors; time in weeks after first application of carcinogen.

Induced Skin Tumors

Experiment 6.—Two groups of 45 JAX-Swiss female mice about 10 weeks old were used. The basic ration was of the same composition as that of experiments 2, 3, and 4. The mice of the control group, K2, were fed 4.5 gm. per day; group K28 was fed the same kind and amount of diet but containing sodium dinitrophenol at the level of 0.27 per cent. Four days after the diets were instituted the mice were given the first application of carcinogen, a single drop of a 0.3 per cent solution of benzpyrene in benzene, to the interscapular area; 52 such applications were given in 21 weeks. The mice fed dinitrophenol consumed nearly all of the 4.5 gm. of daily ration, while the mice of the control group ate an average of 3.3 gm. The dinitrophenol mice drank 30 per cent more water than the controls. Dinitrophenol retarded the growth of the mice despite increased food consumption (Table 3).

The experiment was terminated 42 weeks after the first application of carcinogen inasmuch as few skin tumors appeared after the thirty-fourth week. The data on formation of skin tumors are summarized in Table 3: the incidences were 51 per cent for the control mice, and 43 per cent for the mice in the dinitrophenol group.

Lung Adenoma

Experiments 2, 3, 4, and 6 were performed on strains of mice which spontaneously develop lung tumors (Swiss and ABC). At the termination of those experiments the autopsy observations were used to evaluate the effects of sodium fluoride or dinitrophenol on the incidence of grossly visible primary lung tumors; the few mice bearing other tumors were excluded. Either sodium fluoride or dinitrophenol, incorporated in the diet, caused a significant reduction in the incidence of...
lung tumors (Table 4). These results were obtained in experiments in which there was no noteworthy inhibition in the incidence of either skin tumors or sarcomas.

The lung tumors have been designated as primary rather than spontaneous inasmuch as all the mice were treated with benzpyrene which may have augmented their rate of appearance (5, 6). The lung tumor incidence of group L30, experiment 3, was considerably higher than that of group L30, experiment 2, although the mice were of the same strain, sex, and age. This probably was due to the fact that group L30 was injected with ten times as much carcinogen.

**DISCUSSION**

The results of the experiments included in this publication indicate that, under the selected conditions, the feeding of sodium fluoride or of sodium 90° F., than that of mice at room temperature. In our experiment, the mice housed at 45°–55° F. were given only 3.4 gm. of food daily (on the average about 10 per cent more than that eaten by the mice in 80° F. environment); they would have eaten more if food had been available. Possibly as a partial consequence of this relative restriction of caloric intake, the mice housed in the cold room maintained average body weights of 20 to 22 gm. compared with attained values of 29 to 30 gm. for the control mice.

Mills and co-workers have published results on the effects of the environmental temperature on the formation of tumors in mice (9, 10, 11). Although the factors of the individual experiments differed slightly, in general, groups of mice were housed at 68°, 79°, and 91° F. In early publications, no data on food consumption or body weight were presented, although it was stated that the mice at 91° F. were smaller; later data (12) suggest that both food consumption and body growth were decreased at 91° F. Spontaneous mammary tumors, in both dba and C3H strains, appeared earlier and in greater numbers in mice housed at 68° F. than in mice in the 91° F. room. In our experiment, an 80° F. environment was compared with one of 45°–55° F.; the mice in the latter environment developed considerably fewer tumors. The common point of agreement between the studies of Mills and associates and our own is that inhibition of the formation of spontaneous mammary carcinomas, in mice housed at low or high environmental temperatures, was associated with retarded body growth. Considering both studies, the effect on tumor formation does not appear to be directly related to the total food consumption.

**TABLE 4**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Group</th>
<th>Description</th>
<th>Age of mice (weeks)</th>
<th>Number of mice</th>
<th>Per cent with lung tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (JAX-Swiss)</td>
<td>L30</td>
<td>Control</td>
<td>62</td>
<td>15</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>L7</td>
<td>Dinitrophenol</td>
<td>62</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>L807</td>
<td>Sodium fluoride</td>
<td>14</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>3 (JAX-Swiss)</td>
<td>L50</td>
<td>Control</td>
<td>62</td>
<td>11</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>L407</td>
<td>Sodium fluoride</td>
<td>10</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>4 (JAX-ABC)</td>
<td>L60</td>
<td>Control</td>
<td>60</td>
<td>29</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>L67</td>
<td>Sodium fluoride</td>
<td>25</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>6 (JAX-Swiss)</td>
<td>K2</td>
<td>Control</td>
<td>52</td>
<td>15</td>
<td>53</td>
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<tr>
<td></td>
<td>K28</td>
<td>Dinitrophenol</td>
<td>21</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

2,4 dinitrophenol, or exposing the mice to low environmental temperature significantly inhibits the formation of the spontaneous mammary carcinoma; the feeding of either of the two "growth-retarding chemicals" significantly inhibits the formation of primary lung adenoma; the two chemicals have little effect upon the formation of the induced sarcoma; and dinitrophenol has no significant effect upon the formation of the induced skin tumor.

**Low environmental temperature.**—It is well known that warm-blooded animals tend to increase their food intake when living in the cold. For example, Schwabe, Emery, and Griffith (7) reported that rats housed at 45°–55° F. consumed approximately 30 per cent more food than the controls, yet they experienced some growth retardation. Donhoffer and Vonotzy (8) have shown that the caloric intake of mice is 10 to 20 per cent greater at 50° F., and 10 to 20 per cent lower at 90° F., than that of mice at room temperature. In our experiment, the mice housed at 45°–55° F. were given only 3.4 gm. of food daily (on the average about 10 per cent more than that eaten by the mice in 80° F. environment); they would have eaten more if food had been available. Possibly as a partial consequence of this relative restriction of caloric intake, the mice housed in the cold room maintained average body weights of 20 to 22 gm. compared with attained values of 29 to 30 gm. for the control mice.

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Dinitrophenol.—Administration of dinitrophenol increases the metabolic rate mainly by direct action on the tissues. When fed continually at suitably high levels, it also induces increased food consumption and a loss of body weight (13). Our results are in agreement with these observations in that ingestion of 0.25 per cent sodium 2,4 dinitrophenol (8 to 10 mg. daily) in the ration produced a severe restriction in body growth despite increased food consumption. There was a considerable reduction in the incidences of spontaneous mammary carcinomas and primary lung adenomas but little or no effect upon the rates of appearance or incidences of induced sarcomas or skin tumors. On the other hand, Kreyberg (14) reported that skin tumors induced by tarring appeared at an accelerated rate in mice fed dinitro-o-cresol at levels from 0.1 to 2.0 mg. per day.

Sodium fluoride.—It has been shown (15) that sodium fluoride, fed to rats at levels of 0.1 per cent of the ration, effects a significant retardation in body weight without any accordant change in food consumption. In the sodium fluoride experiments described in the present paper, 0.09 per cent of the salt was incorporated into the diet. The average weights of the several experimental groups were about 10 to 40 per cent less than the weights of the corresponding control groups while the caloric intakes were within 10 per cent, greater or less, of the controls. Sodium fluoride considerably inhibited the incidence of spontaneous mammary carcinomas and of lung adenomas, but not of induced sarcomas. In fact, in the experiments with sarcomas, the fluoride-treated groups of mice had a slightly greater incidence of sarcomas than the mice of the corresponding control groups. The differences in the individual experiments were not of statistically significant magnitude but were consistently in the same direction.

In all of the experiments the mice ingesting 0.09 per cent sodium fluoride drank at least twice as much water as the control mice.

Implications of results.—Two main questions arise from this work: 1) Why did sodium fluoride or dinitrophenol affect the formation of spontaneous mammary carcinomas and lung adenomas differently from that of induced sarcomas and skin tumors? 2) Do the results of the present investigation give any insight into the mechanism by which restricted caloric intake inhibits the formation of tumors?

Feeding of sodium fluoride or dinitrophenol resulted in drastic retardation of body growth. Although, in each experiment, the average weight of the treated mice was considerably less than that of the control mice, the incidence of spontaneous mammary carcinomas and of lung adenomas was markedly reduced, while the formation of sarcomas and of skin tumors, induced by carcinogenic hydrocarbons, was not significantly affected. The discrepant effects of the feeding of sodium fluoride or dinitrophenol on tumor formation are further emphasized by the comparison between their slight influence on the incidences of sarcomas and skin tumors (in experiments 2, 3, 4, and 6) and the considerable reduction in the incidence of primary lung adenomas in the mice of the same groups. There are other instances in which an experimental procedure produced dissimilar effects on tumor formation depending on the neoplasm investigated. This is not unexpected since tumors are different diseases arising in different tissues under different conditions. For example, although caloric restriction inhibits the formation of all tumors tested to the present time, the spontaneously occurring mammary carcinoma, lung adenoma, and hepatoma are affected to a greater degree than are the induced epithelioma or sarcoma of the mouse (2, 16). Fat-enriched diets augment the formation of spontaneous mammary carcinoma and skin tumors induced by carcinogenic hydrocarbons, but have no effect on the incidence of spontaneous lung adenoma or of the sarcoma induced by carcinogenic hydrocarbons (17). Varying the proportion of dietary casein has a pronounced effect on the incidence of spontaneous hepatomas in mice but produces negligible change, if any, in the formation of spontaneous mammary tumors and induced skin tumors (18) or induced sarcomas (18, 19).

There appears to be no simple explanation for the diverse response of different tumor types to the experimental procedures employed in the present study. Possibly the most important determinants are the nature (exogenous or endogenous), potency, and period of action of the carcinogen. It is likely that the spontaneous mammary carcinoma and lung adenoma are produced by mild carcinogenic factors acting over a long period of time, as suggested by their relatively long latent periods. On the other hand, the induced skin tumor and sarcoma, here studied, are produced by relatively large doses of agents exerting their carcinogenic action over a shorter period; under these conditions, the experimental procedures probably are less able to modify the formation of tumors. There is evidence from other studies (20, 21, 22) suggesting that the use of smaller doses of carcinogenic hydrocarbons would increase the sensitivity of the response to the experimental procedures employed. Other factors, also suspect as causes of the diverse
response of various tumors, are the differential actions that both carcinogens and experimental procedures may have on various tissues.

It may be that under other experimental conditions, where low doses of sodium fluoride or dinitrophenol, or housing at slightly below "normal" temperature are employed, mice may consume somewhat more food than the controls and experience no significant restriction in body growth. Under such conditions there may even be some enhancement of tumor production, as suggested by Kreyberg's experience with dinitrocresol (14).

The central purpose of these investigations was not to disclose the effects of sodium fluoride, dinitrophenol, or low environmental temperature on tumor formation; rather, these growth-retarding procedures were utilized to study the mechanism by which caloric restriction inhibits tumor formation. Such investigations could not reveal the finer mechanism of the caloric effect, but might indicate whether decreased caloric intake, accompanying restriction in body weight, or decreased total metabolism is the factor most directly involved.

The results suggest that the amount of food consumed (caloric intake) itself is not the means through which caloric restriction inhibits tumor formation. In the present study the incidences of spontaneous mammary carcinomas and primary lung tumors were strikingly decreased, regardless of whether the food consumption values were greater or less than the corresponding values for control groups; the incidences of induced sarcomas and skin tumors also were not associated with food consumption values.

When the procedures resulted in decreased tumor incidence—in the experiments with the spontaneous mammary carcinoma and primary lung adenoma—the common feature was restricted growth of the animals. In the caloric restriction experiments, as well as those reported here, food intake was inadequate to meet the metabolic requirements of the animal. Neither the rate nor amount of metabolic turnover appears to be the primary factor in the incidence of tumors. What seems to be of significance is the body weight at which a balance is struck between caloric intake and expenditure. If this is struck at a high level of body weight the incidence of tumors is high; if at a low level of body weight the incidence is low. Other dietary modifications, such as limiting the proportion of protein or amounts of riboflavin to levels inadequate for body growth, also result in decreased incidence of these tumors (23, 24, 25, 36). Thus, so far as caloric restriction is concerned, the inhibitory effect on tumor formation may be associated with the limited body weight. On the other hand, the absence in the present study of inhibition of formation of sarcomas or skin tumors, despite considerable reduction in body weight, limits such an interpretation unless recourse is had to explanations special to the experimental procedures or tumors.

**SUMMARY**

The present study was undertaken to determine whether chronic caloric restriction inhibits the formation of tumors through the decreased caloric intake, the associated retardation of body growth, or the reduced total metabolism. Procedures that resulted in retarded body growth despite unchanged or augmented food consumption and total metabolism were investigated: housing mice at low environmental temperature, or feeding them either dinitrophenol or sodium fluoride. Four tumors of the mouse were utilized, but only the mammary carcinoma was investigated with all three experimental procedures.

Under the selected conditions, housing the mice at 45°-55° F., or feeding them either 0.25 per cent sodium 2,4 dinitrophenol or 0.09 per cent sodium fluoride, significantly inhibited the formation of spontaneous mammary carcinomas; the feeding of either of the two chemicals significantly inhibited the formation of primary lung adenomas; the two chemicals had little effect upon the formation of induced sarcomas; and dinitrophenol did not appreciably alter the incidence of induced skin tumors.

The diverse response of the mammary and lung tumors in comparison with the sarcoma and skin tumor is discussed in relation to experiences with procedures other than those employed in this study. The present data suggest that neither the food consumption (caloric intake) nor the amount or rate of metabolic turnover are consistently related to tumor formation. However, the results with the mammary carcinoma and lung adenoma imply that the inhibition of tumor formation brought about by caloric restriction is associated with the low weight of the animals.

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Effect of Low Environmental Temperature, Dinitrophenol, or Sodium Fluoride on the Formation of Tumors in Mice

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