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Effects of Dietary Fat and Dose Level of 7,12-Dimethylbenz(α)-anthracene on Mammary Tumor Incidence in Rats[1]

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SUMMARY

Female Sprague-Dawley rats maintained on a semisynthetic diet containing 20% corn oil developed more mammary tumors after treatment with a single p.o. dose of 7,12-dimethylbenz(α)anthracene than rats treated similarly but fed a low-fat semisynthetic diet. The tumor yield varied with the dose of 7,12-dimethylbenz(α)anthracene, but the rats on high-fat diet developed more tumors at each of 3 dose levels tested. The type of diet fed after administration of 7,12-dimethylbenz(α)anthracene had a greater influence on mammary tumor incidence than did the type fed before the carcinogen was given, indicating that the effect is exerted mainly at the promotional stage of mammary carcinogenesis.

INTRODUCTION

The role of dietary fat in mammary cancer has been under investigation in our laboratory for a number of years (3, 7, 8). In earlier studies, weanling female rats were fed semisynthetic diets containing 0.5% or 20% corn oil by weight and given a single p.o. dose of 10 mg of DMBA[4] in 0.5 ml of sesame oil at 50 to 52 days of age. It was observed that the group on 20% corn oil developed more mammary tumors with a shorter latent period than the group on the low-fat diet (7). A group fed 20% coconut oil gave results intermediate between those of the other 2 groups. In these experiments, an attempt was made to minimize effects of dietary fat on absorption of the carcinogen by placing the animals on commercial diet for 2 days before and 1 day after giving the DMBA. The effect seemed to be independent of caloric intake, which was similar in all 3 groups.

These results confirmed observations in a number of other laboratories that increasing the level of dietary fat enhances the yield of mammary tumors (17). This was observed with tumors induced by various means (5, 6) and also with spontaneous tumors in both rats and mice (1, 13). Dietary fat also consistently enhanced the yield of skin tumors induced by polycyclic hydrocarbons, but it did not appear to increase the incidence of several other kinds of tumors (15).

In our experiments, the mammary tumor incidence reached 70 and 95%, respectively, in the low-fat and high-corn oil groups 3 months after administration of the DMBA. In view of the high incidence in both groups, it seemed worthwhile to explore whether the difference between groups might be accentuated by using a lower dose of the carcinogen, and experiments were therefore carried out with dose levels of 1, 2.5, and 5 mg of DMBA, respectively.

In addition to the evidence that high levels of dietary fat increase the incidence of mammary tumors in experimental animals, there is evidence of a strong positive correlation between dietary fat intake and human mortality from breast cancer, based on data collected from many different countries (3, 9, 18). It may therefore be of practical as well as theoretical interest to determine how dietary fat affects mammary tumor incidence.

Our first approach to this problem was to measure levels of DMBA in mammary tissue of rats on high- and low-fat diets at various time intervals after administration of the carcinogen (8). The results did not support the idea that the higher tumor incidence on the high-fat diet was due to higher tissue levels of DMBA or more prolonged exposure of the tissue to the carcinogen. In a further attempt to gain insight into the mechanism of action, an experiment was carried out in which the high-corn oil diet was fed only before or only after administration of the DMBA. Results of these studies are presented in the present communication.

MATERIALS AND METHODS

Weanling female rats were obtained from Sprague-Dawley, Inc., Madison, Wis. On arrival, they were divided into groups of 30 and were put on semisynthetic diets the same day. They were housed 2 to a cage in a temperature-controlled, well-ventilated room and were weighed once a week.

The low-fat semisynthetic diet consisted of: casein, 18; dextrose, 72; Phillips-Hart (11) salt mix, 4; Cellu flour, 5; and corn oil, 0.5 parts by weight. the high-fat diet contained: casein, 23; dextrose, 46; salt mix, 5; Cellu flour, 5; and corn oil, 20 parts by weight. To each kg of diet were added 15 ml of a mixture of water-soluble vitamins prepared as follows: thiamine hydrochloride, 0.5 g; pyridoxine hydrochloride, 0.5

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g; biotin, 0.02 g; choline chloride, 100 g; and inositol, 10 g, were dissolved in 500 ml of distilled water. Riboflavin, 1.0 g; nicotinic acid, 5.0 g; calcium pantothenate, 5.9 g; and folic acid, 0.05 g, were dissolved in 500 ml of ethanol, and the 2 solutions were mixed and stored in the cold. Fat-soluble vitamins were added to the corn oil to give the following amounts per kg of diet; vitamin A ester (200,000 units/g), 100 mg; DL-α-tocopherol acetate, 110 mg; and vitamin K (2-methylnaphthoquinone), 30 mg. The casein, salt mixture, and vitamins were obtained from Nutritional Biochemical Corp., Cleveland, Ohio; the dextrose was obtained from Ingram and Bell Ltd., Port Credit, Ontario, Canada; the corn oil was obtained from St. Lawrence Starch Co., Ltd., Port Credit, Ontario, Canada; the coconut oil was obtained from Proctor and Gamble, Ltd., Hamilton, Ontario, Canada; and the Cellu flour was obtained from Chicago Dietetic Supply House, Inc., Chicago, Ill.

At 50 days of age, all rats were given a single dose of DMBA in sesame oil by stomach tube (French No. 8 catheter). As in previous experiments, the rats were switched to a standard commercial diet for 2 days before and 1 day after administration of DMBA in order to minimize effects of dietary fat on absorption of the carcinogen.

The DMBA used for these experiments was obtained from Eastman Organic Chemicals, Rochester, N. Y. Thin-layer chromatography on Silica Gel G, developed with Skellysolve B/ether (9/1), showed that some impurities were present in the commercial product, and it was therefore purified by recrystallization from methanol/water (10). Approximately 1.6 g were refluxed with 400 ml of methanol, the solution was filtered, 40 ml of water were added, and the mixture was allowed to cool to room temperature. The precipitated DMBA was filtered and recrystallized once more by the same procedure. The purified product was dried over P_2O_5 and stored in the dark at -15°.

Beginning 1 month after DMBA administration, all rats were palpated every 4 to 5 days for mammary tumors. The positions of the tumors were recorded to serve as a guide for differentiating palpable and nonpalpable tumors at autopsy. Experiments were normally terminated 4 months after DMBA administration. At autopsy, the rats were killed with chloroform, and the tumors were preserved in 10% buffered formalin for sectioning and staining with hematoxylin and eosin.

RESULTS

Effect of Different Dose Levels of DMBA. The cumulative tumor incidence in rats maintained on either 20 or 0.5% corn oil diets and treated with 1, 2.5, or 5 mg of DMBA at 50 days of age is illustrated in Chart 1, and the results at autopsy are summarized in Tables 1 and 2. The groups receiving 2.5 and 5 mg were autopsied 4 months after DMBA administration, as in earlier experiments, but because of the low tumor incidence the experiment with groups receiving 1 mg was continued for 6 months.

The results with the 5-mg dose were quite similar to those obtained earlier with a 10-mg dose. The differences between the high- and low-corn oil groups were significant to the 5% level in nearly all parameters (Table 1). With 2.5 mg of DMBA, the tumor incidence was lower, and the differences between the groups on high-corn oil and low-fat diets were not as consistently significant as with the higher dose. The 1-mg dose of DMBA gave much lower tumor yields, but the high-fat group again developed more tumors than the low-fat group.

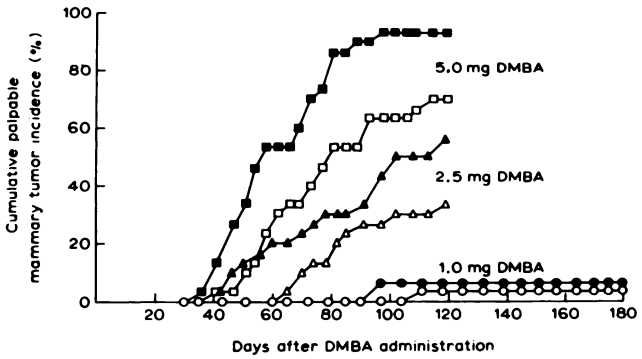

Chart 1. Cumulative palpable mammary tumor incidence in female Sprague-Dawley rats maintained from weaning on semisynthetic diets containing either 20% corn oil (■, ▲, ●) or 0.5% corn oil (□, △, ○). The rats were treated at 50 days of age with a single p.o. dose of 5, 2.5, or 1 mg DMBA in sesame oil. *Curves*, percentage of animals developing palpable tumors as a function of time.

The high-corn oil diet enhanced the yield of fibroadenomas and adenomas as well as the yield of adenocarcinomas (Table 2). In the experiment with 1 mg of DMBA, only 60% of the tumors were adenocarcinomas in both high- and low-fat groups, whereas adenocarcinomas made up more than 90% of the total when the higher doses of DMBA were given.

The rats on high- and low-fat diets had similar growth rates in the above experiments (Table 1). In each case, the final body weight of the group on high-fat diet was slightly greater, but the difference was statistically significant only in the groups treated with 1 mg of DMBA ($p < 0.05$).

Effect of Feeding High-Fat Diet Only before or Only after Administration of DMBA. Two additional dietary groups, L-H and H-L, were included in the experiment in which the rats were treated with 5 mg of DMBA. In these groups, the diet was changed from low fat to high fat and *vice versa* at the time the DMBA was administered. However, as in other experiments, the rats were maintained on commercial diet for 2 days before and 1 day after the DMBA treatment.

The cumulative tumor incidence in the L-H and H-L groups is shown in Chart 2, and the results for the groups fed high-fat (H-H) or low-fat (L-L) diets throughout are included for comparison. Results at autopsy are given in Tables 1 and 2.

It is evident from these results that the groups fed high-fat diet after DMBA had a higher tumor incidence than the groups fed low-fat diet, regardless of the type of diet fed before the DMBA was administered. The rats in the L-H group had a significantly higher incidence of palpable tumors than those in the L-L group, and the latent period was significantly shorter ($p < 0.05$). The L-H group also gave a higher figure in each of the other parameters relating to

Table 1

Mammary tumor incidence in rats on high- and low-corn oil diets following administration of DMBA

Diet	No. of rats	Body weight[a] Initial	Body weight[a] Final	Tumor incidence[b] Total	Tumor incidence[b] Palpable	No. of tumors/rat[a] Total	No. of tumors/rat[a] Palpable	No. of tumors/tumor-bearing rat[a]	Latent period (days)[c]
				1 mg DMBA[d]					
20% corn oil	30	43 ± 0.6	250 ± 4.6	26.6	6.6	0.33 ± 0.11	0.10 ± 0.03	1.25 ± 0.78	97
0.5% corn oil	28	43 ± 0.4	235 ± 4.6	14.3	3.6	0.17 ± 0.02	0.03 ± 0.03	1.25 ± 0.25	111
				2.5 mg DMBA[d]					
20% corn oil	30	40 ± 0.5	245 ± 2.9	76.6	56.6	1.5 ± 0.28	0.9 ± 0.20	1.9 ± 0.26	79.1 ± 4.8
0.5% corn oil	30	40 ± 0.5	238 ± 2.2	43.3	33.3	0.6 ± 0.17	0.4 ± 0.22	1.4 ± 0.15	84.0 ± 3.0
p values				< 0.05		< 0.05			
				5 mg DMBA[d]					
H-H (20% corn oil)	30	51 ± 0.9	250 ± 2.4	96.6	93.3	4.5 ± 0.61	3.0 ± 0.37	4.6 ± 0.61	62.8 ± 2.7
L-L (0.5% corn oil)	30	50 ± 0.6	245 ± 3.4	73.3	70.0	2.4 ± 0.43	1.5 ± 0.28	3.3 ± 0.32	73.2 ± 4.3
p values				< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.1
L-H[e]	30	50 ± 0.9	249 ± 2.7	93.3	93.3	3.7 ± 0.41	2.3 ± 0.28	4.0 ± 0.37	60.0 ± 2.9
H-L	30	50 ± 0.5	251 ± 3.5	73.3	66.6	2.3 ± 0.46	1.6 ± 0.32	3.1 ± 0.37	67.5 ± 2.3

[a] Results expressed as average ± S.E.
[b] Percentage of rats with tumors at autopsy.
[c] Days from DMBA feeding to appearance of first palpable tumor.
[d] The 1-mg and 5-mg doses were administered in 0.25 ml of sesame oil and the 2.5-mg dose was administered in 0.125 ml.
[e] See text for explanation of diets fed.

Table 2

Incidence of different types of mammary tumors in rats on high- and low-corn oil diets following treatment with DMBA

Diet	Adenocarcinomas Palpable	Adenocarcinomas Nonpalpable	Adenocarcinomas Total	Fibroadenomas Palpable	Fibroadenomas Nonpalpable	Fibroadenomas Total	Adenomas Palpable	Adenomas Nonpalpable	Adenomas Total
				1 mg DMBA					
20% corn oil	3	3	6	1	2	3	0	1	1
0.5% corn oil	1	2	3	0	1	1	0	1	1
				2.5 mg DMBA					
20% corn oil	27	14	41	0	1	1	0	2	2
0.5% corn oil	11	6	17	1	0	1	0	0	0
				5 mg DMBA					
H-H (20% corn oil)	88	39	127	3	1	4	1	2	3
L-L (0.5% corn oil)	47	25	72	1	1	2	0	0	0
L-H[a]	68	35	103	2	3	5	1	3	4
H-L	47	20	67	1	1	2	0	0	0

[a] See text for explanation of diets fed.

tumor incidence (Table 1), but the differences were not significant at the 5% level. On the other hand, the percentage of rats with palpable tumors and the number of tumors in the H-L group were significantly lower than in the H-H group ($p < 0.05$). The latent period was also longer, but the difference was not statistically significant. The average body weights of these 4 dietary groups showed no significant differences at the time of autopsy.

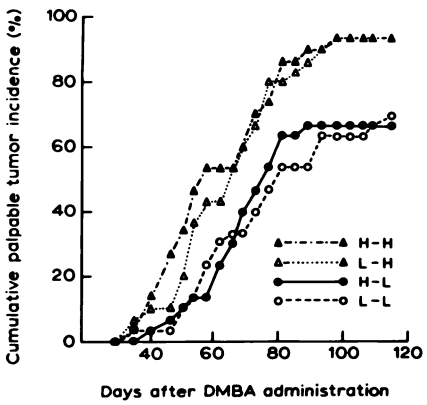

Chart 2. Cumulative palpable mammary tumor incidence in female Sprague-Dawley rats maintained on semisynthetic diets containing either 20% or 0.5% corn oil and treated at 50 days of age with a single p.o. dose of 5 mg DMBA in sesame oil. Groups H-H and L-L were fed high-fat and low-fat diets, respectively, throughout while Groups L-H and H-L were switched from low-fat to high-fat diet and *vice versa* at the time the DMBA was given. *Curves*, percentage of animals developing palpable tumors as a function of time.

The incidence of different types of tumor in the L-H group was similar to that observed for the H-H group, while the incidence in the H-L group resembled that in the L-L group (Table 2).

DISCUSSION

The results of the above experiments provide further evidence that a high-fat diet enhances the yield of mammary tumors in young female rats exposed to DMBA, although the difference in tumor incidence between rats on high- and low-fat diets was not accentuated by using lower doses of the carcinogen (Table 1, Chart 1).

The data also reveal that the high-fat diet was most effective when fed after administration of DMBA (Table 1, Chart 2). This suggests that the effect on tumor incidence was not due to effects of dietary fat on absorption or distribution of the DMBA and is consistent with earlier studies which showed that the administered DMBA did not accumulate in significantly larger amounts in the mammary glands of rats as a result of feeding high-fat diet (8).

The rats on low-fat diet throughout weighed slightly less on the average at autopsy than those on high-fat diet (Table 1), but the differences were in general not statistically significant and the groups fed high-fat diet only before (H-L) or only after (L-H) administration of DMBA grew as well as those on high-fat diet throughout. Therefore, as concluded previously (7), it seems unlikely that the effects on tumor incidence are due to differences in caloric intake.

According to the 2-stage theory of carcinogenesis formulated by Berenblum and Shubik (2), the process can be divided into 2 distinct stages, referred to as the initiating process and the promoting process, respectively. The studies of Dao *et al.* (4), in which mammary grafts were transplanted 24 hr after p.o. administration or 10 min after i.v. injection of DMBA, indicated that the initiation process in mammary carcinogenesis is very rapid. The present results therefore suggest that the effect of dietary fat is exerted primarily at the promotional stage of carcinogenesis. A similar conclusion was reached by Tannenbaum (14) in connection with his studies of the effect of dietary fat on skin tumors induced in mice by benzpyrene.

The exact mechanism by which a high-fat diet promotes tumor formation during the promotional stage is a matter for speculation. Benson *et al.* (1) noted that in histological sections rat mammary tissue appeared more active in animals on high-fat diet compared to those on low-fat diet. Possibly, a high-fat diet stimulates the metabolism of the tissue either by altering hormonal levels in the gland or by some more direct mechanism, and further experiments are being carried out with the aim of providing more information on this aspect of the problem. Studies are also being conducted to obtain more data on the effects of different types of dietary fat.

In our experiments, fat was added to the diets at the expense of carbohydrate, and the observed effects on tumor incidence could as well be due to differences in carbohydrate as in fat intake. An attempt was made to keep the intake of protein, salts, vitamins, and Cellu flour similar on both high- and low-fat diets by increasing the proportions of these components in the high-fat diet to allow for the higher caloric content of fat.

Since any variation in 1 dietary constituent inevitably affects the proportions of others, it is difficult to obtain an unambiguous answer in this type of experiment, but useful information might be gained by keeping the carbohydrate constant and varying the protein inversely with the fat. In earlier experiments by Tannenbaum and Silverstone (16), it was found that varying the casein in the diets of the animals inversely with the carbohydrate, without change in the fat, had little effect on the incidence of spontaneous mammary tumors in mice. Similar results were also obtained by Shay *et al.* (12) in experiments with rats treated with 3-methylcholanthrene.

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