Effects of Thyroxine and Thiouracil on Induction of Skin Tumors in Mice by 9,10-Dimethyl-1,2-benzanthracene and Croton Oil*

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Relatively few studies have been made of the influence of the thyroid gland on the development and growth of tumors. Lerman (15) stated that the thyroid does not greatly influence the onset and growth of either experimental or human cancer, but Rawson (17) recently cited some experimental and clinical observations which suggest that hyperthyroidism may inhibit, while hypothyroidism may enhance, tumor formation.

Kreyberg (14) observed that thyroid administration accelerated skin tumor formation in tarred mice, while Silverstone and Tannenbaum (20) found that thyroid extracts had little or no effect on benzpyrene-induced skin tumors in mice. Epitheliomatous proliferation in the rabbit's ear due to tarring was reported to be inhibited by thyroidectomy (19). The sarcomagenic effect of dibenzanthracene was diminished by co-administration of thyroxine and augmented by thiouracil (1), but methylcholanthrene-induced sarcomas in rats were not influenced by thyroid extracts or thyroidectomy (21). Development and growth of other tumors have been reported to be enhanced, diminished, or not affected by thyroid substances (4, 5, 8, 12, 16, 24).

A preliminary experiment in which urethan was given once, followed by twice-weekly paintings of the skin with croton oil, suggested that the co-administration of thyroxine may inhibit, while thiouracil may enhance the development of skin tumors in mice.1 It was the purpose of the present study to determine the effects of thyroxine and thiouracil on the development of skin tumors in mice, induced by a single application of 9,10-dimethyl-1,2-benzanthracene followed by once-weekly painting with croton oil.

MATERIALS AND METHODS

Female Swiss albino mice of the CF1 strain (Carworth) were used in three separate experiments. The age of the mice in each of the three experiments at the beginning of treatment was as follows: 1, 8-9 weeks old; 2, 11-12 weeks old; 3, 6-7 weeks old. These were divided by weight into uniform groups of about twenty each. The hair was removed with electric clippers from the back of each mouse, and a day later 0.05 ml. of a solution of 9,10-dimethyl-1,2-benzanthracene (DMBA) in distilled benzene was applied over a uniform area of the skin. The length and width of the skin area covered by DMBA during application was measured in 42 mice and found to be 2.94 ± 0.57 X 1.99 ± .043 cm. A different level of DMBA was used in each of the three experiments—namely, 1.0, 0.5, and 0.25 per cent. To prevent evaporation, DMBA was freshly prepared for every two groups in separate vials just prior to application and placed on ice during administration. In all three experiments, thyroxine2 or thiouracil was mixed into the diet in concentrations of 2 mg/kg and 2 gm/kg, respectively. Feeding of these substances was begun 10 days before, on the same day, or 2 weeks after applying DMBA, and continued until the end of each experiment. A complete rat diet and water were available at all times. Body weight was measured once weekly and food intake every 2 weeks. The mice were maintained in an air-conditioned room at a temperature of 75° + 1° F.

1 The thyroxine used in this study was kindly supplied by Dr. A. E. Heming of Smith, Kline & French Labs, Philadelphia, Penn.


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The mice were numbered by ear punch, and as tumors began to appear, each was charted to scale for size and location every 2 weeks. Tumors that regressed before 4 weeks from the time of first appearance were not included in the data. Growth less than 1 mm. in diameter were not recorded. No distinction was made between the various morphological types of tumors, although the majority appeared to be papillomas. Tumors that appeared on the ears in some cases were likewise charted. At the end of each experiment, the diameter of each tumor was measured, and the total tumor cross-sectional area per tumor-bearing mouse was calculated.

It appears doubtful whether either DMBA or croton oil in the amounts employed would have produced tumors. Female Swiss albino mice which were painted twice weekly over the skin with 5 per cent croton oil in liquid paraffin for 20 weeks did not develop tumors, nor did mice painted with croton oil and fed thyroxine or thiouracil at the present dose levels. Saffiotti and Shubik (18) reported that a single painting of the skin of female Swiss albino mice with 1.0 per cent DMBA or with 5 per cent croton oil did not produce tumors during a period of 62 weeks.

**RESULTS**

At the end of 17 weeks fifteen of 21 living mice which received 0.25 per cent DMBA had 86 tumors or an average of 5.7 tumors per tumor-bearing mouse (Table 1). Only three of sixteen living, thyroxine-treated mice had five tumors, while 21 of 21 thiouracil-fed mice had 193 tumors, or 9.2 tumors per tumor-bearing mouse. The average surface tumor area was significantly increased in the mice fed thiouracil and reduced in the mice fed thyroxine as compared with the controls. Tumors first appeared in the controls on the 7th week after DMBA treatment, in the thyroxine-fed mice on the 11th week, and on the 5th week in the thiouracil-treated mice. The tumor of one thyroxine-fed mouse regressed completely after observation for 7 weeks.

The average body weights of the mice were similar at the end of the experiment, although the thyroxine-treated mice consistently outgained the other two groups for the first 10 weeks of the experiment. The average daily amount of food consumed by the controls was 3.5-4.0 gm. each; by the thyroxine-fed mice, 5.3-6.2 gm. each; and by the thiouracil-fed mice, 5.3-6.2 gm. each; and by...
in the ensuing 4 weeks. Thyroxine increased and thiouracil reduced food intake to about the same extent as in the first experiment.

When 1.0 per cent DMBA was administered (Table 3), thyroxine and thiouracil influenced tumor yield similarly but to a lesser extent than in the previous two experiments. These substances were effective whether begun on the same day or 14 days after DMBA application. Thyroxine accelerated and thiouracil retarded body growth for the entire 17-week period of treatment in these mice, which were younger than those of the previous two experiments. The average daily amount of food ingested per mouse for each group was as follows: controls, 3.1-4.0 gm.; thyroxine, 4.5-5.8 gm.; thiouracil, 2.8-3.8 gm.

**DISCUSSION**

In all three experiments, thyroxine inhibited while thiouracil enhanced the development of tumors by DMBA and croton oil. These results are in agreement with our previous experiment in which mice were fed the same levels of thyroxine and thiouracil, but urethan was used as an initiat-

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**TABLE 2**

**EXPERIMENT 2: EFFECTS OF THYROXINE AND THIOURACIL ON SKIN TUMORS IN FEMALE SWISS MICE (CF1) GIVEN .5 PER CENT DMBA AND 5 PER CENT CROTON OIL**

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>NO. MICE</th>
<th>NO. MICE WITH TUMORS</th>
<th>AV. SURFACE TUMOR AREA (SQR. MM.)</th>
<th>MICE WITH TUMOR REJUVENATIONS*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Av. body wt. (gm.)</td>
<td>Total tumors per group</td>
<td>Tumor area (sqr. mm.)</td>
<td>No. tumors</td>
</tr>
<tr>
<td></td>
<td>Begin</td>
<td>17th week</td>
<td></td>
<td>(weeks after DMBA)</td>
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<tr>
<td>None</td>
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<td>19</td>
<td>6 8 10 12 14</td>
<td>17.8 ± 5.2</td>
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<td>82.5</td>
<td>82.5</td>
<td>7 9 12 19 38</td>
<td>3.7</td>
</tr>
<tr>
<td>Thyroxine</td>
<td>18</td>
<td>14§</td>
<td>1 2 2 3 5</td>
<td>7.4 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>82.8</td>
<td>82.8</td>
<td>1 2 2 3 5</td>
<td>1.6</td>
</tr>
<tr>
<td>Thiouracil</td>
<td>19</td>
<td>19</td>
<td>4 7 9 14 16</td>
<td>46.8 ± 10.2</td>
</tr>
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<td></td>
<td>82.7</td>
<td>82.8</td>
<td>5 10 47 71 85 112</td>
<td>7.1</td>
</tr>
</tbody>
</table>

Note: see note, Table 1.
* Tumors which regressed after presence of 4 weeks or more.
† Time when 50 per cent of mice developed tumors.
‡ Thyroxine and thiouracil started same day as DMBA.
§ Two mice killed accidentally.

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**TABLE 3**

**EXPERIMENT 3: EFFECTS OF THYROXINE AND THIOURACIL ON SKIN TUMORS IN FEMALE SWISS MICE (CF1) GIVEN 1.0 PER CENT DMBA AND 5 PER CENT CROTON OIL**

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>NO. MICE</th>
<th>NO. MICE WITH TUMORS</th>
<th>AV. SURFACE TUMOR AREA (SQR. MM.)</th>
<th>MICE WITH TUMOR REJUVENATIONS*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Av. body wt. (gm.)</td>
<td>Total tumors per group</td>
<td>Tumor area (sqr. mm.)</td>
<td>No. tumors</td>
</tr>
<tr>
<td></td>
<td>Begin</td>
<td>17th week</td>
<td></td>
<td>(weeks after DMBA)</td>
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<tr>
<td>None</td>
<td>18</td>
<td>17</td>
<td>6 8 10 12 14</td>
<td>16.6 ± 0.8</td>
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<tr>
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<td>90.5</td>
<td>90.5</td>
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<td>2.5</td>
</tr>
<tr>
<td>Thyroxine</td>
<td>17</td>
<td>14§</td>
<td>2 2 3 5 6</td>
<td>7.1 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>90.5</td>
<td>90.5</td>
<td>2 2 3 5 6</td>
<td>1.2</td>
</tr>
<tr>
<td>Thiouracil</td>
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<td>17</td>
<td>4 6 12 13 14 15</td>
<td>38.8 ± 7.8</td>
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<tr>
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<td>91.1</td>
<td>91.0</td>
<td>4 13 41 56 63 67</td>
<td>4.3</td>
</tr>
<tr>
<td>Thyroxine</td>
<td>17</td>
<td>15</td>
<td>2 4 5 9 10 11</td>
<td>9.6 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>91.4</td>
<td>91.6</td>
<td>2 8 9 9 10 11</td>
<td>1.6</td>
</tr>
<tr>
<td>Thiouracil</td>
<td>18</td>
<td>17</td>
<td>4 6 10 18 17 17</td>
<td>19.8 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>91.9</td>
<td>91.8</td>
<td>5 9 15 35 39 40</td>
<td>8.8</td>
</tr>
</tbody>
</table>

Note: see note, Table 1.
* Tumors which regressed after presence of 4 weeks or more.
† Time when 50 per cent of mice developed tumors.
‡ Thyroxine and thiouracil started same day as DMBA.
§ Three mice killed accidentally.
§ Thyroxine and Thiouracil begun 14 days after DMBA.
ing agent followed by twice-weekly paintings with croton oil. Hyper- and hypothyroidism influenced tumor formation most when the lowest dose of DMBA was employed and least when the highest dose of DMBA was used. This is consistent with the view that maximal doses of carcinogens are apt to obscure the effects of other factors on tumor production (9).

Many of the individual tumors in the controls and particularly in the thiouracil-fed mice grew in size during the experiments, whereas the tumors in the thyroxine-fed mice showed little or no increase in size. Also, of a total of seven mice in the three experiments with complete tumor regression after observation for more than 4 weeks, six were from the thyroxine-treated groups. Thyroxine feeding was as effective in inhibiting tumor development when initiated 14 days after as when begun on the same day as DMBA application.

The total amount of thyroxine ingested by the mice was calculated to be approximately 12 μg daily. Since the daily thyroxine secretion rate of female albino mice is about 2.5 μg/100 gm body weight (11), the mice in this study received about 16–24 times their normal daily secretion rate. This amount proved to be beneficial to body growth during most of the experimental periods and in Experiment 3 during the entire experimental period. Moderate doses of thyroid substances have enhanced body growth in mice for periods of several months or until they reached maturity (11, 18). Although the tumor incidence was decreased, the thyroxine-treated mice ate more and grew at a faster rate than did the controls, particularly in the third experiment. In the thiouracil-treated mice, a depression of food intake or body growth was associated with an increase in tumor incidence. These observations, therefore, constitute an exception to the view that subnormal caloric intake results in inhibition, or excessive food intake in enhancement of tumor development (23).

The mechanisms by which hyper- and hypothyroidism produced their opposite effects on skin tumor development in these mice remain to be elucidated. Skin biopsies taken from CF1 female Swiss albino mice, initially 7–8 weeks old, and fed thyroxine or thiouracil at the present dose levels for 10 days showed that thickness of skin and number of hair follicles were greatly increased by the former and reduced by the latter treatment. Skin biopsies taken from the mice in the third experiment, at the end of 17 weeks, showed that thyroxine stimulated hair growth, while the number of hair follicles and skin thickness were lowered by thiouracil. In all three experiments the hair of the thyroxine-fed mice was longer than in the controls or thiouracil-fed mice. The view that chemical carcinogens act more readily on tissues whose cellular activity has been depressed (10) may be pertinent to the above observations.

Berengrumin (2, 3) suggested that the promotion of tumors is essentially a process of “delayed maturation” of normally developing cells, permitting a “piling up” of dormant tumor cells, which then begin to exhibit the characteristics of established tumors. Since hyperthyroidism can accelerate while hypothyroidism can delay maturation processes (and influence metamorphosis in amphibias), the incidence of skin tumors may have been retarded or promoted accordingly. Another possibility which remains to be investigated is that thyroxine enhanced and thiouracil reduced the elimination of DMBA and croton oil from the skin, thus influencing the amounts of these agents available to stimulate tumor development. Changes in thyroid status can also alter the secretory functions of other endocrine glands, which may in turn influence tumor development. Hyperthyroidism elicits an increase and hypothyroidism a decrease in secretion of adrenal cortical hormones (6, 26), and glucocorticoids such as cortisone can reduce the incidence of chemically induced skin tumors in mice (7, 22).

SUMMARY

1. In three separate experiments, the effects of feeding 2 mg. thyroxine or 2 gm thiouracil/kg diet on induction of skin tumors were studied in young female CF1 Swiss mice. Each mouse received one 0.5-ml. application over the back of 0.25, 0.50, or 1 per cent of 9,10-dimethyl-1,2-benzanthracene (DMBA), followed by once-weekly painting with 5 per cent croton oil in liquid paraffin during a period of 16 or 17 weeks.

2. Tumor incidence per group, average number of tumors per tumor-bearing mouse, and average surface tumor area were significantly reduced in the thyroxine-fed mice and increased in the thiouracil-fed mice. These substances were most effective when the lowest dose of DMBA was used. There was little or no growth of individual tumors after first appearance in the thyroxine-fed mice, whereas most of the tumors in the controls and particularly in the thiouracil-fed mice grew in size during the period of treatment. Most of the tumors appeared to be papillomas as determined by gross and histological examination.

4 A fuller account of the short- and long-term effects of hyper- and hypothyroidism on mouse skin will be published elsewhere.
3. Food intake was greatly increased in the thyroxine-fed mice and moderately reduced in the thiouracil-fed mice. Thyroxine accelerated body growth rate for the first 10–12 weeks of treatment in two experiments, and for the entire 17 weeks of treatment in the third experiment in which the youngest mice were used. Thiouracil reduced body growth only in the latter experiment. Several mechanisms which may explain these results are discussed.

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REFERENCES


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