Ability of Dietary Fat to Overcome the Resistance of Mature Female Rats to 7,12-Dimethylbenz(a)anthracene-induced Mammary Tumorigenesis

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ABSTRACT

The present study is designed to delineate the action of dietary fat on the development of 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary tumors in female Sprague-Dawley rats and to investigate the interdependence of fat intake and age as modifiers of mammary oncogenesis. Compared to rats given DMBA at 50 days of age (immature rats), those exposed to DMBA at 150 days of age (mature rats) were more resistant to the carcinogen irrespective of fat intake, although the incidence of tumors in rats fed a high-fat diet (20% corn oil) still remained substantially higher than in those rats on a low-fat diet (0.5% corn oil) at different doses of DMBA tested. In this experiment, both diets were fed from weaning until termination of the experiment. When adult rats (150 days old) were given 5 mg of DMBA each week for 4 consecutive weeks, tumors appeared earlier, and the proportion of adenocarcinomas to fibroadenomas was higher in the group that was fed the 20% fat diet. Using the adult rats as the model for tumor induction, the contribution of a high dietary fat intake before and after DMBA administration in the promotion of mammary tumorigenesis was assessed. The types of fat intake before and after DMBA were designated LF-LF, LF-HF, HF-LF, and HF-HF, in which LF and HF represent 0.5% and 20% fat diets, respectively. Results of this experiment established the following order of tumor incidence: HF-HF (84.0%) > LF-HF (60.9%) > HF-LF (36.0%) > LF-LF (20.8%). It appears from this study that the level of fat intake after DMBA treatment is more important in determining the subsequent development of mammary cancer than is fat intake prior to DMBA administration, thus confirming earlier observations reported by Carroll and Khor and by Hopkins, Hard, and West. In addition, it was also found that in rats that were fed a 0.5% fat diet from weaning and given a single dose of 5 mg of DMBA at 50 days of age, a transfer to a 20% fat diet as late as 20 weeks after DMBA was able to stimulate a significant increase in the number of rats bearing tumors. It can be concluded from these findings that dietary fat acts preferentially, although by no means exclusively, on the promotion phase of carcinogenesis and that the differential effect of high-fat and low-fat diets on tumorigenesis can be demonstrated regardless of the age at which the carcinogen is given.

INTRODUCTION

Age is an important factor in the induction of mammary cancer by polycyclic hydrocarbons in female rats. Huggins et al. (13) treated Sprague-Dawley rats of various ages with a single dose of 3-methylcholanthrene and reported that rats between 50 and 65 days of age were most susceptible to tumorigenesis. Similar results were obtained by Dao (7) with DMBA2 in Wistar-Furth rats. The tumor incidence varied from 80% in rats given DMBA at 50 days of age to less than 5% in rats given DMBA at 150 days of age. There are other reports that indicate that age at carcinogen administration also affects the proportion of carcinomas versus fibroadenomas (18) as well as the frequency of spontaneous tumor regression (8).

An enhancement of mammary tumorigenesis by dietary fat in rats treated with DMBA has been confirmed by several investigators (3, 5, 12, 15) as well as by our laboratory (14). In view of the observation that adult rats are more resistant to the development of DMBA-induced mammary tumors, it would be of great interest to determine if the promoting effect of a high-fat diet is manifested in rats that are fed the diet since weaning and given DMBA at 150 days of age. The temporal relationship between the introduction of a high-fat diet and DMBA administration should also be explored so as to better delineate the action of dietary fat on the carcinogenic process. Experiments described in the present study are thus designed to provide some insight on the interdependence of dietary fat and age as modifiers of mammary oncogenesis.

MATERIALS AND METHODS

Female Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were fed from weaning (21 days old) either a synthetic high-fat diet or a low-fat diet. In certain experiments, rats on a high-fat diet were switched to a low-fat diet or vice versa during the course of the study, as indicated in the text. The high-fat and low-fat diets contained 20% and 0.5%, respectively, of Mazola corn oil (donated generously by Best Foods, Englewood Cliffs, N. J.) by weight. The composition of the other ingredients was described in a previous publication (14). Since Mazola corn oil contains approximately 0.02% of α-tocopherol (analysis provided by the supplier), the low-fat diet was also supplemented with 40 mg of α-tocopherol (ICN Pharmaceuticals, Cleveland, Ohio) per kg of diet so that the total α-tocopherol content would be similar in both the high-fat and the low-fat diets.

Mammary tumors were induced by i.g. administration of DMBA (Sigma Chemical Co., St. Louis, Mo.) that was dissolved in corn oil at a concentration of 5 mg/ml. A 5-ml syringe and a 5-French stomach tube were used for this purpose, and 5 mg of DMBA were given to each rat. When multiple doses were used, rats were fed 5 mg/week for no more than 4 weeks; the

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2 The abbreviations used are: DMBA, 7,12-dimethylbenz(a)anthracene; i.g., intragastric.
first dose was given when the rats were either 50 or 150 days old. The total dosage is denoted in the text as \( n \times 5 \) mg, in which \( n \) represents the number of weekly administrations. In order to minimize any possible effect of dietary fat on absorption of the carcinogen from the intestine, rats were fasted 12 hr before and after the p.o. feeding of DMBA. Tumor palpation was carried out as described previously (14).

**RESULTS**

Since a number of factors such as rat strain, age, and carcinogen dosage, the schedule of administration, and fat intake are known to affect mammary tumorigenesis, our first experiment was designed to study the effect of multiple doses of DMBA, given initially at 50 or 150 days of age, on mammary tumor development in female Sprague-Dawley rats that were fed either a high-fat diet (20% corn oil) or a low-fat diet (0.5% corn oil) starting from weaning. The experiment was terminated 24 weeks after the first intubation of DMBA. Table 1 shows the final tumor incidence as a function of the DMBA dosage and the time of treatment in each dietary group. Tumor incidence is expressed as the percentage of rats with tumors.

When DMBA was given initially at 50 days of age to rats fed the low-fat diet, the resulting tumor incidence increased in proportion to the dose of DMBA. The incidence was 12.5% with a single dose of 5 mg of DMBA and rose to 92.0% with a total dose of 4 \( \times 5 \) mg. An enhancement of mammary tumorigenesis was observed in rats that were fed the high-fat diet compared to those on the low-fat diet and given the same amount of DMBA. Thus ingestion of a high-fat regimen produced an incidence of 66.7% (a 5-fold increase over the low-fat group) with a dose of 5 mg of DMBA. All of the animals (100%) in the high-fat group developed tumors after being treated with 10 mg of DMBA, in contrast to an incidence of 40% in the low-fat group.

Table 1 also shows that if rats were exposed to DMBA at 150 days of age, they became more resistant to the carcinogen irrespective of fat intake, although the incidence of tumors in rats fed the high-fat diet still remained higher than in those rats on the low-fat diet at all levels of DMBA tested. Thus, when these adult rats were given 5, 10, 15, or 20 mg of DMBA, the corresponding incidences were 0, 4.2, 8.7, and 16.7% in the low-fat group, as compared to incidences of 8.3, 25.0, 52.0, and 78.3% in the high-fat group.

Chart 1 illustrates the time course of tumor development in both low-fat and high-fat groups following a schedule of 4 \( \times 5 \) mg of DMBA treatment, with the initial dose given at 150 days of age. In rats fed the high-fat diet, palpable tumors started to appear 10 weeks after the first dose of DMBA. The incidence continued to rise for 8 more weeks before leveling off at 78.3%. On the other hand, rats fed the low-fat diet started to develop tumors 14 weeks after the first dose of DMBA, and the incidence plateaued 3 weeks later at 16.7%. Results in Table 2 show the total tumor count (both palpable and nonpalpable) as well as the histological type of the tumors obtained in these 2 groups of animals. In the low-fat group, there were 10 palpable tumors and one nonpalpable tumor, of which 6 were adenocarcinomas and 5 were fibroadenomas. A total of 50 palpable and 3 nonpalpable tumors were found in the high-fat group, of which 35 were adenocarcinomas and 18 were fibroadenomas.

In order to evaluate if high dietary fat intake prior to DMBA administration in the adult rats is critical in the promotion of mammary tumorigenesis, another experiment was set up involving 4 groups of rats that were given 4 \( \times 5 \) mg of DMBA starting at 150 days of age and treated as follows: Group 1, a low-fat diet from weaning to termination of the experiment (designated LF-LF); Group 2, a low-fat diet from weaning until the last dose of DMBA and then transfer to a high-fat diet until termination of the experiment (LF-HF); Group 3, the reverse of Group 2, with a high-fat diet first followed by a low-fat diet (HF-LF); and Group 4, feeding of a high-fat diet from weaning to the end of the experiment (HF-HF). All rats were sacrificed 24 weeks after the first dose of DMBA. Results of this study are shown in Table 3.

Compared to rats that were fed the 0.5% fat throughout the experiment, a change of the diet from 0.5 to 20% fat after DMBA treatment was able to increase the incidence of tumor-bearing rats from 20.8 to 60.9% (Group 1 versus Group 2). As a matter of fact, feeding of a high-fat diet after DMBA was only slightly less effective as starting the diet from weaning (Group 2 versus Group 4). On the other hand, a regimen of a high-fat diet before the final DMBA treatment followed by a low-fat diet (HF-LF) resulted in a smaller increase in tumorigenesis when
Rats were given 5 mg of DMBA per week for 4 consecutive weeks; the first dose was given when the rats were 150 days old. They were killed 24 weeks after the first dose of DMBA.

<table>
<thead>
<tr>
<th>Dietary group (% fat)</th>
<th>No. of rats</th>
<th>Tumors/tumor-bearing rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>24</td>
<td>2.8 ± 0.42</td>
</tr>
<tr>
<td>20</td>
<td>23</td>
<td>2.9 ± 0.33</td>
</tr>
</tbody>
</table>

- Numbers in parentheses, percentage of rats with tumors.
- Mean ± S.E.

### Relation of dietary fat feeding and DMBA administration on mammary tumorigenesis in adult female Sprague-Dawley rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Dietary treatment</th>
<th>No. of rats</th>
<th>Body wt at 150 days of age (g)</th>
<th>Final body wt (g)</th>
<th>Tumors/tumor-bearing rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LF-LF</td>
<td>24</td>
<td>304 ± 6.1</td>
<td>328 ± 7.1</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>LF-HF</td>
<td>23</td>
<td>296 ± 7.5</td>
<td>321 ± 7.3</td>
<td>14 (60.9)</td>
</tr>
<tr>
<td>3</td>
<td>HF-LF</td>
<td>25</td>
<td>308 ± 6.8</td>
<td>341 ± 6.6</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>HF-HF</td>
<td>25</td>
<td>308 ± 7.2</td>
<td>336 ± 7.9</td>
<td>55</td>
</tr>
</tbody>
</table>

- LF, 0.5% fat diet; HF, 20% fat diet. Groups LF-LF and HF-HF were fed 0.5 and 20% fat diets, respectively, from weaning to termination of the experiment. Groups LF-HF and HF-LF were switched from 0.5 to 20% fat and vice versa at the time the last dose of DMBA was given.
- Mean ± S.E.

### Effect of a delay feeding of a high-fat diet on subsequent mammary tumor development in rats that were given DMBA at 50 days of age

A group of 60 rats was fed the 0.5% fat diet from weaning (21 days old) and were given 5 mg of DMBA at 50 days of age, of which 58 survived for the next 20 weeks. They were maintained on this diet for 24 weeks up to this point. Of the 52 rats that failed to develop tumors after this period, one-half of them continued to receive the 0.5% fat diet, and the other half were switched to the 20% fat diet. The experiment was terminated 20 weeks later.

### DISCUSSION

There are 3 notable findings in this study concerning the relationship between dietary fat and age at carcinogen administration on mammary carcinogenesis. First, a high-fat intake is able to produce a substantial increase in tumorigenesis in rats that are given DMBA at 150 days of age (adult rats), even compared to those rats that were maintained on the LF-HF schedule (Group 3 versus Group 2). Thus, it can be seen that the time of introduction of a high-fat diet in relation to DMBA administration is important in determining the subsequent tumor incidence. Based on the present experiment, the following order of efficacy was established: HF-HF > LF-HF > HF-LF > LF-LF.

As pointed out in Table 1, when 50-day-old rats fed a 0.5% fat diet were given a single 5-mg dose of DMBA, only a small percentage (12.5%) of the rats developed tumors. In our experience with this particular regimen and this dose of DMBA, tumors started to appear 10 to 12 weeks after DMBA administration and continued to do so for about 4 to 6 weeks (results not shown). We decided to investigate whether a change in the diet from 0.5% to 20% fat 20 weeks after DMBA would further stimulate tumor development in these animals. A third experiment was then initiated involving 60 rats that were fed 0.5% fat from weaning and given a single dose of 5 mg of DMBA at 50 days of age. During the next 20 weeks after DMBA, 6 rats developed tumors and 2 died (with no tumor). At this point, the remaining 52 rats were divided into 2 groups; the first group continued to receive the 0.5% fat diet, and the second group was fed the 20% fat diet. The experiment was terminated 20 weeks later. Results of this study are shown in Table 4.

In rats that were switched from the 0.5% to the 20% fat diet, tumors started to appear 7 weeks after the transfer, with more developing in the next 7 weeks. When the experiment was terminated 20 weeks after the change of diet, 6 of 26 rats developed a total of 9 tumors. During the same period, one rat developed a tumor in the group that received the 0.5% fat diet.
Although these animals are more resistant to the carcinogen than are those that are treated at 50 days of age (immature rats). In other words, the differential effect of high-fat and low-fat diets in tumorigenesis can be demonstrated regardless of age at which the carcinogen is given. Second, with the adult rats as the model for tumor induction, our experiment shows that the level of fat intake after DMBA treatment is more important in determining the subsequent development of mammary cancer. Third, a change of the diet from 0.5 to 20% fat 20 weeks after DMBA administration (at 50 days of age) results in a further stimulation of tumor appearance compared to those rats that are fed the 0.5% fat diet throughout the experiment. Thus, it can be concluded from these observations that dietary fat acts preferentially, although by no means exclusively, on the promotion phase of carcinogenesis. This effect of dietary polyunsaturated fat has been reported previously in DMBA-induced mammary tumors in rats (2, 12) and mice (10) and in a transplantable mammary adenocarcinoma in mice (11).

With similar dietary regimens and rats that were treated with DMBA at 50 days of age, Carroll and Khor (2) have examined the effect of feeding a high-fat diet before and after DMBA administration. They reported that the fat intake prior to DMBA had negligible effect. According to their study, it was the level of dietary fat given after the carcinogen that determined the final tumor incidence. Results from our study shown in Table 3 tend to disagree with their conclusion, since we found that, provided the animals were fed a high-fat diet prior to DMBA, a transfer to a low-fat regimen after DMBA could still lead to an enhancement in tumorigenesis, although to a smaller degree. The physiological mechanism by which dietary fat influences the susceptibility of the mammary gland to carcinogenic injury remains to be elucidated. The effect of fat intake on the pharmacokinetics and metabolism of the carcinogen may also be a contributing factor and should be investigated.

In a later publication, Carroll and Khor (4) showed that the yield of mammary tumors was increased only if the rats were switched to a high-fat diet 1 or 2 weeks after given DMBA. If the transfer was delayed for 4 weeks, little or no effect was seen. In the experiment described in Table 4, we demonstrated that a change to a high-fat diet as late as 20 weeks after DMBA administration was able to produce a significant increase in the number of rats bearing tumors. The reason for the discrepancy between these 2 studies is not apparent. If dietary fat influences primarily the developmental or promotional phase of carcinogenesis, as suggested by Carroll and Khor (2), it seems inconsistent that these authors would find that the introduction of a high-fat diet more than 3 weeks after the carcinogen had a negligible effect. Based on our observations, it would be more logical to infer that ingestion of a high-fat diet provides a favorable milieu for the subsequent proliferation of dormant transformed cells.

The prevailing environment that would enhance DMBA-induced tumor development as a result of a high-fat intake may include elevated circulating prolactin (6, 14) and estrogens, since the growth of these tumors is dependent on hormones. The effect of dietary fat may also conceivably be mediated through the maintenance and induction of prolactin receptors (16) or the cellular immune system with the possible involvement of prostaglandins (9) or lymphocyte dysfunction (17).

There is one other interesting point in Table 2 that deserves some attention, specifically, the distribution of adenocarcinomas and fibroadenomas. In rats that are fed either a high-fat or a low-fat diet and are given DMBA when they are 50 days old, over 90% of the tumors obtained are adenocarcinomas. This observation has been confirmed by several investigators (2, 5, 15), including this author (14). However, when rats were treated at 150 days of age, we found that the proportion of adenocarcinomas to fibroadenomas decreased substantially; the ratio was ~1:1 in the low-fat group and ~2:1 in the high-fat group. This is in agreement with an earlier report by Meranze et al. (18) that more benign lesions were obtained in older rats. It remains to be determined whether the augmented ratio of adenocarcinomas to fibroadenomas in the high-fat group is a coincidence or due to the increased stimulation by estrogens and prolactin as discussed above.

The 0.5% corn oil used in the low-fat diet, although only marginal in the adequacy of essential fatty acids, did not seem to inhibit the growth of the animals (refer to body weights of Groups 1 and 4 in Table 3). They did not manifest any signs of fatty acid deficiency such as rough hair coat, scaly feet, necrosis of tail, or hyperemia of the renal tissues even after 50 weeks on this diet. The reason for using the 0.5% fat diet in this study instead of a 5% fat diet (which approximates the fat content in laboratory chow) is because of the very low tumor incidence produced by the former dietary regimen, thus enabling us to magnify the difference in tumor incidence between the 20% fat group and the 0.5% fat group.

As pointed out in a recent report by Newberne et al. (19), the formulations of high-fat and low-fat diets used in this study may lead to small changes in the consumption of protein, vitamins, and minerals in addition to changes in fat and carbohydrate intake, since the diets are not isocaloric in nature. In their opinion, this actually results in the comparison of the effects of 2 different diets rather than of the effects of dietary fat. We have measured the food intake of rats fed the high-fat and low-fat rations in our experiment. The average amounts of food consumption daily per rat in the high-fat and low-fat groups were about 13 and 16 g, respectively. This represented a very similar caloric intake between these 2 groups. Although there was a 20% difference in the ingestion of the other nutrients besides fat and carbohydrate, the total lipid consumption in the high-fat (20%) group was approximately 30 to 35 times that of the low-fat (0.5%) group. We therefore justifiably believe that our diets were adequate to reflect a preponderant change in fat intake.

In conclusion, it should be put in perspective that the implication of this study is not without its parallel in human mammary cancer. Descendants of Japanese immigrants to the United States show an increase in breast cancer rate, but this increase is not seen among the immigrants themselves (1). Adaptation to a western diet (presumably a higher fat intake) may be partially responsible for this disease. Thus, the influence of a dietary factor operating either early in life or continuously over a longer life span may be critical in determining the risk of a particular generation to breast cancer.

REFERENCES

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Effect of Dietary Fat and Age on Mammary Tumorigenesis

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