Influence of Thyroid Hormone on the Formation of Induced Skin Tumors in Mice*

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Chronic caloric restriction inhibits the formation of various tumors of the mouse (1, 2). The restriction in caloric intake produces, among many changes, a decreased total metabolism and decreased body weight of the animals. In order to acquire information as to which of these—caloric intake, total metabolism, or body weight—might be most directly related to tumor formation, diverse experimental procedures have been studied: feeding of dinitrophenol or sodium fluoride, and the housing of the mice at low environmental temperatures (3).

The present study supplements the above experiments and has a similar objective; in this instance thyroid hormone was fed to mice in a dose that caused increased food consumption and increased metabolism, and only a slight retardation of body growth. The experiments were designed to also reveal the effects of administration of thyroid on the different stages of carcinogenesis.

The concept of discrete stages in the genesis of tumors has been introduced through a variety of studies (4, 5, 6, 7, 8) indicating that the formation of induced skin tumors proceeds through at least 2 stages: a) initiatory changes induced by the action of a carcinogen on normal cells; and b) the promotion or development of these "biased" cells, under necessary conditions, into neoplastic cells that grow into a visible tumor. These two stages can be separated roughly by the experimental technique of applying a carcinogen for a short time and terminating these applications before tumors arise in any of the experimental animals. One may arbitrarily regard the consequent intervals—a) that encompassing the application of the carcinogen, and b) the subsequent period during which grossly visible tumors appear—as corresponding respectively to the stage of initiation and the stage of development. This technique has been employed to demonstrate that the inhibitory ac-

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This general knowledge of the stages of carcinogenesis has been briefly reviewed, because the present study was in part based upon these considerations. The experiments were designed to study the effects on skin tumor formation of thyroid hormone when administered a) throughout the experiment, b) only during the interval of cutaneous application of carcinogen, and c) only during the period of tumor appearance.

METHODS

The mice employed were adult dba strain males bred in our laboratories. Litter mates were distributed between the several groups of each experiment so far as possible. From the time of weaning until transfer to the experimental diets they were fed a commercial ration, Purina fox chow checkers. In all experiments the animals were housed in groups of 5 in cages with solid bottoms. The carcinogen was a 0.3 per cent solution of 3,4-benzpyrene in acetone; at semi-weekly intervals a single drop (0.02 cc.) was applied to the interscapular area by means of a dropping pipet.

The experimental diets were composed of Purina fox chow meal, skimmed milk powder, and cornstarch, and were prepared as in earlier work (9). The thyroid extract (Proloid)1 was incorpo-

1 Generously donated by The Maltine Company, N.Y. Proloid is a thyroglobulin equivalent to an equal weight of Armour's U.S.P. thyroid; 1 grain of Proloid corresponds to 169 micrograms of crystalline thyroxine in the thyroidectomized rat assay. It is also stated to have less toxic action than other thyroid preparations.
rated in the diets at the level of 0.04 per cent, inasmuch as previous experience had indicated that this concentration increased caloric intake by approximately 50 per cent, without a drastic effect on body weight. Where the design of the experiment called for changing the diet, this was done a few weeks after the final application of the carcinogen.

The general care of the animals, examination and evaluation of tumors and other pathology, and recording were carried out as previously described (9).

EXPERIMENTS

Experiment 1. Three month old dba males were distributed into 3 groups of 50 mice each. The diets were composed of Purina fox chow meal, 55 per cent; skimmed milk powder, 20 per cent; and cornstarch, 25 per cent. The ration at the level of 4 gms. daily per mouse was the control diet. The same ration at the level of 6 gms. and containing 0.04 per cent thyroid extract, was the thyroid diet. These amounts were approximately at the ad libitum level. Group h-CC2 was fed the control diet, and groups h-TC, and h-TT were fed the thyroid diet. Three weeks after institution of the experimental diets, treatment with carcinogen was begun. Fourteen applications of the solution of benzpyrene were given at semi-weekly intervals over a period of 6 1/2 weeks; 3 weeks after the final application, the mice of group h-TC were transferred from the thyroid ration to the control ration; the rations of groups h-CC and h-TT were not changed. Thus group h-CC was fed the control diet, and groups h-TC, and h-TT were fed the thyroid diet. During the course of the experiment the caloric intake was dependent on the ration fed. The mice on the control diet consumed, on the average, 3.9 to 4.0 gms. daily; those on the thyroid diet, 5.4 to 6.0 gms. In the same order, they drank approximately 3 cc. and 6 cc. of water daily. Although they consumed nearly 50 per cent more food, the mice on the thyroid diet weighed less than those on the control diet (Table 1a).

The formation of skin tumors is illustrated in Figure 1 and the data summarized in Table 1b. The results are discussed together with those of the following experiment.

Experiment 2. Two hundred dba male mice, approximately 2 1/2 months of age, were divided into 4 equivalent groups of 50 each. The ration consisted of Purina fox chow meal, 50 per cent; skimmed milk powder, 25 per cent; cornstarch, 22.5 per cent; and brewers yeast, 2.5 per cent. This ration, compared with that of Experiment 1, contains a higher proportion of protein, mineral, and fat and a supplementary source of B-vitamins. The control diet consisted of 4.0 gms. of the ration daily, the thyroid diet of 6.0 gms. of the ration containing 0.04 per cent thyroid extract.

Four weeks after initiation of the experimental diets, the mice were given the first of 12 semi-weekly applications of the benzpyrene solution—over a period of 5 1/2 weeks. Three weeks after the final application of carcinogen the diets were either

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1 For convenience, the experimental groups are designated as follows: The initial letter designates the experiment. The first letter after the dash indicates the diet during the period of carcinogen application, C (control) or T (thyroid). The second letter after the dash indicates the diet during the period of tumor appearance, again either C or T.

3 Anheuser-Busch Strain K—generously donated by Anheuser-Busch Inc.
changed or continued according to the design of the experiment as tabulated:

<table>
<thead>
<tr>
<th>DIETARY REGIMEN DURING</th>
<th>Period of carcinogen application</th>
<th>Period of tumor appearance*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>(–4 to 84 weeks)</td>
<td>(84 weeks to end of experiment)</td>
</tr>
<tr>
<td>n-CC†</td>
<td>control</td>
<td>control</td>
</tr>
<tr>
<td>n-TT</td>
<td>thyroid</td>
<td>thyroid</td>
</tr>
<tr>
<td>n-CT</td>
<td>control</td>
<td>thyroid</td>
</tr>
<tr>
<td>n-TC</td>
<td>thyroid</td>
<td>control</td>
</tr>
</tbody>
</table>

* Time is indicated in weeks after initial application of carcinogen.
† See footnote number 2.

The study was terminated 48 weeks after the initial application of carcinogen inasmuch as subsequent to this time there occurred a sharp increase in the death rate of the mice ingesting the thyroid diet. In the first 48 weeks of the experiment, there were only 2 to 5 deaths in a group, and most of these were caused by spontaneous lymphomata.

The mice receiving the control diet consumed from 3.7 to 4.0 gms. of food daily, those on the thyroid diet from 5.2 to 6.0 gms. The water consumption of the latter was from 25 to 50 per cent greater than that of the mice on the control ration. As in Experiment 1, the mice ingesting the thyroid diet weighed less than those on the control diet despite the increase in caloric consumption of approximately 40 per cent (Table 2a). On the average, the hyperthyroid mice were only about 2 per cent smaller in body length.

With respect to the formation of skin tumors, the results of Experiment 2 (Table 2b, Figure 2) agree with those of Experiment 1 (Table 1b, Figure 1) in the following particulars: The initial tumors appeared earlier in those groups receiving the thyroid diets during the period of application of carcinogen (h-TT and n-TC compared with h-CC; and n-CC and n-CT). The tumor incidences among the mice fed the thyroid diet only during the period of carcinogen application, followed by the control diet during the period of tumor formation (groups h-TC and n-TC), were slightly greater than those of the mice on the control diets throughout the experiment. Tumor incidences among the mice fed the thyroid diet throughout (h-TT and n-TT) were at first slightly greater than among the control mice, but at about the thirtieth week there was a relatively sharp reduction of tumor formation among the hyperthyroid mice, and by the time the experiments were terminated these groups had slightly lower tumor incidences than the controls. As a consequence of this early augmentation and later repression of tumor formation the mean times of tumor appearance were shorter in groups h-TC and n-TC than in the corresponding control groups. The effect of feeding the thyroid diet only during the period of tumor appearance (i.e. preceded by the control diet during the period of ap-

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TABLE 2a

GROWTH OF MICE IN EXPERIMENT 2

<table>
<thead>
<tr>
<th>GROUP</th>
<th>MEAN BODY WEIGHT (GRAMS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-CC</td>
<td>23</td>
</tr>
<tr>
<td>n-TT</td>
<td>22</td>
</tr>
<tr>
<td>n-CT</td>
<td>23</td>
</tr>
<tr>
<td>n-TC</td>
<td>23</td>
</tr>
</tbody>
</table>

* n-CC: control diet throughout; n-TT: thyroid diet throughout; n-CT: control diet until 84 weeks, thyroid diet subsequently; n-TC: thyroid diet until 84 weeks, control diet subsequently.

TABLE 2b

EFFECT OF FEEDING THYROID HORMONE DURING THE DIFFERENT STAGES OF CARCINOGENESIS ON THE FORMATION OF INDUCED SKIN TUMORS. (EXPERIMENT 2)

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NUMBER OF MICE WITH SKIN TUMORS</th>
<th>TIME OF APPEARANCE OF TUMORS (WEEKS)</th>
<th>FREE AND ALIVE AT END OF EXPERIMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-CC</td>
<td>40</td>
<td>15 11 22 25 28 28 31 32 33 35 35 37 38 38</td>
<td>17</td>
</tr>
<tr>
<td>n-TT</td>
<td>40</td>
<td>22 22 22 22 22 22 22 22 22 22 22 22 22 22</td>
<td>21</td>
</tr>
<tr>
<td>n-CT</td>
<td>40</td>
<td>34 40 40 40 40 40 40 40 40 40 40 40 40 40</td>
<td>21</td>
</tr>
<tr>
<td>n-TC</td>
<td>40</td>
<td>34 60 90 90 90 90 90 90 90 90 90 90 90 90</td>
<td>12</td>
</tr>
</tbody>
</table>

* See footnote, Table 2a, for description of dietary regimen.
† Number of mice obtained by adjustments for deaths of animals without tumors (15).
‡ Time in weeks after initial application of carcinogen.
§ End of experiment.
plication of carcinogen) is illustrated in Experiment 2, group n-CT. There was initially some inhibition of tumor formation (ending at about the twenty-seventh week) followed by an acceleration which raised the tumor incidence to that of the mice fed the thyroid diet throughout.

The values given for skin tumor formation in the tables and figures represent the total number and per cent of mice that developed skin tumors (papillomas and carcinomas). Approximately 70 per cent of the papillomas eventually became carcinomas, and the incidence of mice with carcinomas varied in the same order as given for total skin tumors.

DISCUSSION

The literature contains few reports on the effects of the administration of thyroid hormone on the genesis of neoplasms. Nowak and Ciechanowski (19) observed no change in the rate of appearance of tar-induced skin tumors in rabbits. On the other hand, Kreyberg (13) noted an acceleration of skin tumor formation in tarred mice. Smith and associates (14) reported no effect of the hyperthyroid state on the formation of induced sarcomas in rats; the result was attributed in part to the high dose of carcinogen employed. We have unpublished experiments in which mice injected with moderate doses of carcinogen were given thyroid extract in rations fed ad libitum; there were no noteworthy effects on sarcoma formation.

In the present experiments the striking augmentation of caloric intake and metabolic activity produced by the thyroid extract was not accompanied by a spectacular effect upon the incidence or time of appearance of skin tumors induced by the application of carcinogen. The data suggest, however, a small differential action of thyroid hormone during the two different stages of carcinogenesis. When fed during the period of application of carcinogen (initiation stage) it exerted a stimulating action on the formation of skin tumors, principally evidenced by the invariably earlier appearance of the first tumors in the four groups fed thyroid in this period, and by the higher incidence of tumors in the two groups fed thyroid extract during this period only. When administered during the period of tumor appearance (development stage), thyroid extract exerted some retarding effect on skin tumor formation, as indicated by the lower incidence of tumors. If these observations are valid, it would be expected that the feeding of thyroid extract throughout the entire experiment (both stages) would result in a balancing of the two effects. To a degree, the results are in agreement with this expectation.

The mechanisms of these actions are obscure. The administration of thyroid hormone has many effects upon the animals: increased food consumption, increased metabolism, a relative loss in body weight, increased heat loss, peripheral dilatation of blood vessels, shifts and changes in protein and fat storage, etc. It is possible that the accelerating action on skin tumor formation during the initiation stage of carcinogenesis is due to local changes at the site and time of carcinogen application, possibly even affecting the tissue dose of carcinogen. Thus the augmented tumor formation reported by Kreyberg may have been due to protracted tar application (for 6 months), which contrasts with the relatively short period of carcino
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on tumor formation mainly during the second stage of carcinogenesis (9).

The most pertinent finding is that the considerable augmentation of food intake and metabolic activity, produced by thyroid extract, resulted in only a small effect upon tumor formation. These data support the conclusions arrived at in related investigations (3) concerned with dinitrophenol, sodium fluoride, and low environmental temperature: It is not the level of caloric intake or total metabolic turnover but rather the body weight level at which a balance is struck between caloric intake and utilization that is a significant factor in the genesis of some mouse tumors.

SUMMARY

1. Mice fed diets containing 0.04 per cent thyroid extract (Proloid) consumed 40 to 50 per cent more food, yet weighed about 10 per cent less, than the control mice.

2. The striking augmentation of caloric intake and metabolic activity did not produce a large effect upon the incidence or mean time of appearance of skin tumors induced by the application of 3,4-benzpyrene.

3. The data suggest, however, a small differential effect of thyroid hormone on the two stages of carcinogenesis: stimulating in the stage of initiation and retarding in the stage of development.

4. The results give indirect supporting evidence that the inhibition of tumor formation produced by caloric restriction is more related to the low body weight of the animal than to the actual level of caloric intake or metabolic activity.

REFERENCES


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