The Genesis and Growth of Tumors

VI. Effects of Varying the Level of Minerals in the Diet*

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Investigations concerning the influence of nutrition on the genesis and growth of neoplasms have emphasized the significance of the level of caloric intake and the proportions of dietary fat, protein, and vitamins (13). Little attention has been given recently to the possible role of the salt content of the diet in modifying the rate of formation or growth of tumors. In the earlier literature there are occasional reports that certain inorganic ions might be of specific importance, but generally these dealt with marked deficiencies or toxic excesses. There are no definitive reports, however, dealing with the influence of variations in the proportion of dietary salts (as a group) within what might be considered physiological limits. The lack of such studies and also the desire to complete the broad outlines of an over-all program on nutrition in relation to neoplasia (7-10, 12) prompted the present experiments. The results indicate that varying the salt content of the diet from 2 to 8 per cent has no noteworthy effect upon the relative frequency or rate of formation of the spontaneous mammary carcinoma and carcinogen-induced skin tumors of the mouse.

METHODS

The mice were of inbred strains, raised in our laboratory. At weaning they were divided into three groups, litter-mate distribution being employed as far as possible. They were housed five to a cage and were fed Purina Laboratory Chow Checkers until institution of the experimental diets.

In all experiments the mice received a partially purified diet which, excluding the salts, was composed of:

- Casein, vitamin-free 22 per cent
- Gelatin 2
- Partially hydrogenated cottonseed oil 5
- Cornstarch 71

In addition, the daily ration was supplemented with 0.09 ml. of yeast extract and the following amounts of crystalline B vitamins: thiamine, 28 μg; riboflavin, 8 μg; pyridoxine, 24 μg; pantothenate, 92 μg; niacin, 80 μg; choline, 2,000 μg. Ten U.S.P. units of Vitamin A, 1 U.S.P. unit of Vitamin D, and 0.4 mg. of Vitamin E were incorporated into the daily ration.

For the mineral component of the diet, Wesson’s modification of the Osborne-Mendel salt mixture was utilized (14). It is a standard, adequate mixture made up as follows:

<table>
<thead>
<tr>
<th>Salt</th>
<th>Gm/kilo</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>105</td>
</tr>
<tr>
<td>KCl</td>
<td>180</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>310</td>
</tr>
<tr>
<td>Ca₃(PO₄)₂</td>
<td>149</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>210</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>90</td>
</tr>
<tr>
<td>MnSO₄</td>
<td>0.2</td>
</tr>
<tr>
<td>FePO₄·4H₂O</td>
<td>14.7</td>
</tr>
<tr>
<td>K₃Al(SO₄)₄·24H₂O</td>
<td>0.09</td>
</tr>
<tr>
<td>CuSO₄·5H₂O</td>
<td>0.39</td>
</tr>
<tr>
<td>NaF</td>
<td>0.57</td>
</tr>
<tr>
<td>KI</td>
<td>0.05</td>
</tr>
</tbody>
</table>

This salt mixture was added to the basic ration at levels of 2, 4, or 8 per cent. Thus, each experiment included three groups of mice which were fed diets identical in composition and caloric content but differing in mineral content. The 2, 4, and 8 per cent levels were considered to represent low, moderate, and high proportions of dietary minerals, and, for simplicity, these terms will be used in referring to the rations and the respective experimental groups of mice.

In the first experiments with the spontaneous mammary carcinoma (Exp. 1) and the induced skin tumor (Exp. 3), the mice were fed ad libitum, and there were differences in food intake and body weights among the three groups of a study. Such differences were prevented, for all practical purposes, in Experiments 2 and 4, begun about 1 year later. This was accomplished by feeding the three groups of an experiment equicaloric amounts at slightly below ad libitum levels. Although there is relatively good agreement between the findings of the four experiments, those of the latter two are more specific.

The mice were allowed drinking water ad libitum. The routine execution of the experiments—preparation and storage of the diets, determination of food consumption and body weights, inspection for physical condition of the mice, gross

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recognition of tumors, and histologic examination of lesions—are described in previous publications (5, 10).

EXPERIMENTS AND RESULTS

GENESIS OF SPONTANEOUS MAMMARY CARCINOMA

Experiment 1.—Three groups, each consisting of 47 DBA female mice 21–26 weeks of age, were placed on partially purified diets which differed only in mineral content—2, 4, or 8 per cent. The diets were offered ad libitum. Within a few weeks the groups began to display differences in caloric intake that persisted throughout the experiment. The mean daily consumptions were 9.8, 9.4, and 8.9 Calories for the low-, moderate-, and high-salt groups, respectively. There were consonant differences in mean body weight (Table 1).

In each group five mice were alive and free of tumors at the termination of the experiment. The control of food intake resulted in body weights of a similar order.

In each group five mice were alive and free of tumors at the termination of the experiment. Some particulars of the study and the results are listed in Table 1. Here, too, no noteworthy differences were observed between low-, moderate-, and high-salt groups as to the relative frequency and times of appearance of mammary carcinomas, nor was there an influence on the incidence of mice with more than one mammary tumor.

GENESIS OF SKIN TUMORS INDUCED BY CARCINOGENIC HYDROCARBONS

Experiment 3.—Three groups of 50 C3H male mice, 12–14 weeks of age, were placed on partially purified rations which differed only in mineral content—2, 4, or 8 per cent. The mice, fed ad libitum, consumed an average of 14.3, 13.9, and 13.4 Calories, respectively.

Five weeks after institution of the experimental diets the animals received the first application of the carcinogen, a single drop of a 0.1 per cent acetone solution of 20-methylcholanthrene on the interscapular area. They were given a total of seventeen applications at twice-weekly intervals. The experiment was ended 51 weeks after the first application of the carcinogen, at which time 0, 9, and 13 nontumorous mice were alive. The incidences of total skin tumors (papillomas and carcinomas) were 90, 69, and 58 per cent, respectively: 72, 56, and 44 per cent. Although the divergence in tumor in-

The study proceeded without untoward events and was terminated when the mice were 110 weeks old; at this time five, four, and three mice, respectively, were alive and without mammary carcinoma. The data on incidence of tumors and the average time at which they appeared are given in Table 1. It is obvious that there were no significant variations in the relative frequency and rate of formation of spontaneous mammary carcinoma between the groups receiving the diets containing 2, 4, or 8 per cent minerals. About one-fifth of the mice in each group developed multiple mammary tumors.

Experiment 2.—This study was patterned after Experiment 1, with the following pertinent differences: 65 DBA female mice, 10–13 weeks of age, constituted each group; isocaloric food consumption was achieved by feeding the rations at the slightly restricted level of 9.0 Calories per day. The control of food intake resulted in body weights of a similar order.

In each group five mice were alive and free of tumors at the termination of the experiment. Some particulars of the study and the results are listed in Table 1. Here, too, no noteworthy differences were observed between low-, moderate-, and high-salt groups as to the relative frequency and times of appearance of mammary carcinomas, nor was there an influence on the incidence of mice with more than one mammary tumor.

TABLE 1

<table>
<thead>
<tr>
<th>EXPERIMENT</th>
<th>MEAN DAILY CALORIC INTAKE</th>
<th>MEAN BODY WEIGHT (GM.)</th>
<th>PER CENT MICE WITH TUMORS</th>
<th>MEAN TIME OF TUMOR APPEARANCE (WEEKS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Mammary carcinoma</td>
<td>2</td>
<td>9.8</td>
<td>27</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>9.4</td>
<td>25</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>8.9</td>
<td>25</td>
<td>29</td>
</tr>
<tr>
<td>2. Mammary carcinoma</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>9.0</td>
<td>29</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>8.0</td>
<td>27</td>
<td>26</td>
</tr>
<tr>
<td>3. Skin tumors</td>
<td>2</td>
<td>14.3</td>
<td>53</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>15.0</td>
<td>53</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>15.4</td>
<td>53</td>
<td>54</td>
</tr>
<tr>
<td>4. Skin tumors</td>
<td>2</td>
<td>11.2</td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>11.2</td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>11.2</td>
<td>54</td>
<td>54</td>
</tr>
</tbody>
</table>

* Number of mice adjusted for deaths of nontumor animals (1).
† Weeks after initial application of carcinogen.
cidence is statistically significant, its biological pertinence must be evaluated in light of the concomitant decrease in food consumption and body weight values (Table 1). This is considered in the discussion.

**Experiment 4.**—The conditions were similar to those employed in Experiment 3, except for the following differences: 65 DBA male mice, 9–12 weeks of age, composed each group; isocaloric food consumption was achieved by feeding the mice restricted rations containing only 11.2 Calories per day (this resulted in comparable mean body weights); after the mice were on their experimental diets for 11 weeks, the applications of carcinogen were begun. Eighteen applications, each consisting of a single drop of a 0.3 per cent solution of 3,4-benzpyrene in acetone, were given at twice-weekly intervals.

The experiment was concluded 51 weeks after the first application of the carcinogen. The results are shown in Table 1. In contrast to the findings of Experiment 3, varying the mineral content of the diet from 2 to 4 to 8 per cent had no significant effect upon the incidence of total skin tumors or upon their average time of appearance. Not listed in the table are the percentages of mice with carcinoma; by the end of the experiment, 51 weeks, these were 56, 56, and 53, respectively, for the groups consuming 2, 4, and 8 per cent dietary minerals.

**GENESIS OF SPONTANEOUS BENIGN HEPATOMAS**

Separate studies on the influence of the mineral content of the diet on the formation of spontaneous benign hepatomas were not conducted. However, inasmuch as C3H and DBA male mice on partially purified diets develop these neoplasms in appreciable frequency, the animals of Experiments 3 and 4 were routinely examined for hepatomas at autopsy. The incidences are shown in Table 2.

Although, in both studies, there was a decreasing incidence of neoplasms with the increasing proportion of dietary minerals, the differences are of small magnitude. It is concluded, tentatively, that varying the dietary salts from 2 to 8 per cent produces no important effect on the incidence of spontaneous benign hepatomas of the mouse.

**TUMOR GROWTH AND METASTASES**

The actual rate of increase in the size of tumors was not determined. However, it was considered that an acceptable criterion of tumor growth is the survival time of the host—i.e., the interval between appearance of the neoplasm and death of the animal. Appropriate data have been gathered on spontaneous mammary carcinomas in C3H and DBA mice, indicating that the coefficient of linear correlation between the rate of tumor growth and the survival time of the host is approximately $-0.5$. In Experiment 1 the mean survival times were 8.9 weeks $\pm 0.65$, 9.3 $\pm 0.74$, and 9.3 $\pm 0.65$ for the low, moderate, and high-salt groups, respectively; in Experiment 2 the values were 8.2 $\pm 0.78$, 10.2 $\pm 0.78$, and 10.9 $\pm 0.92$. It is our opinion that alterations in dietary minerals, within the range utilized in these experiments, had no effect upon the growth of mammary neoplasms.

That the tumor-host relationship was not affected is also suggested by the data on grossly visible metastases to the lungs. In both experiments with mammary carcinomas, the relative frequency of metastases was approximately 10 per cent and did not vary with the salt content of the diet. In Experiment 4, the relative frequencies of metastases from skin carcinomas were 27, 25, and 26 per cent, respectively, for the low-, moderate-, and high-salt groups.

**DISCUSSION**

There is no reliable evidence that the genesis and growth of neoplasms are influenced significantly and specifically by altering the proportions of inorganic components natural to the diet. The literature contains many contradictory reports, most of which are based on studies that suffered from a variety of technical difficulties. Stern and Willheim reviewed the evidence and aptly concluded that there are few claims that have been verified without doubt (6). A principal error has been the failure to consider the role of the changes in nutritional state that usually accompany deficiencies or toxic excesses of dietary components. When such relationships were evaluated they generally sufficed to explain any observed effects on neoplasia. For example, injurious levels of fluoride definitely retarded the genesis of mammary carcinoma and lung tumors, but apparently no more than might be expected from the decrease in food intake and body weight (11). A diet deficient in potassium hindered the development of
tumor implants (2); again, the effect could be largely explained by the retarded growth of the deficient mice.

In the experiments reported in this publication, proportions of dietary minerals from 2 to 8 per cent were chosen, since it was reasoned that these levels approximate the lower and upper limits of the physiological range; furthermore, they are not trivial differences with respect to the animal. This is substantiated by studies on the influence of various salt levels on body and bone growth (3, 4) and by data of the present experiments. When fed ad libitum, the 8 per cent salt diet, as compared to the 2 per cent salt diet, resulted in suppression of food intake and body weight. In addition, the relative ad libitum water consumptions of the 2, 4, and 8 per cent salt groups were approximately 0.8:1.0:1.2.

The diets contained the minerals in Wesson's modification of Osborne-Mendel's salt mixture, as well as the inorganic ions present in the other dietary constituents. The salt mixture contains those trace inorganic elements for which need has been established; others not specifically incorporated into the mixture (cobalt and zinc) are probably present in trace amounts in both the salt mixture and in the other components of the diet. In the studies reported, no objective evidence of deficiencies was observed. At any rate, the findings and conclusions of this publication are related only to the stated conditions of the experiments.

It is likely that the results of Experiment 3 are valid—that there was a decreasing incidence of tumors with increasing proportions of dietary minerals. However, the results are in logical order and relation to the decreasing caloric consumption and body weight values, which in themselves may explain the findings (19). In fact, this interpretation seems so reasonable to us that the absence of a similar trend in Experiment 1 was unexpected.

With respect to the specific influence of the level of salt intake, however, principal attention must be given Experiments 2 and 4, in which food intakes were kept isocaloric, and resulting body weights were similar. In these studies no significant modification of the genesis of neoplasms occurred as a consequence of varying the mineral content of the diet from 2 to 4 to 8 per cent.

SUMMARY

The influence of varying the salt content of the diet on the genesis and growth of the spontaneous mammary carcinoma and carcinogen-induced skin tumors of the mouse was investigated. The studies utilized partially purified rations containing a standard salt mixture at levels of 2, 4, or 8 per cent of the diet—considered to be the physiological range for dietary minerals. When the experimental conditions were controlled so that the groups of mice were equivalent as to mean caloric intake and body weight, no significant differences were observed in the incidences or mean times of appearance of the neoplasms, or in their rate of growth.

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