

# Dietary Lipid Effects on the Growth, Membrane Composition, and Prolactin-Binding Capacity of Rat Mammary Tumors<sup>1,2,3</sup>

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**ABSTRACT**—The effects of qualitative and quantitative differences in dietary lipid on the growth of *N*-methyl-*N*-nitrosourea (CAS: 684-93-5)-initiated mammary tumors were evaluated in inbred female (F344) rats. Specific prolactin-binding measurements and qualitative lipid analyses were performed on both tumor and hepatic microsomes from these animals. Our results indicated that 1) when the polyunsaturated lipid component (corn oil) of the diet exceeded 3%, it was the quantitative level of total lipid, rather than the level of polyunsaturated lipid alone, that best correlated with the observed reduction in tumor latent period; 2) when the polyunsaturated lipid content of the diet fell below 3%, there was a decrease in tumor incidence and an increase in the mean latent period; 3) only those changes in tumor development that occurred when the dietary polyunsaturated lipid content was below 3% were associated with alterations in specific prolactin binding capacity.—JNCI 1984; 73:185-191.

Previous studies have documented that qualitative and quantitative differences in dietary fat intake can affect the growth of mammary tumors in rats (2-7). These effects are thought to be mediated by events occurring during the promotional phase of tumor development, but the exact mechanisms responsible for these effects are unknown (8, 9). In recent studies investigators have indicated that certain modifications in dietary lipid can cause alterations in the prolactin-binding capacities of mammary tumor microsomes and have suggested that these changes might be a significant factor in regulating mammary tumor growth (10). Therefore, the objective of these experiments was to determine more precisely the relative effects of graded differences in dietary lipid on mammary tumor growth and to observe whether parallel alterations in the prolactin-binding capacities of host tissue could be correlated with these changes.

## MATERIALS AND METHODS

**Treatment groups.**—Female F344 rats (Charles River, Kingston, Ontario, Canada) were divided into 5 diet groups. Each diet had a specific lipid content that either was distinctive qualitatively or quantitatively from the others. The exact compositions of four of these diets are listed in table 1. The formulas for these semisynthetic diets were chosen purposely to follow closely those of previously established experimental diets (5, 8, 10-12). They were designed to provide an equivalent ratio of protein calories to total calories. The vitamin and mineral contents of all of the diets were sufficient to meet all growth requirements and were not increased in the high-fat diets. The fifth diet used in these experiments, Formulab Chow 5008 (Ralston Purina Co., St. Louis, Mo.), was selected to represent a standard, commercially available rat diet. It contains approximately 6.5% lipid.

The corn oil, choline, and alphacel used in these semisynthetic diets were purchased from ICN Nutritional Biochemicals Corp., Cleveland, Ohio. All other ingredients except the tallow came from Teklad, Inc., Madison, Wis. The tallow, the lipid profile of which has been defined by Hopkins and Carroll (12), was donated by Canada Packer's Ltd., Toronto, Ontario.

**Tumor induction and ovariectomy.**—At 51 days of age the animals started their special diets 2 days before the initial administration of the carcinogen MNU (ICN Life Sciences Group, K & K Laboratories, Plainview, N.Y.). Following the method of Gullino et al. (13), we dissolved the MNU in water (10 mg/ml) and administered an iv dose of 5 mg/100 g body weight to the rats. Similar doses again were administered to the rats at 79 and 107 days of age. On the day following the last dose of carcinogen, a portion of the animals on regular laboratory chow underwent ovariectomy. All animals were maintained under identical environmental conditions, and the development of mammary tumors was monitored weekly. When each animal's tumor reached a size of approximately 1-2 cm in diameter, the unanesthetized animal was killed by cervical transection early in the day during diestrus. Immediately afterward an autopsy was performed, and samples of tumor and liver were snap frozen in liquid nitrogen and stored at -80°C.

**Subcellular fractionation.**—The frozen tissue samples were homogenized initially in a tissumizer (Tekmar Co., Cincinnati, Ohio) and then differentially separated into specific subcellular fractions by centrifugation with use of previously described methods (10). The resultant

**ABBREVIATIONS USED:** BSA = bovine serum albumin; hGH = human growth hormone; MNU = *N*-methyl-*N*-nitrosourea; oPRL = ovine prolactin; TM=0.025 M Tris-HCl-0.1 M MgCl<sub>2</sub>.

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TABLE 1.—*Diet composition*

Component	0.5% corn oil	3% corn oil	3% corn oil + 17% tallow	20% corn oil
Corn oil, 100% pure, vitamin-free	5 g/kg	30 g/kg	30 g/kg	200 g/kg
Beef tallow	0	0	170 g/kg	0
Casein, vitamin-free	180 g/kg	186 g/kg	230 g/kg	230 g/kg
Dextrose	720 g/kg	685 g/kg	455 g/kg	455 g/kg
Rogers Harper vitamin mix	5 g/kg	5 g/kg	5 g/kg	5 g/kg
Rogers Harper salt mix	50 g/kg	50 g/kg	50 g/kg	50 g/kg
Vitamin B <sub>12</sub> , 10 µg/ml water	5 ml/kg	5 ml/kg	5 ml/kg	5 ml/kg
Choline, 0.20 g/ml water	15 ml/kg	15 ml/kg	15 ml/kg	15 ml/kg
Alphacel	50 g/kg	50 g/kg	50 g/kg	50 g/kg

microsomal membrane fraction was then divided into two portions; one portion was saved for lipid analysis by storage at -80°C in a gaseous nitrogen atmosphere, while the remainder was resuspended in TM buffer and used for prolactin receptor analysis.

**Hormone iodination and receptor-binding studies.**—The hGH and oPRL were obtained from the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, Bethesda, Md. The hGH was iodinated initially with carrier-free <sup>125</sup>I (Amersham Corp., Arlington Heights, Ill.) by a lactoperoxidase method, which has been previously described (10, 14). This <sup>125</sup>I-labeled hGH then was used to measure the specific prolactin-binding capacities of the sample microsomes, according to the basic method of Posner et al. (15), Posner (16), and Shiu and Friesen (17). In these experiments, the <sup>125</sup>I-labeled hGH was incubated with 150 µg microsome-membrane protein in a final volume of 0.5 ml of 0.2% BSA-TM buffer (pH 7.55) for 22 hours at 24°C. Parallel incubations were performed in the presence of excess unlabeled oPRL (1 µg) to determine nonspecific binding. The assay was terminated by the addition of 3 ml of cold 0.1% BSA-TM buffer, and the bound and free labeled hormones were separated by centrifugation (16). Specific hormone binding was calculated by subtraction of the nonspecific counts from the total radioactivity bound.

**Qualitative fatty acid analysis.**—The lipids from the microsome-membrane fractions were obtained initially by application of the methodology of Folch et al. (18). They were subsequently methylated and stored as has been described previously (10). At the time of final analysis, these methylated lipids were dried down under gaseous nitrogen and redissolved in 25–50 µl n-hexane. Two microliters of this solution were then injected in a gas chromatograph (Hewlett-Packard 5830-A) containing a 6-foot glass column packed with 10% SP-2330 on a 100/120 chromosorb W acid-washed support (Supelco, Inc., Bellefonte, Pa.). The retention times of the individual fatty acids and the integrated areas under each peak were obtained directly from the computer associated with the instrument. We determined the identity of the observed peaks by comparing them with known standards (Supelco, Inc. and Sigma Chemical Co., St. Louis, Mo.) run at the time of assay.

**Data analysis.**—All results are reported as the mean

plus or minus the standard error of the mean. They have been statistically evaluated by Dr. C. Chuang of the Biostatistics Department, the University of Rochester, with use of the forms of analysis specifically listed below each of the tables.

## RESULTS

### Autopsy Data

The autopsy findings on the experimental animals used in this study are presented in table 2. Although some differences in body weights do exist among the different treatment groups, no clinical or autopsy evidence suggests that this was due to any dietary deficiency in the 0.5% corn oil group. Instead, these differences seemed to be due largely to the specific effects of ovariectomy on weight gain and the heavier tumor burdens carried by the groups on the higher fat diets.

The mean values for tumor incidence, tumor number, and tumor burden varied quite remarkably among the different treatment groups. Tumor incidence was reduced greatly in the ovariectomized animals and those on the 0.5% corn oil diet. In the other groups there appeared to be a positive correlation between the total amount of lipid in the diet and the mammary tumor burden. There was no major difference noted in this parameter, however, between the rats on the 3% corn oil plus 17% tallow diet and those on the 20% corn oil diet.

The mean age of death, which was essentially a measure of the duration of the latent period of tumor growth, also varied markedly among the different treatment groups. The 0.5% corn oil diet group and the ovariectomized group had the oldest ages of death, whereas the high-fat diet groups had the youngest ages of death. These data, when taken together with the previously presented results, indicated that within the nonovariectomized groups there was a direct association between the quantitative amount of fat in the diet and the ability of the host to develop a greater tumor burden in a shorter period of time.

### Liver and Mammary Tumor Membrane Binding Data

The results of the studies on the relative binding capacities of the different hepatic and mammary tumor

TABLE 2.—Autopsy data

Diet group	No. of rats	Age at death, days <sup>a</sup>	Body weight, g <sup>a</sup>	Tumor incidence, %	Tumor No. <sup>a</sup>	Tumor burden <sup>a</sup>
Corn oil, 0.5%	9	257.9 ±3.7	176.9 ±2.7	44	.4 ±.2	.23 ±0.15
Corn oil, 3%	6	224.0 ±16.1	185.0 ±3.4	100 <sup>b</sup>	1.8 ±.3	3.03 <sup>c</sup> ±1.25
Corn oil, 20%	9	205.8 <sup>d</sup> ±12.7	202.2 <sup>d,e</sup> ±7.0	100 <sup>b</sup>	3.3 <sup>d,e</sup> ±.5	6.20 <sup>c</sup> ±1.21
3% corn oil + 17% tallow	11	202.7 <sup>d</sup> ±10.7	191.9 ±4.9	100 <sup>b</sup>	2.3 <sup>d,f</sup> ±.4	7.90 <sup>c</sup> ±2.64
Rat chow, ovary intact	8	207.5 <sup>d</sup> ±9.2	191.4 ±2.5	75	1.8 <sup>f</sup> ±.5	3.72 ±1.43
Rat chow, ovariectomized	8	251.3 <sup>f,g,h</sup> ±8.0	214.1 <sup>d,e,g,h</sup> ±5.0	25 <sup>i,j,k,l</sup>	.3 <sup>f</sup> ±.2	.22 <sup>m,n,o</sup> ±0.18

<sup>a</sup>Values are means ± SE; data are from all animals in each group.<sup>b</sup>Difference from 0.5% corn oil group significant at  $P<.05$  by chi-square analysis.<sup>c</sup>Difference from 0.5% corn oil group significant at  $P<.05$  by Kruskal-Wallis analysis.<sup>d</sup>Difference from 0.5% corn oil group significant at  $P<.05$  by Neuman-Keul analysis.<sup>e</sup>Difference from 3% corn oil group significant at  $P<.05$  by Neuman-Keul analysis.<sup>f</sup>Difference from 20% corn oil group significant at  $P<.05$  by Neuman-Keul analysis.<sup>g</sup>Difference from 3% corn oil + 17% tallow group at  $P<.05$  by Neuman-Keul analysis.<sup>h</sup>Difference from rat chow group significant at  $P<.05$  by Neuman-Keul analysis.<sup>i</sup>Difference from 3% corn oil group significant at  $P<.05$  by chi-square analysis.<sup>j</sup>Difference from 20% corn oil group significant at  $P<.05$  by chi-square analysis.<sup>k</sup>Difference from 3% corn oil + 17% tallow group significant at  $P<.05$  by chi-square analysis.<sup>l</sup>Difference from rat chow group significant at  $P<.05$  by chi-square analysis.<sup>m</sup>Difference from 3% corn oil group significant at  $P<.05$  by Kruskal-Wallis analysis.<sup>n</sup>Difference from 20% corn oil group significant at  $P<.05$  by Kruskal-Wallis analysis.<sup>o</sup>Difference from 3% corn oil + 17% tallow group significant at  $P<.05$  by Kruskal-Wallis analysis.

microsome fractions are presented in table 3. These data indicated that the hepatic microsomes from the 0.5% corn oil group and the ovariectomized group that had the lowest values for specific prolactin binding also had the highest mean ages of death (tumor latency), whereas the hepatic microsomes from the 20% corn oil group, the 3% corn oil plus 17% tallow group, and the ovary intact rat chow (6.5% lipid) group, which had higher values for specific prolactin binding, had lower values for age of death. It was noted particularly, however, that, despite a consistent correlation between the total amount of fat in the diet and an earlier age of death in all ovary intact

groups, the specific prolactin binding values did not seem to vary significantly once a group's diet contained more than 3% corn oil.

Although limited by the lack of tumor tissue from the ovariectomized and 0.5% corn oil groups, the results of the hormone-binding studies on the mammary tumor microsomes were generally consistent with those obtained from the hepatic microsomes. Specifically, these data demonstrated that there were no major differences in prolactin-binding capacities of the tumor microsomes from any of the groups on diets containing more than 3% corn oil. When considered together, these hepatic and

TABLE 3.—Prolactin binding capacity of hepatic and mammary tumor microsomes

Group	Specification	0.5% corn oil	3% corn oil	20% corn oil	3% corn oil + 17% tallow	Rat chow, ovary intact	Rat chow, ovariectomized
Hepatic microsomes, fmol/mg protein	No.	9	6	9	11	8	8
	Mean	19.50	21.22	30.11 <sup>a</sup>	30.06 <sup>b,c</sup>	28.35 <sup>b,d</sup>	14.63 <sup>e,f,g</sup>
	SE	2.82	4.22	4.05	1.87	4.11	2.35
Tumor microsomes, fmol/mg protein	No.		3	9	8	4	
	Mean		58.84	58.62	61.40	63.74	
	SE		6.52	2.25	3.56	4.57	

<sup>a</sup>Difference from 0.5% corn oil group significant at  $P<.05$  by Kruskal-Wallis analysis.<sup>b</sup>Difference from 0.5% corn oil group significant at  $P<.01$  by Kruskal-Wallis analysis.<sup>c</sup>Difference from 3% corn oil group significant at  $P<.05$  by Kruskal-Wallis analysis.<sup>d</sup>Difference from 3% corn oil group significant at  $P<.01$  by Kruskal-Wallis analysis.<sup>e</sup>Difference from 20% corn oil group significant at  $P<.01$  by Kruskal-Wallis analysis.<sup>f</sup>Difference from 3% corn oil + 17% tallow group significant at  $P<.01$  by Kruskal-Wallis analysis.<sup>g</sup>Difference from rat chow group significant at  $P<.01$  by Kruskal-Wallis analysis.

TABLE 4.—*Fatty acid composition of lipids extracted from liver microsomes*

Fatty acid	Formula <sup>a</sup>	0.5% corn oil (8)	3% corn oil (6)	20% corn oil (9)	3% corn oil + 17% tallow (9)	Rat chow, ovary intact (8)	Rat chow, ovariectomized (8) <sup>b,c</sup>
Palmitic	16:0	20.19±1.02	19.23±1.42	14.79±0.62 <sup>d,e</sup>	14.31±0.74 <sup>d,f</sup>	15.61±0.51 <sup>d</sup>	17.91±0.64 <sup>g,h,i</sup>
Palmitoleic	16:1	5.43±0.36	3.24±0.77 <sup>j</sup>	0.45±0.35 <sup>d,e,g,k</sup>	0.60±0.21 <sup>d,e,l</sup>	0.81±0.21 <sup>d,f</sup>	1.31±0.19 <sup>d</sup>
Stearic	18:0	18.28±0.89	19.26±1.37	22.11±0.78 <sup>d,f</sup>	24.42±0.93 <sup>d,f</sup>	24.12±0.61 <sup>d,f</sup>	22.03±0.61 <sup>i,j</sup>
Oleic	18:1	24.13±1.35	17.89±1.42 <sup>j</sup>	8.36±0.53 <sup>d,e</sup>	16.85±1.54 <sup>d,g</sup>	12.18±0.71 <sup>d,e,g,l</sup>	12.56±0.59 <sup>d,e,g,l</sup>
Linoleic	18:2	2.57±0.07	8.40±1.22 <sup>d</sup>	15.45±1.23 <sup>d,e</sup>	7.42±1.08 <sup>d,g</sup>	11.13±0.27 <sup>d,e,g,h</sup>	12.55±0.42 <sup>d,e,h,i,k</sup>
Arachidonic	20:4	18.03±1.22	20.32±1.67	24.39±1.67 <sup>d,f</sup>	23.22±0.99 <sup>j</sup>	19.90±0.73 <sup>g,l</sup>	18.24±0.73 <sup>g,h</sup>
Docosatetraenoic	22:4	0.63±0.05	1.19±0.17 <sup>d</sup>	1.55±0.08 <sup>d,f</sup>	0.89±0.16	0.52±0.07 <sup>e,g,h</sup>	0.42±0.07 <sup>e,g,h</sup>
Docosapentaenoic	22:5 (n-6)	5.80±0.38	6.31±0.54	5.84±0.32	5.25±0.44	0.34±0.08 <sup>d,e,g,h</sup>	0.19±0.07 <sup>d,e,g,h</sup>
Docosapentaenoic	22:5 (n-3)	0.05±0.03	0.21±0.03 <sup>d</sup>	0.31±0.04 <sup>d,f</sup>	0.31±0.02 <sup>d,f</sup>	1.32±0.03 <sup>d,e,g,h</sup>	1.50±0.04 <sup>d,e,g,h</sup>
Docosahexanoic	22:6	1.63±0.13	2.44±0.21 <sup>j</sup>	3.97±0.28 <sup>d,e</sup>	5.59±0.31 <sup>d,e,g</sup>	11.06±0.31 <sup>d,e,g,h</sup>	10.14±0.35 <sup>d,e,g,h</sup>

<sup>a</sup>No. of C-atoms:No. of double bonds. Numbers in parentheses indicate the location of the first double bond from the methyl end of the fatty acid.

<sup>b</sup>Values are means ± SE.

<sup>c</sup>Numbers in parentheses in the box head are numbers of samples analyzed.

<sup>d</sup>Difference from 0.5% corn oil group significant at  $P<.01$  by Kruskal-Wallis analysis.

<sup>e</sup>Difference from 3% corn oil group significant at  $P<.01$  by Kruskal-Wallis analysis.

<sup>f</sup>Difference from 3% corn oil group significant at  $P<.05$  by Kruskal-Wallis analysis.

<sup>g</sup>Difference from 20% corn oil group significant at  $P<.01$  by Kruskal-Wallis analysis.

<sup>h</sup>Difference from 3% corn oil + 17% tallow group significant at  $P<.01$  by Kruskal-Wallis analysis.

<sup>i</sup>Difference from rat chow (ovary intact) group significant at  $P<.05$  by Kruskal-Wallis analysis.

<sup>j</sup>Difference from 0.5% corn oil group significant at  $P<.05$  by Kruskal-Wallis analysis.

<sup>k</sup>Difference from 20% corn oil group significant at  $P<.05$  by Kruskal-Wallis analysis.

<sup>l</sup>Difference from 3% corn oil + 17% tallow significant at  $P<.05$  by Kruskal-Wallis analysis.

mammary tumor microsome data indicated that when normal female rats were maintained on a 0.5% corn oil diet there was an inverse relationship between the duration of the tumor latent period and membrane prolactin-binding capacity but that once the corn oil content of the diet reached or exceeded 3% this relationship seemed to be lost.

#### Qualitative Fatty Acid Profiles

The mean qualitative profiles of the fatty acids extracted from the liver and mammary tumor microsomes are recorded in tables 4 and 5, respectively. In general, the microsomes from both organ sites tended to have similar patterns of fatty acid distribution, even though the absolute percentages of the specific fatty acids were not exactly the same. The only exceptions to this generalization were the disproportionately greater contributions of stearic acid (18:0) to the lipid profiles of the liver microsomes and of oleic acid (18:1) to the lipid profiles of the mammary tumor microsomes.

Upon comparative analysis, it was readily apparent that the fatty acid profiles of the lipids extracted from the microsomes of the 0.5% corn oil diet group were significantly different from those of the other dietary groups. Specifically, it was observed that the liver microsomes from both the groups on the highest fat diets (20% corn oil and 3% corn oil + 17% tallow) had greater percentages of their total lipid content distributed in the stearic acid (18:0), linoleic acid (18:2), arachidonic acid (20:4), docosapentaenoic acid (22:5, n-3), and docosahexanoic acid (22:6) fractions than did those of the 0.5%

corn oil group; conversely, the microsomes from these high-fat groups had significantly lesser percentages of their total lipid in the palmitic acid (16:0), palmitoleic acid (16:1), and oleic acid (18:1) fractions than did those of the 0.5% corn oil group. Likewise, the mammary tumor microsomes of these 2 high-fat groups also were noted to have a greater percentage of their lipid in the linoleic acid (18:2) and docosatetraenoic acid (22:4) fractions and a lesser percentage in the palmitic acid (16:0) and palmitoleic acid fractions than the microsomes of the high-fat groups. Finally, whereas the distribution pattern for the fatty acids extracted from the liver and tumor microsomes of the 3% corn oil group was largely intermediate between those of the highest and lowest fat groups, the lipid profiles of the intact and ovariectomized animals maintained on identical rat chow diets revealed relatively few differences. Only a slightly higher percentage of palmitic acid (16:0) and linoleic acid (18:2) combined with a slightly lower percentage of stearic acid (18:0) was found in the hepatic membranes of the ovariectomized group.

#### Indices of Membrane Lipid Desaturation

Inasmuch as the physical-chemical behavior of a membrane is thought to be greatly influenced by the degree of desaturation of its lipid components, we believed that it would be particularly helpful to examine this aspect of molecular structure in the microsomal membranes used in this study. Therefore, we calculated the molar ratio of oleic acid (18:1) to linoleic acid (18:2) and the mean number of double bonds per mole of fatty

TABLE 5.—*Fatty acid composition of lipids extracted from mammary tumor microsomes*

Fatty acid	Formula <sup>a</sup>	Mole percent of total lipid <sup>b,c</sup>				
		0.5% corn oil (4)	3% corn oil (5)	20% corn oil (8)	3% corn oil + 17% tallow (10)	Rat chow, ovary intact (6)
Palmitic	16:0	25.08±1.25	19.69±1.63 <sup>d</sup>	16.33±1.96 <sup>e</sup>	17.16±1.96 <sup>d</sup>	20.12±1.43 <sup>d</sup>
Palmitoleic	16:1	4.76±0.61	2.49±1.03	0.49±0.19 <sup>e,f</sup>	0.99±0.19 <sup>e</sup>	1.41±0.21 <sup>d,g</sup>
Stearic	18:0	8.91±0.91	10.43±1.48	11.15±1.35	13.14±0.65 <sup>d</sup>	11.15±0.75
Oleic	18:1	34.4±2.48	26.09±2.60 <sup>e</sup>	18.48±2.68 <sup>e,f</sup>	29.15±1.65 <sup>d,g</sup>	24.39±2.40 <sup>h</sup>
Linoleic	18:2	2.12±0.67	5.96±1.67 <sup>d</sup>	15.82±1.67 <sup>e</sup>	4.29±0.34 <sup>d,g</sup>	7.39±1.04 <sup>d,h,i</sup>
Arachidonic	20:4	12.25±2.16	16.47±2.51	17.37±1.28	17.82±1.46	15.47±1.32
Docosatetraenoic	22:4	3.49±0.35	5.99±1.07 <sup>d</sup>	7.45±1.28 <sup>d</sup>	5.68±0.62 <sup>d</sup>	7.32±1.01
Docosapentaenoic	22:5 (C-6)	4.47±0.83	5.19±1.32	3.58±0.57	3.42±0.43 <sup>e,g,j</sup>	0 <sup>e,g,i,j</sup>
Docosapentaenoic	22:5 (C-3)	0.15±0.15	0.21±0.13	1.19±0.22 <sup>d,f,k</sup>	0.51±0.08	1.53±0.19 <sup>e,i,j</sup>
Docosahexaenoic	22:6	0.93±0.63	1.16±0.19	1.49±0.21	1.83±0.20 <sup>f</sup>	3.08±0.25 <sup>d,g,i,j</sup>

<sup>a</sup> No. of C-atoms:No. of double bonds. Numbers in parentheses indicate the location of the first double bond from the methyl end of the fatty acid.

<sup>b</sup> Values are means ± SE.

<sup>c</sup> Numbers in parentheses in the boxhead are numbers of samples analyzed.

<sup>d</sup> Difference from 0.5% corn oil group significant at  $P<.05$  by Kruskal-Wallis analysis.

<sup>e</sup> Difference from 0.5% corn oil group significant at  $P<.01$  by Kruskal-Wallis analysis.

<sup>f</sup> Difference from 3% corn oil group significant at  $P<.05$  by Kruskal-Wallis analysis.

<sup>g</sup> Difference from 20% corn oil group significant at  $P<.01$  by Kruskal-Wallis analysis.

<sup>h</sup> Difference from 20% corn oil group significant at  $P<.05$  by Kruskal-Wallis analysis.

<sup>i</sup> Difference from 3% corn oil + 17% tallow group significant at  $P<.01$  by Kruskal-Wallis analysis.

<sup>j</sup> Difference from 3% corn oil group significant at  $P<.01$  by Kruskal-Wallis analysis.

<sup>k</sup> Difference from 3% corn oil + 17% tallow significant at  $P<.05$  by Kruskal-Wallis analysis.

acid for each microsome sample by using the qualitative data we have obtained previously by gas chromatography. The results of these calculations are presented in table 6.

These data showed that, although the absolute values for these indices of desaturation were not exactly the same for the lipids from the hepatic and tumor microsomes in the same treatment group, the comparative changes in these values between individual treatment

groups followed a similar pattern in both tissues. This pattern indicated that the degree of desaturation of the microsomal lipids was correlated positively with the amount of corn oil in the diet. The microsomes of the 0.5% corn oil group had the highest ratio of oleic acid (18:1) to linoleic acid (18:2) as well as the lowest mean number of double bonds per mole of membrane fatty acid. The values for this latter index of desaturation were seen to be 16-21% greater in the 3% corn oil plus 17%

TABLE 6.—*Indices of membrane lipid desaturation*

Diet group	Liver microsomes <sup>a</sup>			Mammary tumor microsomes <sup>a</sup>		
	No. <sup>b</sup>	Moles of oleic acid(C18:1) Mole of linoleic acid(C18:2)	No. of double bonds Mole of fatty acid	No. <sup>b</sup>	Moles of oleic acid(C18:1) Moles of linoleic acid(C18:2)	No. of double bonds Mole of fatty acid
Corn oil, 0.5%	8	9.44±0.59	1.48±0.06	4	21.28±5.49	1.35±0.06
Corn oil, 3%	6	2.18±0.23 <sup>c</sup>	1.71±0.09	5	4.90±0.62 <sup>d</sup>	1.64±0.15
Corn oil, 20%	9	.58±0.04 <sup>c,e</sup>	1.98±0.05 <sup>c,f</sup>	8	1.37±0.20 <sup>c</sup>	1.81±0.10 <sup>c</sup>
3% corn oil + 17% tallow	9	2.56±0.29 <sup>c,g</sup>	1.90±0.05 <sup>c</sup>	10	6.93±0.23 <sup>d</sup>	1.65±0.10 <sup>d</sup>
Rat chow, ovary intact	8	1.09±0.05 <sup>c,e,g,h</sup>	1.92±0.04 <sup>c</sup>	6	3.39±0.15 <sup>d</sup>	1.58±0.06 <sup>d</sup>
Rat chow, ovariec-tomized	8	1.00±0.03 <sup>c,e,g,h</sup>	1.82±0.04 <sup>c,i</sup>			

<sup>a</sup> Values are means ± SE.

<sup>b</sup> No. of samples analyzed.

<sup>c</sup> Difference from 0.5% corn oil group significant at  $P<.01$  by Kruskal-Wallis analysis.

<sup>d</sup> Difference from 0.5% corn oil group significant at  $P<.05$  by Kruskal-Wallis analysis.

<sup>e</sup> Difference from 3% corn oil group significant at  $P<.01$  by Kruskal-Wallis analysis.

<sup>f</sup> Difference from 3% corn oil group significant at  $P<.05$  by Kruskal-Wallis analysis.

<sup>g</sup> Difference from 20% corn oil group significant at  $P<.01$  by Kruskal-Wallis analysis.

<sup>h</sup> Difference from 3% corn oil + 17% tallow group significant at  $P<.01$  by Kruskal-Wallis analysis.

<sup>i</sup> Difference from 20% corn oil group significant at  $P<.05$  by Kruskal-Wallis analysis.

tallow group and 34–36% greater in the 20% corn oil group. Both of the treatment groups on normal rat chow had values 17–30% greater than those of the 0.5% corn oil group. Ovariectomy alone produced no significant change in the desaturation indices of the liver microsomes. Collectively, these results indicated that the microsomes from each of the dietary groups had distinctive indices of lipid desaturation and that the lipids extracted from the microsomes of the 0.5% corn oil group were significantly more saturated than those from any of the other groups.

## DISCUSSION

The ability of dietary fat to enhance mammary tumor growth in rodents has been documented by many investigators (2–7, 19). This relationship is particularly interesting because of its relative specificity for mammary tumor growth and its unique restriction to dietary lipids (3, 20–22). Whether all dietary lipids are equally capable of stimulating mammary tumor development has been a difficult question to answer. Early reports suggested that diets high in unsaturated lipid were more effective than those with comparable levels of saturated lipid (11, 23); but more recently Hopkins and Carroll (12) and Carroll and Davidson (19) have proposed that, once there is a minimum of 3% unsaturated fat in the diet, it is the sum total of both saturated and unsaturated fat that best correlates with the acceleration of tumor development.

Precisely how dietary fats enhance mammary tumor development is not well understood. The evidence suggests that its major effect is on the promotional stage of tumor development rather than on the initiation stage, but very little is known about the specific biochemical processes that are involved directly (8, 9). One hypothesis is that dietary lipids influence tumor development either directly or indirectly through cell membrane modifications (10, 19). It is well documented that dietary lipids can significantly alter the lipid composition of mammary tumor membranes, and it seems very probable that such modifications may greatly influence the ability of certain cell lines to respond to their physiologic environment.

Within this context the hormonal status of the host appears to play a critical role in facilitating lipid-dependent mammary tumor development (24). Some uncertainty remains as to the number of hormones that are importantly involved, but the reports that prolactin-inhibiting drugs, estrogen antagonists, and ovariectomy can greatly reduce or totally abolish the effect of dietary lipid on tumor development have indicated that prolactin and estrogen are of primary importance (7, 25). Exactly how these hormones facilitate lipid-dependent tumor development is unclear; however, the lack of any evidence that animals on high-fat diets have elevated prolactin or estrogen levels leads us to believe that the individual tumor cells have acquired an enhanced sensitivity to their normal hormone environment.

In earlier studies we examined the possibility that lipid modifications in the tumor membrane could affect specific prolactin-receptor binding. Our results clearly demonstrated that the lactogenic binding capacities of mammary tumor and liver microsomes of Buffalo rats maintained on a 20% corn oil diet were significantly greater than those obtained from rats on a 0.5% corn oil diet (10). However, because from these data we were unable to distinguish whether the observed differences represented an actual increase in the binding of the microsomes of the high-fat animals or a relative decrease in the binding capacities of the microsomes of the low-fat animals, we designed the present study to examine this question. F344 rats were chosen for this study because previous observations had indicated that their mammary tumor development might be even more sensitive to dietary lipid than that of the Buffalo strain (7).

The autopsy results confirmed this anticipated sensitivity to dietary lipid and, in fact, showed it to be so pronounced in the 0.5% corn oil and ovariectomized groups that we could collect only a limited amount of tumor tissue for biochemical analysis from these groups. However, despite this restriction we were able to make several important observations. First, we were able to establish clearly that by manipulating the lipid content of the diet we could either selectively increase or decrease the duration of tumor latent period relative to rats on normal rat chow. Second, we were able to show that the duration of tumor latency for rats on a 3% corn oil plus 17% tallow diet was essentially the same as for those on a 20% corn oil diet.

The prolactin-binding data reaffirmed the fact that the microsomes of the 0.5% corn oil group had significantly lower binding capacities than those of the higher fat groups and indicated that this difference was the result of an absolute decrease in the specific binding of the low-fat group. Surprisingly, however, despite obvious differences in their rates of tumor development, the microsomes of the high-fat groups demonstrated no absolute increase in the amount of prolactin binding relative to the regular rat chow group.

The chromatographic analyses demonstrated that the qualitative fatty acid profiles of the microsomes from each treatment group were distinctive. No obvious correlation was found between the rate of tumor development and the qualitative distribution of any single fatty acid, but in the nonovariectomized rats it was apparent that the microsomal lipids from the group with the least tumor development and lowest prolactin binding (0.5% corn oil group) had a significantly lower percentage of double bonds than those from any of the other groups.

We believe these individual results collectively provide some important insights into the general relationships between dietary lipids and mammary tumor growth. They firmly establish the concept that, once there is at least 3% of a polyunsaturated vegetable oil such as corn oil in the diet, it is the sum content of the saturated and unsaturated lipid present that correlates best with mammary tumor development. Additionally, these

results confirm that variations in dietary lipid do modify the qualitative fatty acid profiles of both normal and neoplastic membranes in the host and that some of these modifications are associated with alterations in the prolactin-binding capacities of these membranes. The observation that these reductions in prolactin binding are associated only with those membrane changes that occur when the polyunsaturated lipid content of the diet falls below 3%, however, suggests that there is a critical level of lipid desaturation that must be reached before the membrane becomes fluid enough to allow optimal prolactin binding (26). If this critical level of desaturation is not achieved, decreased hormone binding occurs together with delayed mammary tumor development. We believe this biochemical relationship may explain why Hopkins and Carroll (12) originally found a minimum dietary requirement of 3% polyunsaturated lipid before growth became directly dependent on the total amount of fat in the diet.

The fact that quantitative increases in dietary fat above the required 3% polyunsaturated lipid minimum continue to be associated with enhanced tumor development in the absence of any accompanying changes in prolactin binding capacity implies that there also must be other lipid-dependent mechanisms involved. In this regard several investigators have reported recently that tumor prostaglandin metabolism may be importantly influenced by dietary lipid (27, 28).

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