Interaction of Dietary Fat and the Thymus in the Induction of Mammary Tumors by 7,12-Dimethylbenz(a)anthracene

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ABSTRACT

The interaction of dietary fat and the thymus in the induction of mammary tumors by dimethylbenz(a)anthracene has been examined in female Sprague-Dawley rats. In these experiments, rats fed diets of 0.5% (low fat), 5% (normal fat), or 20% (high fat) corn oil from weaning (21 days of age) were thymectomized or sham thymectomized at 35 days of age and were given 5 mg of dimethylbenz(a)anthracene at 55 days of age. Thymectomy exerted a protective effect in rats fed low and normal fat diets, and this was not reversed by Thymosin Fraction V. In high fat-fed rats, tumorigenesis was increased compared to the low fat groups, and in addition, the protective effect of thymectomy was absent. This differential effect of thymectomy could not be explained on the basis of changes in prolactin concentration, since prolactin levels were decreased in all dietary groups. Neither diet nor thymectomy affected corticosterone levels or the estrus cycle of mature rats. Peripheral blood lymphocytes were, however, decreased by both thymectomy and increasing the fat content of the diet. It is hypothesized that the promoting effect of dietary fat on dimethylbenz(a)anthracene-induced mammary tumorigenesis is mediated via the immune system, although a role for the endocrine system still cannot be ruled out.

INTRODUCTION

Recent observations support an important role for diet in the etiology of breast cancer. In particular, epidemiological studies have shown that there is a positive association between the incidence and mortality of breast cancer in women and the intake of dietary fat (1, 7, 19, 24, 26). This correlation has been supported by a large number of animal studies which demonstrate an increased incidence of both spontaneous and carcinogen-induced mammary tumors in rodents fed diets rich in fat, especially polyunsaturated fat (9, 10, 31, 34, 41, 62, 65). The evidence strongly suggests that dietary fat acts at the promotional stage of carcinogenesis (8, 31–33), and it has been proposed that this change in the hormonal environment of the host, specifically an elevation of serum prolactin levels, might be responsible for this effect (11, 34). Other possibilities include an effect of dietary fat on the immune system (20, 22, 43), prostaglandins (30), or through maintenance and induction of prolactin receptors (42).

There is some experimental evidence which indicates that the thymus can modulate mammary tumorigenesis. For example, neonatal thymectomy has been shown to decrease the incidence and increase the latency period of spontaneous (58), transplantable (3), and chemical- or virus-induced mammary tumors (28, 44, 45, 53, 55, 56, 60, 66). In addition, the incidence of mammary tumors is decreased in mice treated with antithymocyte serum (5). Furthermore, it has been observed clinically that patients with myasthenia gravis have a high incidence of breast cancer and that the incidence is dramatically reduced in patients who have undergone thymectomy (50, 51).

In this paper, we have examined the interaction between dietary fat and the thymus on the induction of mammary tumors by DMBA. An initial experiment demonstrated that tumor incidence was markedly reduced in thymectomized rats fed laboratory chow, and subsequent experiments were undertaken to see if thymectomy would alter the effectiveness of dietary fat as a promoter of DMBA-induced mammary carcinogenesis. We also investigated the ability of Thymosin Fraction V to reverse the effects of thymectomy.

MATERIALS AND METHODS

Animals and Treatment. Female Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were housed in a temperature (22°C) and light-controlled (12 hr of light and 12 hr of dark) room with food and water available ad libitum. In the initial experiment, rats were fed laboratory chow (Teklad, Madison, Wis) throughout the course of the experiment. They were thymectomized or sham thymectomized at 34 to 36 days of age and were given a single i.g. intubation of 10 mg of DMBA (Sigma Chemical Co., St. Louis, Mo.) dissolved in corn oil at a concentration of 10 mg/ml at 50 to 55 days of age. Thymectomy was performed by making a midline longitudinal incision in the area above the sternum, separating the fascia to expose the thymus, and then aspirating the thymic lobes through a glass cannula using gentle manipulation and constant suction. The site was inspected for residual thymic tissue before closure. Sham-thymectomized rats were opened in the same way, but the incision was closed without removal of the thymic lobes.

In the first dietary fat experiment, hereafter called Experiment 1, rats were placed on a diet of either 0.5% (low fat) or 20% (high fat) corn oil starting at weaning (21 days of age). The composition of the diets is given in Table 1. Within each dietary group, the rats were either thymectomized or sham thymectomized at 35 days of age and were...
given 5 mg DMBA (in 1 ml corn oil), as above, at 55 days of age. The second dietary fat experiment (Experiment 2) was the same as the first except that a third dietary group, 5% corn oil (normal fat), was also added. In addition, half of the thymectomized rats on the 5% corn oil diet received Thymosin Fraction V, 10 mg/kg i.p., 3 times per week starting the day after thymectomy. Thymosin Fraction V was prepared by Hoffman-La Roche Inc. (Nutley, N. J.) and was a generous gift of Dr. Allan Goldstein, George Washington University Medical Center, Washington, D. C.

Rats were weighed and palpated weekly for the presence of mammary tumors. Each tumor location was recorded, and the size was measured with a vernier caliper in 2 perpendicular dimensions. At autopsy, the tumors were excised and weighed, fixed in buffered formalin, and sectioned for histological examination. In Experiment 1, the spleen, thymus (if present), liver, kidney, ovary, and uterus were removed and weighed at autopsy. In all experiments, completeness of thymectomy was confirmed at autopsy.

Prolactin Assay. Blood for prolactin assay was taken in the afternoon (between 1 and 3 p.m.) of proestrus in order to catch the peak level of circulating prolactin. In Experiment 1, neck blood was collected into heparinized tubes after decapitation of unanesthetized rats and was centrifuged, and the plasma was stored at −70°C until assay. Sacrifice was increased to 60,000 cpm. Samples were assayed at 4 dilutions, was centrifuged, and the plasma was stored at −70°C until assay. In Experiment 2, blood was drawn from the orbital sinus of rats lightly anesthetized with ether, through heparinized capillary tubes and was collected into heparinized centrifuge tubes. After centrifugation, the plasma was stored at −70°C. In this experiment, blood was taken 140 noon (between 1 and 3 p.m.) of proestrus in order to catch the peak

Corticosterone Assay. In Experiment 1, blood was obtained for corticosterone assay in the same way as it was drawn for prolactin assay. Hence, these measurements were made in rats that were in the afternoon of proestrus. In Experiment 2, neck blood was obtained after decapitation of the rats and was collected in nonheparinized tubes. After centrifugation, the serum was stored at −70°C. Sacrifice was done between the hr of 1 and 3 p.m., 160 to 180 days after DMBA administration and was done without regard to the stage of the estrus cycle.

Corticosterone in plasma (Experiment 1) or serum (Experiment 2) was measured by the procedure described by Henning (27). This assay is based on corticosterone binding to rat corticosteroid-binding globulin and is very sensitive if careful attention is paid to the rate and method by which dextran-coated charcoal is added.

Estrus Cycle. For determination of proestrus in rats that were to be bled for prolactin assay, rats were monitored for a minimum of 2 complete cycles. In addition, the effect of thymectomy and dietary fat on the estrus cycle was determined in 10 rats from each group of Experiment 1. This was done starting at 160 days after DMBA administration. Vaginal smears were taken between 8 and 11 a.m. and were analyzed according to the method of Bukovsky et al. (6). This technique uses a scale of 1 to 10, based on the number of different cell types observed. To prevent introduction of bias, slides were examined blind, without knowledge of dietary group or presence or absence of the thymus. Animals were examined for 14 days.

WBC and Differential WBC Count. For determination of WBC count, tail vein blood (20 μl) was diluted with 10 ml isotonic (Coulter Diagnostics, Hialeah, Fla). Three drops of Zat-Isotol II (Coulter Diagnostics) were added to lyse RBC, and the WBC were counted in a Coulter Counter, Model Zbl. To determine the differential WBC count, a drop of tail vein blood was smeared on a glass slide and dried, and the slide was stained in an Amos automatic stainer.

Statistical Analysis. Differences in tumor incidence were analyzed by χ² analysis according to the method of Peto (52). The data in Table 5 were analyzed using Student’s range test (23). All other data were analyzed by the Kruskal-Wallis nonparametric analysis of variance with multiple group comparisons (14).

RESULTS

Effect of Thymectomy on DMBA-induced Mammary Carcinogenesis in Rats Fed Laboratory Chow. An initial experiment was undertaken to see if thymectomy would alter DMBA-induced mammary carcinogenesis in rats fed laboratory chow. As can be seen from Table 2, thymectomy at 34 to 36 days of age markedly reduced the total number of mammary tumors, expressed as tumors per rat, in rats given DMBA. In addition, tumor incidence and the number of tumors per tumor-bearing rat were decreased in thymectomized rats, and the average tumor-free period was increased.

Effect of Thymectomy on DMBA-induced Mammary Carcinogenesis in Rats Fed Different Levels of Dietary Fat. Since thymectomy was so effective in reducing tumorigenesis in rats fed laboratory chow, we undertook an experiment to determine whether thymectomy might reduce the high incidence of DMBA-induced mammary tumors in rats fed a high fat diet. In this experiment, rats were fed either a diet with 0.5% corn oil

| Table 1
<p>| Composition of diets |</p>
<table>
<thead>
<tr>
<th>Dietary component</th>
<th>Low fat diet (g/100 g)</th>
<th>Normal fat diet (g/100 g)</th>
<th>High fat diet (g/100 g)</th>
</tr>
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<tbody>
<tr>
<td>Corn oil ^a</td>
<td>0.6</td>
<td>5.0</td>
<td>20</td>
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<tr>
<td>Casein ^b</td>
<td>20</td>
<td>21.1</td>
<td>24.8</td>
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<tr>
<td>Dextrose ^c</td>
<td>72.3</td>
<td>66.3</td>
<td>46.2</td>
</tr>
<tr>
<td>Salt mix ^d</td>
<td>5.0</td>
<td>5.3</td>
<td>6.2</td>
</tr>
<tr>
<td>Vitamin mix ^e</td>
<td>2.2</td>
<td>2.3</td>
<td>2.7</td>
</tr>
</tbody>
</table>

^a Mazola corn oil (H. Levit and Son, Inc., Buffalo, N. Y.). ^b Vitamin-free casein (Teklad, Madison, Wis.). ^c Dextrose (Federal Bakers Supply, Buffalo, N. Y.). ^d Rogers and Harper salt mix (Teklad). ^e Vitamin diet fortification mixture (ICN Pharmaceuticals, Cleveland, Ohio).

| Table 2
<p>| Effect of thymectomy on DMBA-induced mammary carcinogenesis in rats fed laboratory chow |</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Tumor incidence (%)</th>
<th>No. of palpable tumors</th>
<th>Palpable tumors/rat</th>
<th>Palpable tumors/ tumor-bearing rat</th>
<th>Tumor-free days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham thymectomy</td>
<td>12</td>
<td>91.7</td>
<td>29</td>
<td>2.42 ± 0.53 ^d</td>
<td>2.64 ± 0.53 ^d</td>
<td>94 ± 11</td>
</tr>
<tr>
<td>Thymectomy</td>
<td>20</td>
<td>70.0</td>
<td>17</td>
<td>0.85 ± 0.15 ^c</td>
<td>1.21 ± 0.11 ^c</td>
<td>133 ± 8 ^c</td>
</tr>
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</table>

^d These are minimal values; for rats without tumor at sacrifice (165 days after DMBA), the value of 165 was arbitrarily assigned so that an average of tumor-free days for the entire group could be calculated.

^c Mean ± S.E.

^e Statistically different compared to control (p < 0.01).
(low fat) or a diet with 20% corn oil (high fat) from weaning and were thymectomized or sham thymectomized at 35 days of age. The cumulative palpable mammary tumor incidence is shown in Chart 1. Rats fed the high fat diet had a higher tumor incidence throughout the entire experimental period than did rats on the low fat diet, independent of the presence or absence of the thymus. Moreover, the number of tumors per rat and the number of tumors per tumor-bearing rat were significantly increased, and the latent period was decreased in rats fed the high fat diet (Table 3).

In addition to differences between the low and high fat groups, a difference in tumor incidence between the 2 low fat groups became evident as early as 96 days after DMBA administration and was maintained throughout the experiment (Chart 1). When analyzed by the method of Peto (52), this decrease in tumor incidence in thymectomized rats fed the low fat diet was statistically significant (p < 0.05) at each time point. Table 3 shows the tumor data at autopsy. Both tumor incidence (palpable and nonpalpable tumors) and the number of tumors per rat were decreased by thymectomy; only the former was statistically significant. No effect of thymectomy was observed on the number of tumors per tumor-bearing rat in the low fat group; however, thymectomized rats showed a longer tumor-free period.

In contrast to the protective effect of thymectomy in rats fed a low fat diet, thymectomized rats fed a high fat diet were somewhat worse off than were their sham-operated counterparts. The cumulative tumor incidence (Chart 1) in the high fat, thymectomized group was consistently higher throughout the experiment than was that in the high fat control group, although this difference was not statistically significant. Final tumor incidence at autopsy was 77.4 and 94.7% for the sham-thymectomized and thymectomized groups, respectively (Table 3). The mean number of tumors per rat was increased significantly in both thymectomized and thymectomized groups, a difference in tumor incidence between the 2 low fat groups became evident as early as 96 days after DMBA administration and was maintained throughout the entire experimental period than did rats on the low fat diet, independent of the presence or absence of the thymus. Moreover, the number of tumors per rat and the number of tumors per tumor-bearing rat were significantly increased, and the latent period was decreased in rats fed the high fat diet (Table 3).

In order to confirm that high levels of dietary fat abolished the protective effect of thymectomy, a second experiment was initiated, similar to Experiment 1 except that a normal fat (5% corn oil) group was also included. In addition, Thymosin Fraction V was administered to half the normal fat, thymectomized group to determine if this thymic hormone influenced tumor induction and growth. The number of rats in each of these groups is shown in Table 3.

Table 3

<table>
<thead>
<tr>
<th>Group</th>
<th>Tumor Incidence (%)</th>
<th>No. of Tumors</th>
<th>Tumors/rat</th>
<th>Tumors/tumor-bearing rat</th>
<th>Tumor-free days</th>
</tr>
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<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low fat, sham thymectomy</td>
<td>27</td>
<td>37.0</td>
<td>13</td>
<td>0.48 ± 0.14e</td>
<td>1.30 ± 0.21f</td>
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<td>Low fat, thymectomy</td>
<td>38</td>
<td>16.7d</td>
<td>9</td>
<td>0.25 ± 0.11f</td>
<td>1.50 ± 0.34</td>
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<tr>
<td>High fat, thymectomy</td>
<td>31</td>
<td>77.4d</td>
<td>72</td>
<td>2.32 ± 0.36f</td>
<td>3.00 ± 0.37f</td>
</tr>
<tr>
<td>High fat, thymectomy (+ Thymosin Fraction V)</td>
<td>38</td>
<td>94.7d</td>
<td>136</td>
<td>3.58 ± 0.45g &amp;</td>
<td>3.78 ± 0.45g</td>
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</table>

Experiment 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Tumor Incidence (%)</th>
<th>No. of Tumors</th>
<th>Tumors/rat</th>
<th>Tumors/tumor-bearing rat</th>
<th>Tumor-free days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low fat, sham thymectomy</td>
<td>32</td>
<td>21.9</td>
<td>11</td>
<td>0.34 ± 0.13</td>
<td>1.57 ± 0.30</td>
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<td>Low fat, thymectomy</td>
<td>24</td>
<td>8.3</td>
<td>3</td>
<td>0.13 ± 0.09</td>
<td>1.50 ± 0.50</td>
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<td>Normal fat, sham thymectomy</td>
<td>24</td>
<td>54.2d</td>
<td>19</td>
<td>0.79 ± 0.20</td>
<td>1.46 ± 0.24</td>
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<tr>
<td>Normal fat, thymectomy</td>
<td>31</td>
<td>25.8d</td>
<td>11</td>
<td>0.38 ± 0.13</td>
<td>1.38 ± 0.26</td>
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<tr>
<td>Normal fat, thymectomy (+ Thymosin Fraction V)</td>
<td>28</td>
<td>21.4d</td>
<td>7</td>
<td>0.25 ± 0.10</td>
<td>1.17 ± 0.17</td>
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<td>High fat, thymectomy</td>
<td>34</td>
<td>67.6d</td>
<td>54</td>
<td>1.59 ± 0.28</td>
<td>2.35 ± 0.31</td>
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<tr>
<td>High fat, thymectomy (+ Thymosin Fraction V)</td>
<td>30</td>
<td>80.0d</td>
<td>60</td>
<td>2.00 ± 0.35 &amp;</td>
<td>2.50 ± 0.41</td>
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* Includes palpable and nonpalpable tumors discovered at autopsy.

† For calculation, see Table 2, Footnote a, with the exception that, in this case, rats without tumor at sacrifice were assigned a value of 180 days (Experiment 1) or 180 days (Experiment 2).

‡ Mean ± S.E.

§ Statistically different from sham-thymectomized group fed the same diet (p < 0.05).

** Statistically different from sham-thymectomized group fed the same diet (p < 0.01).

Statistically different from sham-thymectomized group fed the low fat diet (high fat versus low fat, p < 0.01; normal fat versus low fat, p < 0.05).

Statistically different from thymectomized group fed the low fat diet (p < 0.01).

Statistically different from sham-thymectomized group fed the normal fat diet (p < 0.05).

Statistically different from thymectomized group fed the normal fat diet (p < 0.001).
The results with the normal fat animals were quite similar to those seen in animals fed laboratory chow. As can be seen in Chart 3, a significant difference in palpable tumor incidence ($p < 0.05$) occurred between the normal fat, sham-thymectomized and thymectomized rats throughout the experiment. There was little difference in the palpable tumor incidence between the 2 low fat groups or between the 2 high fat groups; however, with the inclusion of nonpalpable tumors detected at autopsy, the final tumor incidence (including both palpable and nonpalpable tumors) was decreased in the low and normal fat thymectomized groups compared to the corresponding sham-operated controls (only the latter is statistically significant, $p < 0.05$), but was unchanged or actually slightly elevated in the high fat group (Table 3). Similar results were observed when the total number of tumors was examined; the number of tumors per rat was decreased by thymectomy in both low and normal fat groups (for the latter, $p < 0.05$) but was unchanged or slightly increased by thymectomy in rats fed the high fat diet. It is of interest that, in the sham-operated rats, the feeding of a 5% corn oil diet resulted in an increase in both tumor incidence and in the number of tumors per rat when compared to rats fed the 0.5% corn oil diet; however, thymectomy was able to restore both parameters to the levels observed in the low fat, sham group. Table 3 shows that the number of tumors per tumor-bearing rat and the latency of tumor appearance were not significantly altered by thymectomy in any of the dietary groups.

Effect of Thymosin Fraction V on DMBA-induced Mammary Carcinogenesis in Thymectomized Rats Fed a Normal Fat Diet. The administration of Thymosin Fraction V starting immediately after thymectomy at 35 days of age delayed the onset of tumor formation in this group of rats when compared to sham-thymectomized rats fed the same diet (Chart 4; Table 3). Such an effect was also apparent when the thymosin-treated group was compared to the untreated thymectomized group (Chart 4); however, when the data were expressed as the mean number of tumor-free days (Table 3), this latter difference was not statistically significant. It should be noted, however, that, at sacrifice, only one-fourth of the rats in each of these latter 2 groups actually had tumors, and this difference might have been significant if the experiment had been carried out for a longer period of time. Of interest was the inability of Thymosin Fraction V, at this dose schedule, at least, to reverse the protective effect of thymectomy. Table 3 shows that Thymosin Fraction V did not restore either the tumor incidence or the number of tumors per rat to the levels observed in the sham-thymectomized control group and, in fact, both parameters were actually slightly decreased (although not significantly) when compared to the untreated thymectomized rats.

In all the experiments reported in this paper, greater than 90% of the tumors were adenocarcinomas, and neither dietary fat nor thymectomy affected the ratio of malignant to benign tumors.

Plasma Prolactin Concentrations. Since changes in prolactin concentration might explain the discrepancy between the effects of thymectomy at different dietary fat levels, we measured plasma prolactin concentrations at proestrus in thymectomized and sham-thymectomized rats fed low, normal, or high fat diets. As can be seen from Chart 5, thymectomy significantly
decreased \((p < 0.05)\) prolactin concentration in all rats, regardless of the type of diet fed. Furthermore, Thymosin Fraction V did not restore prolactin levels in thymectomized rats fed the normal fat diet. The high fat, sham-thymectomized groups had higher plasma prolactin levels than did the corresponding groups fed low or normal fat diets; however, this increase was not statistically significant. Of interest was the lack of correlation between plasma prolactin concentration and tumor incidence; calculation of the correlation coefficient between these 2 parameters in Experiment 2 yielded an \(r\) value of 0.317.

**Plasma Corticosterone Concentrations.** Since there appears to be an interrelationship between the thymus and the adrenals (17, 18) and since an inhibitory effect of glucocorticoids on DMBA-induced mammary tumors has been demonstrated (2), it was reasonable to suppose that altered corticosterone levels might explain the effects of thymectomy on the incidence of mammary tumors. As can be seen in Chart 6, corticosterone levels were increased by thymectomy at all levels of dietary fat; however, this increase was not statistically significant. Thymosin Fraction V also had no statistically significant effect on corticosterone levels. In addition, no correlation was observed between tumor incidence and plasma corticosterone levels \([r = 0.173\text{ (Experiment 2), data not shown}]\) or between corticosterone and dietary fat intake (Chart 6).

**Total and Differential WBC Count.** Table 4 shows that no meaningful differences in total WBC levels were observed in response to dietary fat or thymectomy, although the thymectomized rats tended to have lower levels than did the sham-thymectomized rats in the low and normal dietary fat groups. A major effect of thymectomy was to lower the total lymphocyte count (Table 4). This was observed in all dietary groups with only one exception; the discrepancy between Experiments 1 and 2 in terms of the effect of thymectomy on lymphocyte levels in rats fed the high fat diet (a decrease in Experiment 1, no change in Experiment 2) is not readily explainable. Interestingly, Thymosin Fraction V was able to restore lymphocyte levels to those observed in the sham-thymectomized group. High dietary fat significantly reduced lymphocyte levels in Experiment 2 but not in Experiment 1. In Table 4, Experiment 2, it can be seen that there is an inverse correlation between dietary fat intake and peripheral blood lymphocyte concentra-

![Chart 5. Plasma prolactin concentrations at proestrus in thymectomized (TX) or sham-thymectomized (STX) rats fed different levels of dietary fat. TX + T, thymectomized rats given Thymosin Fraction V, 10 mg/kg i.p., 3 times per week, starting the day after thymectomy. These measurements were made on 8 to 15 (Experiment 1) or 8 to 11 (Experiment 2) rats/group. Exp., Experiment.](chart5.png)

![Chart 6. Corticosterone concentrations in thymectomized (TX) or sham-thymectomized (STX) rats fed different levels of dietary fat. TX + T, thymectomized rats given injections of thymosin Fraction V. These measurements were made on 18 to 23 (Experiment 1) or 18 to 30 (Experiment 2) rats/group. Exp., Experiment.](chart6.png)

<table>
<thead>
<tr>
<th>Experiment 1</th>
<th>WBC</th>
<th>Lymphocytes</th>
<th>Neutrophils</th>
<th>Eosinophils</th>
<th>Monocytes</th>
<th>Basophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low fat, sham thymectomy</td>
<td>12954 ± 1510</td>
<td>9152 ± 1036</td>
<td>3169 ± 490</td>
<td>196 ± 26</td>
<td>396 ± 68</td>
<td>41 ± 14</td>
</tr>
<tr>
<td>Low fat, thymectomy</td>
<td>8607 ± 779</td>
<td>5046 ± 458</td>
<td>2930 ± 349</td>
<td>285 ± 50</td>
<td>326 ± 46</td>
<td>24 ± 11</td>
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<tr>
<td>High fat, sham thymectomy</td>
<td>11254 ± 972</td>
<td>8024 ± 686</td>
<td>3279 ± 313</td>
<td>264 ± 64</td>
<td>323 ± 44</td>
<td>33 ± 13</td>
</tr>
<tr>
<td>High fat, thymectomy</td>
<td>9661 ± 832</td>
<td>5354 ± 428</td>
<td>3712 ± 456</td>
<td>306 ± 48</td>
<td>250 ± 33</td>
<td>41 ± 12</td>
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<table>
<thead>
<tr>
<th>Experiment 2</th>
<th>WBC</th>
<th>Lymphocytes</th>
<th>Neutrophils</th>
<th>Eosinophils</th>
<th>Monocytes</th>
<th>Basophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low fat, sham thymectomy</td>
<td>10415 ± 1466</td>
<td>6414 ± 1020</td>
<td>3052 ± 430</td>
<td>443 ± 90</td>
<td>513 ± 88</td>
<td>27 ± 19</td>
</tr>
<tr>
<td>Low fat, thymectomy</td>
<td>9722 ± 877</td>
<td>6657 ± 608</td>
<td>2381 ± 346</td>
<td>248 ± 79</td>
<td>350 ± 56</td>
<td>6 ± 6</td>
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<tr>
<td>Normal fat, sham thymectomy</td>
<td>7920 ± 478</td>
<td>4921 ± 337</td>
<td>2338 ± 200</td>
<td>166 ± 28</td>
<td>352 ± 55</td>
<td>17 ± 10</td>
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<tr>
<td>Normal fat, thymectomy</td>
<td>9841 ± 736</td>
<td>6112 ± 671</td>
<td>3063 ± 306</td>
<td>257 ± 31</td>
<td>351 ± 69</td>
<td>33 ± 11</td>
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<tr>
<td>High fat, sham thymectomy</td>
<td>8147 ± 801</td>
<td>5383 ± 345</td>
<td>2853 ± 465</td>
<td>198 ± 26</td>
<td>373 ± 40</td>
<td>11 ± 8</td>
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<td>High fat, thymectomy</td>
<td>8665 ± 852</td>
<td>5631 ± 581</td>
<td>2856 ± 385</td>
<td>376 ± 131</td>
<td>388 ± 51</td>
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</table>

1. Mean ± S.E. of 20 (Experiment 1) or 15 (Experiment 2) rats/group.
2. Statistically different from animals fed the same diet \((p < 0.05)\).
3. Statistically different from animals fed the same diet \((p < 0.001)\).
4. Statistically different from sham-thymectomized animals fed the low fat diet \((p < 0.05)\).
5. Statistically different from thymectomized animals fed the low fat diet \((p < 0.001)\).
6. Statistically different from untreated thymectomized group fed the normal fat diet \((p < 0.05)\).
7. Statistically different from sham-thymectomized animals fed the low fat diet \((p < 0.01)\).
8. Statistically different from thymectomized animals fed the normal fat diet \((p < 0.05)\).
Effect of dietary fat on the age of first estrus and the day of vaginal opening

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>Day of vaginal opening (age in days)</th>
<th>First day of estrus (age in days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low fat</td>
<td>36.5 ± 0.7a,c</td>
<td>40.8 ± 0.7a,c</td>
</tr>
<tr>
<td>Normal fat</td>
<td>34.3 ± 0.6</td>
<td>37.6 ± 0.8c</td>
</tr>
<tr>
<td>High fat</td>
<td>32.6 ± 0.6</td>
<td>34.6 ± 0.8c</td>
</tr>
</tbody>
</table>

- a Mean ± S.E. of 20 rats/group. Rats were placed on diets at 21 days of age.
- b Significantly different from the normal fat and high fat groups (p < 0.05).
- c The mean values are all significantly different from one another (p < 0.05).

Thymectomy in rats at 35 days of age can offer substantial protection from the carcinogenic effects of DMBA. In the experiments reported in this paper, thymectomy was done around the time of onset of sexual maturity. At this time, rats fed the high fat diet had had their first estrus cycle, while rats fed the low and normal fat diets had not (Table 5; Ref. 21). The fact that the thymus had already "seen" estrogen prior to the high tumor incidence observed in rats fed diets high in fat could be dramatically altered. Surprisingly, this was not observed and, in fact, tumorigenesis was actually slightly increased in thymectomized rats fed a high fat diet when compared to sham-thymectomized rats on the same diet. This was in contrast to the protective effect of thymectomy in rats fed a low fat (0.5%), normal fat (5%), or laboratory chow diet.

Thymosin Fraction V is one of several thymic hormones and is composed of a family of small polypeptides ranging in size from 1000 to 15,000 (63). It is a proven immunorestorative agent in many animal models (63) and is currently being used clinically where it has been shown to increase the survival time of patients with small-cell carcinoma of the lung (63). When it was administered to thymectomized rats in the present studies at a dose which at least partly restored lymphocyte levels, it did not reverse the protective effect of thymectomy and, in fact, tumor incidence and total tumor number were even further decreased beyond that seen with thymectomy alone. Of even more significance was the fact that thymosin seemed to delay the time of tumor appearance.

As suggested by our data, the differential effect of thymectomy on DMBA-induced mammary tumorigenesis in rats fed different levels of dietary fat does not appear to be mediated by a change in the concentrations of either prolactin or corticosterone. Thymectomy at 35 days of age significantly decreased plasma prolactin concentrations in all dietary groups; accompanying the decrease was a slight, although not statistically significant, increase in corticosterone levels. Importantly, while prolactin and corticosterone levels in high fat-fed thymectomized rats were not different than in low fat-fed or normal fat-fed thymectomized animals, tumor incidence was significantly different. Why thymectomy should alter prolactin and corticosterone levels is not known; however, a thymus-pituitary-adrenal axis has been demonstrated (15-18). Chen et al. (12) found that adenectomy could stimulate DMBA-induced mammary tumor growth and elevate serum prolactin levels. In contrast, dexamethasone both inhibited tumor growth and decreased serum prolactin (2). Changes in other pituitary hormones have also been reported following thymectomy (15, 48).

Recently, Berczi and Nagy (4) found that hypophysectomized rats had an impaired immune response to various antigenic stimuli. When pituitary hormones were administered to hypophysectomized animals, only prolactin could restore immunocompetence. The mechanism of prolactin regulation of the immune response needs to be further explored.

Other investigators have reported ovarian dysgenesis in rats and mice after neonatal thymectomy, although this was not observed in mice thymectomized after 7 days of age (25, 49, 57). It is unlikely that a change in ovarian function mediated the effect of thymectomy in the present experiments, however, since thymectomy did not alter the estrus cycle of the rat when measured 160 days after DMBA administration (although changes at earlier time periods cannot be ruled out).

It is likely that the time of thymectomy is quite critical in terms of subsequent mammary tumor development (29, 54). In the experiments reported in this paper, thymectomy was done around the time of onset of sexual maturity. At this time, rats fed the high fat diet had had their first estrus cycle, while rats fed the low and normal fat diets had not (Table 5; Ref. 21). The fact that the thymus had already "seen" estrogen prior to...
thymectomy in the high fat-fed rats, with resulting immunologi-
cal and/or endocrine changes, could have important implica-
tions for mammary tumor development. Estrogens, for exam-
ple, have been shown to decrease thymus weight and depress
the immune response (13, 37, 61, 64). Michael et al. (47) have
also shown that plasma levels of thymosin \( \alpha \) (one of the
peptides in Thymosin Fraction V) were decreased following a
single injection of estradiol.

An important finding in this study is that high levels of dietary
fat can still enhance tumorigenesis in rats, the prolactin levels
of which have been decreased as a result of thymectomy. Chan
et al. (11) had reported previously that prolactin levels were
raised at proestrus in rats fed a 20% saturated fat (lard) diet
when compared to rats fed a 0.5% lard diet and had suggested
that this increase in prolactin might mediate the promoting
effect of dietary fat on DMBA-induced mammary tumorigenesis.
Similar observations were made by Ip et al. (34) using a corn
oil diet. In the experiments reported here, although an increase
in prolactin was observed when the low and high fat diets were
compared, the difference was not statistically significant. That
increase in prolactin secretion is not the sole mechanism
responsible for the promotional effect of dietary fat, however,
is suggested by both the work reported in this paper and also
the earlier paper of Ip et al. (34). In the latter case, it was found
that low fat-fed rats with high prolactin levels as a result of
median eminence lesions still had a significantly lower tumor
incidence than did rats fed a high fat diet (34). In this paper,
we report that, in high fat-fed rats, prolactin levels were de-
creased 50% in thymectomized rats, but tumor incidence was
unchanged.

The protection against the carcinogenic effect of DMBA
afforded to thymectomized rats fed low and normal fat diets
could be due to decreased prolactin levels. We cannot rule out,
however, that an alteration in T-cell populations due to thymec-
tomy did not cause inhibition of tumorigenesis. Thymectomy
did decrease total lymphocyte levels; however, we have no
information on T-cell levels and subpopulations. Evidence in
mice suggests that adult thymectomy decreases suppressor T-
cell activity with a concomitant rise in helper and cytolytic T-
cell activity (36, 38–40, 59); this could explain the results
observed in the low and normal fat-fed rats. However, additional
changes would be necessary to explain the loss of effect in
rats fed the high fat diet. There is some evidence which
suggests that high fat-fed animals have altered lymphocyte
function (20, 22, 43). For example, the blastogenesis of spleen
lymphocytes to the mitogen concanavalin A was suppressed in
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Dietary Fat, the Thymus, and Mammary Cancer
Interaction of Dietary Fat and the Thymus in the Induction of Mammary Tumors by 7,12-Dimethylbenz(a)anthracene

David A. Wagner, Paul H. Naylor, Untae Kim, et al.


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