ABSTRACT

We have studied the effects of hypothyroidism on the induction phase and growth phase of mammary cancer induced in the rat by 7,12-dimethylbenz(a)anthracene (DMBA). Moderate hypothyroidism was initiated before administration of DMBA by treatment with propylthiouracil (PTU) (0.5 to 2.0 mg/100 ml), and PTU was continued for 4 days after DMBA. Four months later, the tumor incidence in this group, 89 of 116 (77%), did not differ significantly from that in the control group, 81 of 115 (70%). Inasmuch as it is very probable that tumor induction occurs within 3 days after DMBA administration, it appears that hypothyroidism does not affect induction. When moderate hypothyroidism was initiated by administering PTU starting 4 days after DMBA, the final tumor incidence was 64 of 121 (53%) as compared to 82 of 119 (69%) in the control rats (p < 0.01), but in two similar, smaller studies we did not find an effect of hypothyroidism on tumor incidence. Thus, it appears that hypothyroidism does not enhance, and may suppress, promotion or growth of DMBA-induced cancer. When hypothyroidism was initiated before the DMBA dose and maintained continually thereafter, the final tumor incidence was 70 of 118 (59%), which did not differ significantly from the incidence in the controls of 82 of 119 (69%). Severe hypothyroidism was produced by administering PTU (4.0 mg/100 ml) before DMBA and continuing it thereafter, and the final tumor incidence was 3 of 45 (7%) as compared to 68 of 108 (63%) in the controls (p < 0.01). However, rats given the same PTU regimen, but maintained euthyroid by administration of thyroid hormone, had a tumor incidence of 35 of 45 (78%), indicating that PTU per se does not inhibit tumorigenesis. Moderate hypothyroidism produced by iodine deficiency or by PTU did not affect the tumor latent period, and moderate PTU-induced hypothyroidism did not affect the histological differentiation or estrogen dependence of the tumors. Overall, this study provides strong evidence against previous claims that hypothyroidism enhances DMBA-induced carcinogenesis.

INTRODUCTION

In recent years, there has been considerable controversy concerning the possibility that hypothyroidism may predispose to the development of breast cancer in women (3, 8, 14, 19, 23–25). Relevant to this controversy, a number of investigators have examined the effect of experimentally induced hypothyroidism on the development of mammary cancer in rats following the administration of the carcinogen DMBA. The results of these studies have been variable, inasmuch as in some studies it appeared that hypothyroidism caused an increase in the incidence of DMBA-induced mammary cancer (7–9, 15, 16), in others there was a decreased incidence (6, 17, 22, 27), and in still others hypothyroidism had no significant effect (20, 27). There are at least 4 possible reasons for the diversity of the obtained results.

First, in some of the studies, the rats were rendered hypothyroid before the administration of DMBA and maintained hypothyroid for the duration of the study (6–9, 16, 20, 27), whereas in others the thyroid was not suppressed or ablated until after administration of DMBA (15, 17, 22, 27). It appears likely that when a single dose of DMBA is administered, the resultant induction of preneoplastic changes in the mammary gland occurs very rapidly, probably within 24 h (4, 11, 28). Therefore, if hypothyroidism increases the susceptibility of the mammary gland to the carcinogenic influence of DMBA, this effect presumably would be observed only in rats who were hypothyroid at the time of the administration of the DMBA.

Second, the published studies vary with regard to the severity of the hypothyroidism that was induced. This is important, for severe hypothyroidism in the rat causes a sharp drop in food intake and in weight gain (16, 21, 22), and it is known that a decrease in food intake markedly suppresses the growth of DMBA-induced mammary cancer (18). Thus, in the severely hypothyroid, anorectic rat, the suppressant effect of decreased food intake might mask a stimulatory effect of hypothyroidism per se on tumor development.

Third, in most of the previous investigations, the number of rats studied was relatively small, and, consequently, the investigators might have failed to observe a statistically significant effect of hypothyroidism on tumor development, even though a significant effect might have been evident in a larger study (12).

Fourth, in most of the studies, the hypothyroid state was produced by the administration of PTU or 131I, and it is possible that either agent might have an effect on the inducibility or growth of DMBA-induced cancer independent of its effect on thyroid function.

In the present study, we examined the effect of PTU-induced hypothyroidism on DMBA-induced carcinogenesis in the mammary tissue of the rat. In order to avoid some of the limitations of previous investigations, we took the following 4 steps. (a) Separate studies were done on rats who were hypothyroid only during the induction period, on rats in whom hypothyroidism was initiated after the induction period, and on rats maintained in the hypothyroid state during the induction period and for the duration of the study. (b) Tumorigenesis was studied in rats with moderate PTU-induced hypothyroidism and normal weight gain, in iodine-deficient rats with moderate hypothyroidism and normal weight gain, and in rats with more severe PTU-induced hypothyroidism. (c) The number of rats studied was relatively
large. (d) Tumor development was studied in rats who were given PTU but were maintained euthyroid by concomitant administration of T4 and T3. In addition, we examined the effect of PTU-induced hypothyroidism on the estrogen dependence and histological appearance of DMBA-induced mammary tumors.

MATERIALS AND METHODS

Female Sprague-Dawley rats were obtained from Taconic Farms, Germantown, N. Y., and maintained on Wayne Lab Blox chow (Allied Mills, Chicago, Ill.). DMBA was obtained from Eastman Organic Chemicals, Rochester, N. Y., and PTU, T4, and T3 were obtained from Sigma Chemical Co., St. Louis, Mo. Plasma T4 and T3 were determined by radioimmunoassay using kits obtained from Pantex, Santa Monica, Calif. Remington low-iodine diet was obtained from ICN Nutritional Biochemicals, Cleveland, Ohio.

DMBA was dissolved in sesame oil in a concentration of 5 mg/ml and administered by stomach tube when the rats were 50 to 60 days old. Food was taken from the animals at approximately 3 hr before tube feeding, and food and water were provided again at approximately 2 hrs after tube feeding. Rats from the various experimental groups were fed in an alternating sequence. Inasmuch as several hundred rats were studied in some of these experiments, the tube-feeding process as well as the subsequent palpation of the animals for tumors was facilitated by anesthetizing the animals for about 1 min by placing them for about 45 sec in a cylinder flushed with 50% CO2-50% O2.

Hypothyroidism was induced by adding PTU to the drinking water. In most of the studies, the concentration of PTU was periodically adjusted between 0.5 and 2.0 mg/100 ml based on the plasma T4 levels and body weights in an effort to maintain significant hypothyroxinemia without causing impairment of weight gain, while in one experiment 4.0 mg/100 ml was administered. (See Tables 1 and 2 for the concentrations of PTU used in each experiment.) In all of the studies, plasma T4 and T3 levels were determined periodically on 0.5-ml samples of blood obtained by incision of the tail vein under ether anesthesia in 4 to 7 rats in each group. In those experiments in which PTU was discontinued 4 days after administration of DMBA, plasma T4 and T3 levels were always normal within 10 days of discontinuation of the PTU and remained normal thereafter. In those studies in which PTU therapy was initiated after administration of DMBA, the plasma T4 level was decreased in every instance by at least 60% (p < 0.01) within 2 weeks and was found to be significantly decreased when checked at monthly intervals.

To avoid observer bias in examining the animals for tumors, the cages were coded and randomized on the days of palpation so that the investigators did not know the experimental group to which the animals belonged. In calculating tumor incidence, the rats that had tumors at the time of palpation plus tumor-bearing rats that had died previously constituted the numerator, while all of the surviving animals plus those that had died bearing tumors constituted the denominator.

The statistical significance of the differences in tumor incidence was calculated by a standard method for determining the significance of the difference between 2 proportions (5), whereas the significance of all other differences was determined by Student’s t test (10).

RESULTS

Experiment 1: Effect of Hypothyroidism Maintained Only during the Induction Period on Tumor Incidence. In this study, we investigated the incidence of DMBA-induced tumors in rats who were maintained hypothyroid during the presumed induction phase (first 4 days following the administration of DMBA) but were allowed to return to the euthyroid state early in the subsequent growth phase. Two hundred thirty-four rats were given 10 mg of DMBA at 51 days of age. Group A (control group) was not given PTU, while Group B (PTU pre-DMBA) was given PTU from 17 days before the DMBA until 4 days after the DMBA. All of the animals were palpated for tumors at 2, 3, and 4 months after the administration of the carcinogen, and there was no significant difference in tumor incidence between the 2 groups at any point. At 4 months, there were more tumors per affected rat in the PTU-treated group, but the difference was quite small (Table 1).

Experiment 2: Effects of Hypothyroidism Initiated Post-DMBA and of Hypothyroidism Initiated Pre-DMBA and Maintained Post-DMBA on Tumor Incidence (DMBA Dose 10 mg). Three hundred seventy-five rats were given 10 mg DMBA at 56 days of age. Group A (control group) did not receive PTU. Group B (PTU post-DMBA) was started on PTU 4 days after the DMBA dose, and Group C (PTU pre- and post-DMBA) was started on PTU 14 days before the DMBA dose. Four months after the administration of DMBA, the tumor incidence in the rats that were made hypothyroid pre- and post-DMBA (Group B) was 53%, which was significantly lower than the 69% incidence in the control group (p < 0.01) (Table 1). The incidence of tumors in the rats that were hypothyroid pre- and post-DMBA (Group C) was 59%, which was not significantly different from that of the control group. At 2 and 3 months after DMBA, the tumor incidence in Group B was also significantly lower than in the control group, whereas Group C and the control group did not differ significantly.

Experiment 3: Effects of Hypothyroidism Initiated Post-DMBA and of Hypothyroidism Initiated Pre-DMBA and Maintained Post-DMBA on Tumor Incidence (DMBA Dose 6.5 mg). If hypothyroidism has an effect on DMBA-induced carcinogenesis, this effect might be most apparent in an experiment in which the dose of DMBA is small and the period of observation relatively long. Therefore, in this study, the experimental design was similar to that of Experiment 2, but the dose of DMBA, which was administered at 58 days of age, was only 6.5 mg rather than 10 mg, and the experiment was terminated 7 months after administration of DMBA rather than after 4 months. Group A, the control group, did not receive PTU. Group B (PTU post-DMBA) was started on PTU 3 days after the dose of DMBA, while Group C (PTU pre- and post-DMBA) was started on PTU 14 days prior to the DMBA dose. Four months following the administration of DMBA, the incidence of tumors was very low in all 3 groups, and there was no significant difference between the groups (Table 1). Seven months after the DMBA, the tumor incidence in the rats that were made hypothyroid post-DMBA (31%) and in the rats that were hypothyroid pre- and post-DMBA (36%) did not differ significantly from the incidence in the control group (38%), and the number of tumors per affected rat was similar in all 3 groups.

Experiment 4: Effects of Hypothyroidism Initiated Only during the Induction Period, and of Hypothyroidism Initiated
Table 1

Effect of PTU given pre-DMBA, post-DMBA, or pre- and post-DMBA on the incidence of mammary tumors

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Plasma T&lt;sub&gt;4&lt;/sub&gt; (µg/100 ml) Pre-DMBA</th>
<th>End</th>
<th>Plasma T&lt;sub&gt;3&lt;/sub&gt; (ng/100 ml) Pre-DMBA</th>
<th>End</th>
<th>Body wt (g) Start</th>
<th>End</th>
<th>Rats with tumors/total&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Tumors/affected rat&lt;sup&gt;a&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>Experiment 1&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>A. Controls</td>
<td>4.6 ± 0.3&lt;sup&gt;c&lt;/sup&gt; (5)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.1 ± 0.3 (6)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>85 ± 3 (5)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>111 ± 11 (6)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>108 ± 2 (21)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>256 ± 5 (21)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>81/115 (70)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.4 ± 0.2&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>B. PTU pre-DMA</td>
<td>0.5 ± 0.0 (5)</td>
<td>5.5 ± 0.5 (6)</td>
<td>12 ± 2&lt;sup&gt;d&lt;/sup&gt; (5)</td>
<td>99 ± 6 (6)</td>
<td>109 ± 2 (21)</td>
<td>246 ± 6 (21)</td>
<td>89/116 (77)</td>
<td>3.0 ± 0.2&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td>Experiment 2&lt;sup&gt;f&lt;/sup&gt;</td>
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<tr>
<td>A. Controls</td>
<td>3.4 ± 0.3 (5)</td>
<td>5.2 ± 0.1 (5)</td>
<td>61 ± 5 (5)</td>
<td>81 ± 6 (5)</td>
<td>143 ± 1 (24)</td>
<td>285 ± 5 (23)</td>
<td>82/119 (69)</td>
<td>2.2 ± 0.2&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>B. PTU post-DMA</td>
<td>0.5 ± 0.0&lt;sup&gt;g&lt;/sup&gt; (5)</td>
<td>58 ± 3 (5)</td>
<td>42 ± 8 (5)</td>
<td></td>
<td>139 ± 2 (24)</td>
<td>275 ± 6 (24)</td>
<td>64/121&lt;sup&gt;h&lt;/sup&gt;</td>
<td>1.7 ± 0.2&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>C. PTU pre- and post-DMA</td>
<td>1.1 ± 0.4&lt;sup&gt;g&lt;/sup&gt; (5)</td>
<td>1.4 ± 0.4 (5)</td>
<td>41 ± 11 (5)</td>
<td>73 ± 11 (5)</td>
<td>147 ± 2 (23)</td>
<td>267 ± 6&lt;sup&gt;j&lt;/sup&gt; (21)</td>
<td>70/118 (59)</td>
<td>2.1 ± 0.2&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td>Experiment 3&lt;sup&gt;f&lt;/sup&gt;</td>
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<tr>
<td>A. Controls</td>
<td>4.2 ± 0.1 (4)</td>
<td>4.2 ± 0.4 (5)</td>
<td>74 ± 5 (5)</td>
<td></td>
<td>132 ± 1 (27)</td>
<td>279 ± 10 (21)</td>
<td>10/64 (16)</td>
<td>1.3 ± 0.2&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>B. PTU post-DMA</td>
<td>0.6 ± 0.0&lt;sup&gt;g&lt;/sup&gt; (6)</td>
<td>51 ± 5&lt;sup&gt;g&lt;/sup&gt; (5)</td>
<td></td>
<td></td>
<td>130 ± 1 (27)</td>
<td>230 ± 6 (21)</td>
<td>6/64 (9)</td>
<td>1.2 ± 0.2&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>C. PTU pre- and post-DMA</td>
<td>0.5 ± 0.0&lt;sup&gt;g&lt;/sup&gt; (4)</td>
<td>0.5 ± 0.0&lt;sup&gt;g&lt;/sup&gt; (5)</td>
<td>28 ± 10&lt;sup&gt;g&lt;/sup&gt; (5)</td>
<td></td>
<td>131 ± 1 (60)</td>
<td>232 ± 7 (21)</td>
<td>9/56 (16)</td>
<td>1.2 ± 0.1&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td>Experiment 4&lt;sup&gt;f&lt;/sup&gt;</td>
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<tr>
<td>A. Controls</td>
<td>3.5 ± 0.1 (2)</td>
<td>4.3 ± 0.3 (8)</td>
<td>54 ± 8 (4)</td>
<td>72 ± 6 (8)</td>
<td>138 ± 2 (26)</td>
<td>243 ± 4 (32)</td>
<td>22/33 (67)</td>
<td>2.0 ± 0.2&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>B. PTU pre-DMA</td>
<td>1.0 ± 0.0&lt;sup&gt;g&lt;/sup&gt; (3)</td>
<td>4.6 ± 0.4 (8)</td>
<td>22 ± 7&lt;sup&gt;g&lt;/sup&gt; (3)</td>
<td>61 ± 9 (6)</td>
<td>134 ± 2 (26)</td>
<td>233 ± 4 (29)</td>
<td>24/32 (75)</td>
<td>1.8 ± 0.2&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>C. PTU post-DMA</td>
<td>0.7 ± 0.1&lt;sup&gt;g&lt;/sup&gt; (6)</td>
<td>25 ± 10&lt;sup&gt;g&lt;/sup&gt; (8)</td>
<td></td>
<td></td>
<td>136 ± 2 (24)</td>
<td>211 ± 5&lt;sup&gt;j&lt;/sup&gt; (32)</td>
<td>27/36 (75)</td>
<td>1.9 ± 0.2&lt;sup&gt;e&lt;/sup&gt;</td>
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</table>

<sup>a</sup>Four months after the dose of DMBA (given once between 50 to 56 days of age).
<sup>b</sup>Mean ± S.E.
<sup>c</sup>Numbers in parentheses, number of rats tested.
<sup>d</sup>Numbers in parentheses, percentage of rats with tumors.
<sup>e</sup>P < 0.05 for difference from controls.
<sup>f</sup>All rats received 10 mg DMBA. Group B was started on PTU (0.5 mg/100 ml) 17 days before DMBA; treatment was changed to 2.0 mg/100 ml 7 days before DMBA and was discontinued 4 days after DMBA.
<sup>g</sup>PTU pre-DMA.
<sup>h</sup>PTU post-DMA.
<sup>i</sup>PTU pre- and post-DMA.
<sup>j</sup>All rats received 10 mg DMBA. Group B was started on PTU (0.7 mg/100 ml) 4 days after DMBA. Group C was begun on PTU (0.5 mg/100 ml) 14 days before DMBA, and treatment was changed to 0.7 mg/100 ml 4 days after DMBA.
<sup>k</sup>PTU pre-DMA.
<sup>l</sup>PTU post-DMA.
<sup>m</sup>PTU pre- and post-DMA.
Post-DMBA on Tumor Incidence and Tumor Latent Period (DMBA Dose 13.5 mg). A relatively large dose of DMBA (13.5 mg) was administered to all of the rats in this study at 50 days of age. Group A (control group) did not receive PTU. Group B (PTU pre-DMBA) was given PTU from 13 days before the DMBA dose until 4 days after the DMBA dose. Group C (PTU post-DMBA) started on PTU 4 days after the DMBA dose. Starting 1 month after the administration of DMBA, the animals were examined weekly for tumors over a 3-month period. Four months following the administration of DMBA, the tumor incidence, number of tumors per affected rat, and mean latent period were similar in the 3 groups (Table 1).

Experiment 5: Effects of Moderate and Severe Hypothyroidism and of PTU per se, Independent of Its Thyroidal Effect, on DMBA-induced Carcinogenesis. Two hundred forty-nine rats were divided into a control group numbering 111 rats and 3 experimental groups each containing 46 rats, and all were given 10 mg of DMBA at 55 days of age. Group A (control group) was not given PTU. Group B (moderately hypothyroid group) was given PTU (0.5 to 0.7 mg/100 ml) from 7 days before the DMBA until the end of the study, while Group C (severely hypothyroid group) was given PTU (4.0 mg/100 ml) during the same period. Group D (euthyroid PTU group) was given PTU (4.0 mg/100 ml) during the same period, but the drinking water was also supplemented with T4 and T3 in concentrations which were adequate to maintain normal plasma levels of T4 and T3. The rats were examined for tumors at 2, 3, and 4 months after the administration of DMBA.

The rats given 0.5 to 0.7 mg PTU per 100 ml (moderate hypothyroidism) had substantial decreases in plasma T4 and T3 levels compared to that of the control rats but gained almost the same amount of weight as the controls (Table 2). The rats given 4.0 mg PTU per 100 ml (severe hypothyroidism) had an even more profound decrease in the plasma T4 level, as well as in the T3 level on the one occasion it was measured, and their weight gain was decreased by 86% as compared to the controls. In contrast, plasma T4 and T3 levels were normal at the end of the study in the rats receiving PTU (4.0 mg/100 ml) plus T4 and T3 (Table 2, Group D). Plasma T4 and T3 levels were also determined in 4 to 6 Group D rats and control rats on 7 different occasions during the study (approximately every 2 weeks). The mean of the 7 values for T4 levels was 4.8 ± 0.2 (S. E.) µg/100 ml in the control group and 5.1 ± 0.5 µg/100 ml in Group D, while the mean T3 levels were 73 ± 6 ng/100 ml in the controls and 74 ± 9 ng/100 ml in Group D. The incidence of tumors in the moderately hypothyroid group was 79%, which did not differ significantly from the 63% incidence in the control group. The rats receiving PTU (4 mg/100 ml) that were allowed to become severely hypothyroid had a mean tumor incidence that was markedly less than that in the control group (7% versus 63%, p < 0.01), but the tumor incidence in the euthyroid PTU-treated group (79%) was not significantly different from that of the control group. It is concluded from the latter observation that the marked decrease in tumor incidence in the rats receiving PTU (4 mg/100 ml) without thyroid hormone supplementation was due to the resultant hypothyroidism rather than to an anti-neoplastic effect of PTU per se.

Experiment 6: Effect of Hypothyroidism Induced by Iodine Deficiency on Tumor Incidence and on the Tumor Latent Period. Thirty-six rats were divided into 2 equal groups and started on a Remington low-iodine diet at 37 days of age. Group A (control group) was provided with deionized water containing sodium iodide (0.15 mg/100 ml). Group B (iodine-deficient group) was given deionized water devoid of sodium iodide. At 50 days of age, both groups were given 15 mg of DMBA. Starting 3 weeks after the DMBA, the rats were examined for tumors every 4 days for the ensuing 2 months.

At the termination of the experiment, the iodine-deficient animals had a significant decrease in their plasma T4 levels but normal plasma T3 levels and normal weight gain compared to the controls (Table 3), as was expected from the work of others (13). The tumor incidence and tumor latent period in the iodine-deficient group did not differ significantly from that of the control group.

Experiment 7: Effect of Oophorectomy on DMBA-Induced Tumors in Hypothyroid and Euthyroid Rats. Five months after the administration of DMBA to the rats that were described in Experiment 5, we took 21 tumor-bearing rats from Group A (control group) and 21 tumor-bearing rats from Group B (moderately hypothyroid group) and removed their ovaries bilaterally under ether anesthesia. The tumors were measured with callipers just prior to and 1 month after oophorectomy. The surface area of each tumor was calculated by multiplying the longest dimension of the tumor by the width of the tumor. In rats that had more than one tumor, total tumor surface area was calculated by adding the areas of the individual tumors.

During the 1 month following bilateral oophorectomy, there was regression of at least one tumor per rat in 20 of the 21 hypothyroid rats and in all 21 control rats, with regression being defined as a decrease of 50% or more in the surface area of the tumor. Total regression of at least one tumor occurred in 10 of the 21 animals in each group. Mean tumor surface area in the control rats decreased from 679 ± 111 to 116 ± 28 sq mm (83% decrease), while in the hypothyroid rats the decrease was from 608 ± 126 to 123 ± 36 sq mm (80% decrease). Thus, there was no evidence of a difference in estrogen dependence of the tumors between the 2 groups.

Experiment 8: Histological Appearance of DMBA-induced Tumors in Hypothyroid and Euthyroid Rats. Four months following the administration of DMBA in Experiment 4, one or more tumors from each tumor-bearing rat was placed in formalin, sectioned, and stained with hematoxylin and eosin. The degree of histological differentiation was then graded by using the system of Archer and Orlando (1). The slides were coded so that the investigators did not know the source of the tumors at the time of microscopic examination. As seen in Table 4, there was no major difference between the control group and the 2 hypothyroid groups with regard to the distribution of tumors in the various histological subdivisions.

DISCUSSION

According to prevailing concepts, carcinogens effect a pre-neoplastic change in cells, which occurs relatively rapidly ("induction phase") (2). The preneoplastic cells then must replicate a number of times before becoming neoplastic ("promotion phase"), and the neoplastic cells then multiply and give rise to tumors ("growth phase"). It appears very probable that following the i.g. administration of a single dose of DMBA, the preneoplastic changes are initiated within 3 days, since the carcinogen reaches its peak concentration in mammary tissue within 12 hr after administration and most of it has disappeared.
from the mammary tissue within 3 days after administration (11). Indeed, there are studies suggesting that induction of preneoplastic changes by DMBA occurs in less than 24 hr following exposure of the mammary tissue to the carcinogen (4, 28). In the present study, when PTU-induced hypothyroidism was initiated in rats prior to the i.g. administration of a single dose of DMBA and the PTU was stopped 4 days after the DMBA dose, there was no significant increase in the incidence of tumors 4 months later (Experiments 1 and 4). This observation indicates that hypothyroidism does not enhance the induction of mammary cancer by DMBA.

We also observed that when hypothyroidism was initiated soon after the presumed induction period and maintained for the rest of the study (PTU post-DMBA group in Experiments 2, 3, and 4), there was either a modest decrease in the incidence of palpable tumors as compared to the controls 4 months later (Experiment 2) or no significant difference (Experiments 3 and 4). We cannot explain why hypothyroidism appeared to suppress tumor development in Experiment 2 and not in the other studies. However, the observations taken together indicate that hypothyroidism does not enhance the promotional or growth phases of DMBA-induced carcinogenesis and may variably interfere with one or both of these phases. Consistent with this conclusion, rats made hypothyroid before administration of DMBA and maintained hypothyroid throughout the remainder of the study also did not have an increased incidence of tumors (Experiments 2, 3, and 5). The fact that hypothyroidism did not cause an increase in tumor incidence cannot be ascribed to poor nutrition in the hypothyroid animals, since the techniques we used for producing moderate hypothyroidism did not substantially impair weight gain in most of our studies (Tables 1 and 2).

We considered the possibility that PTU per se might suppress induction or growth of mammary cancer and thereby obscure a stimulatory effect of hypothyroidism on some phase of DMBA-induced carcinogenesis. In investigating this possibility, we found that even a relatively large concentration of PTU (4.0 mg/100 ml) administered before and after DMBA did not decrease the incidence of tumors as long as the rats were maintained euthyroid by administration of exogenous thyroid hormone (Experiment 5). However, when severe hypothyroidism was produced by administration of PTU (4.0 mg/100 ml) without exogenous thyroid hormone, there was a marked decrease in the incidence of tumors (7% in the hypothyroid group versus 63% in the controls) (Experiment 5). This decrease may have been secondary to the poor food intake of the severely hypothyroid rats in this study (18). There have been previously published observations which appear to conflict with our finding that hypothyroidism does not enhance DMBA-induced development of mammary carcinoma. Eskin et al. (9) administered PTU to 6 rats starting 4 days before administration of DMBA and noted that the treated rats and 10 control rats all had tumors after 60 days, but that the tumors developed significantly earlier in the PTU-treated rats. However, when these investigators subsequently repeated the study, using 50 PTU-treated and 50 control rats, the median time of onset between the 2 groups did not differ substantially (8). Further, when we examined this point (Experiment 4), we did not observe a significant difference in the control and hypothyroid rats with regard to the time of tumor development. Eskin (8) also noted that 9 rats put on a Remington iodine-
deficient diet 4 days prior to administration of DMBA all developed tumors after 60 days, as did 10 control rats, but the mean latent period for tumor development was significantly less in the iodine-deficient rats. However, interpretation of this experiment is complicated by the fact that the control rats received a standard diet rather than a Remington diet supplemented with iodide; therefore, the iodine-deficient and control rats differed significantly with regard to their intake of nutrients other than iodine. In the present study (Experiment 6), we compared rats receiving a Remington iodine-deficient diet with rats receiving the same diet supplemented with iodide and found that the latent period for the development of DMBA-induced tumors was similar in the 2 groups.

Gruenstein et al. (16) thyroidectomized rats 7 days before the administration of DMBA and did not note a difference in tumor incidence between the thyroidectomized and control rats 50 weeks later, but they did find that the thyroidectomized rats had a significantly higher tumor incidence than did a group of euthyroid rats pair-fed to the thyroidectomized rats. However, the pair-fed animals weighed substantially less than did the thyroidectomized animals during a 3-month period towards the end of the study, and since the pair-fed animals were fed only once daily, they presumably had a different feeding pattern than did the thyroidectomized animals, which were fed ad libitum. Davidson et al. (7) and Grice et al. (15) noted “significant” increases in tumor incidence in DMBA-treated rats that were made hypothyroid with 131I, but neither the number of rats studied nor the degree of statistical significance is mentioned in these reports. Thus, there appears to be no convincing evidence in the literature that hypothyroidism enhances DMBA-induced tumorigenesis.

In the present study, we have also found that there is no difference in estrogen dependence between the DMBA-induced tumors of hypothyroid rats and those of euthyroid rats. Further, the tumors of the hypothyroid rats did not appear more anaplastic on histological examination.

In summary, in the present study, we have observed that hypothyroidism does not enhance the induction or growth of DMBA-induced mammary carcinoma in the rat and does not alter its estrogen dependence or differentiation.

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