are in contrast to results of earlier studies on whole livers, which showed that under similar conditions, the percentage of stearic acid decreases. There were no changes in fatty acid percentage composition in RBC ghost cells after 5–9 weeks of exposure. These studies also indicate that there were striking differences in the fatty acid composition of membranes of various organelles in the cell.

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Mammary Gland Fatty Acids in Rats of Different Susceptibility to Mammary Carcinoma Induction* (32632)

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Shay *et al.* first demonstrated that oral (intragastric) administration of 3-methylcholanthrene to Wistar rats induced mammary carcinomas (1). Huggins and associates (2) later showed the exquisite and invariable sensitivity of the young female Sprague-Dawley rat to mammary carcinoma induction by optimum doses of either 3-methylcholanthrene or 7,12-dimethylbenz(*a*)anthracene; and Sydnor *et al.* (3) subsequently demonstrated the relative resistance of the female Long-Evans rat.

Since the bulk of rat mammary gland is adipose tissue and since the polycyclic hydrocarbon carcinogens are strongly lipophilic and hydrophobic, the lipid composition of mammary gland may be important in mammary carcinogenesis. The lipid composition of the induced carcinomas and of the mammary tissue of the Sprague-Dawley (Holtzman) rat has been published (4). Data on the mammary fat composition of some additional strains of rats are presented below.

Methods. All rats, except those of the Sprague-Dawley strain, used in these experiments were bred in this institution by brother–sister mating, and were maintained under identical environmental conditions. Osborne-Mendel, Fischer, and Marshall strains can be considered to be genetically homogeneous. The Long-Evans strain was derived from a single male and two females obtained from the Diablo farms. The Sprague-Dawley rats were purchased from the Holtzman Co., Madison, Wisconsin. The animals re-

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ceived a diet of standard Chow pellets (Purina Chow Mills, Davenport, Iowa) supplemented by lettuce twice and pork liver once each week. The animals were not fasted. Immediately after decapitation the abdominal and inguinal mammary glands were removed and the lipid was extracted by the method of Bligh and Dyer (5). The preparation of methyl esters and gas-liquid chromatography was carried out exactly as before (4) including use of (a) a 10 foot × ⅛ inch stainless steel column of 12% DEGS polyester on 60–70 mesh Anakrom A, (b) hydrogen flame ionization detector, and (c) a disk integrator for area measurements under each peak. Chromatographic peaks were identified by comparison of retention times with standards and from graphs relating log of retention time to carbon number and degree of unsaturation. The degree of unsaturation of C_{20} and C_{22} fatty acids was not determined. Quantitative results were checked with National Heart Institute reference mixtures B, C, and D and the results agreed with the stated composition with a relative error less than ± 2% for major components and less than ± 5% for minor components. Mammary tissue from two males and two females of each strain was analyzed and in each case at least three chromatograms were prepared. The data presented below (Table I) represent the mean values obtained. Phospholipid levels were estimated by digesting dried aliquots of the lipid extracts and analyzing for phosphorous (4): a conversion factor of 840 mg of phospholipid per mmole of lipid phosphorous was used.

Results and Discussion. With respect to the fatty acid compositions of the mammary glands (Table I), an analysis of variance was used to determine whether there was evidence of significant strain, sex, or sex-strain differences (Table II). No strain difference at the 5% level of significance was noted but there was a significant sex difference for the 16:1 and 18:2 fatty acids with male rats of the Sprague-Dawley, Long-Evans and Fischer strains having a somewhat greater proportion of 18:2 and a lower proportion of 16:1 fatty acids than did the females. A sex-strain interaction effect approached significance at the 5% level for the 18:2, 18:1, and 16:1 fatty acids. Intragastric instillation of a single dose of dimethylbenz(*a*)anthracene (100 mg/kg body weight) induces carcinomas in the mammary glands of 90–100% of female Sprague-Dawley and Osborne-Mendel rats but in only about 10% of Long-Evans, Fischer, and Marshall rats (Dr. Katherine Sydnor, personal communication). A further analysis testing explicitly for a difference between the highly susceptible strains (Sprague-Dawley and Osborne-Mendel) and the relatively resistant strains (Long-Evans, Fischer, and Marshall) was carried out and there was no indication of a significant difference in the proportions of the major groups of fatty acids.

A rat mammary gland can be considered to be a branching tube of epithelium (arising from a main excretory duct) sheathed by connective tissue and extending into a large pad of adipose tissue. Approximately 95% of the mammary and adipose lipid is triglyceride (4), thus mammary fatty acids are predominately of triglyceride origin. The phospholipid levels of mammary glands from rats of all five strains are quite low (Table I); the phospholipid levels increase with the proliferation of epithelium during pregnancy and lactation (4). Although the polycyclic hydrocarbon carcinogens (depending on route and manner of administration) can induce various types of neoplasms in various organs, it is remarkable that an oily solution of these carcinogens administered intragastrically produces almost exclusively mammary cancers in rats of susceptible strains. One would expect that the lipophilic polycyclic hydrocarbons would be concentrated in the fat depots and this has been demonstrated by spectrofluorometric (6) and by radioisotope methods (7). On theoretical grounds the composition of lipid and mammary fat could influence the carcinogen solubility in the tissue as well as the formation of reactive lipid peroxides from polyunsaturated fatty acids. Since the fatty acid compositions of mammary fat from all five strains are so similar, it seems unlikely that carcinogen solubility in mammary gland could account for strain differences in mammary cancer induction. The question of lipid peroxide formation is more difficult. High

TABLE I. Proportions of Fatty Acids in the Mammary Glands of Male and Female Rats of Different Strains.

Strain	Individual fatty acids (%)												mg PL/ 100 mg TL[c]
	10:0	12:0	14:0	14:1	16:0	16:1	[a]	18:0	18:1	18:2	20 uns[b]	22 uns[b]	
Male													
Holtzman	0.3	0.8	3.3	0.7	18.1	6.2	0.7	8.0	30.1	26.9	4.2	0.3	1.5
	0.4	0.8	3.2	0.7	18.9	5.6	0.6	7.6	31.0	27.6	3.3	0.7	1.0
Long-Evans	0.6	2.0	3.5	0.8	21.4	6.8	0.8	4.3	32.1	26.8	1.8		2.5
	0.2	0.2	2.4	0.4	19.4	4.2		8.3	34.2	30.4	0.4		
Fischer	0.2	0.2	2.2	0.3	21.6	4.8	0.3	6.5	34.6	27.1	1.7		1.7
	0.2	0.3	2.1	0.5	22.1	4.5	0.4	6.5	33.8	27.6	1.7		
Marshall		0.2	2.3	0.4	21.1	7.4	0.5	8.4	34.0	23.0	2.3		0.9
	0.3	0.5	3.2	0.8	20.4	6.2	0.6	7.5	31.3	26.6	2.9		0.9
Osborne-Mendel	1.1	2.7	5.0	1.5	18.5	6.0	0.8	5.5	31.5	25.7	1.8		1.5
	0.2	0.4	2.4	0.7	19.2	7.1	0.9	6.8	33.7	25.9	3.0		
Female													
Holtzman	0.3	0.8	3.2	0.4	20.9	6.7	0.5	6.1	33.5	25.4	2.4	0.1	1.0
	0.3	0.6	3.0	0.3	20.9	8.1	0.5	5.4	33.3	24.3	3.3		1.6
Long-Evans	0.4	1.1	3.2	0.9	22.7	8.7	0.5	5.3	32.3	22.5	2.4		0.8
	0.6	2.9	6.0	0.4	18.2	7.8	0.5	4.8	33.1	24.7	0.8		
Fischer		0.3	2.3	0.6	20.5	5.8	0.9	8.5	33.3	24.6	3.2		1.4
		0.2	2.8	0.7	19.9	7.2	0.6	6.7	33.7	25.3	2.3		
Marshall	0.2	0.4	3.3	0.8	19.5	5.9	0.9	7.5	31.5	25.6	3.7	0.9	1.1
	0.2	0.4	2.3	0.8	18.5	6.2	0.7	8.6	31.3	26.2	3.1	0.6	0.7
Osborne-Mendel	1.0	3.4	5.2	0.9	18.1	6.1	0.7	5.8	31.1	25.8	2.0		1.0
	0.5	1.5	3.6	0.8	18.9	7.2	0.9	6.0	32.2	25.3	3.4		

[a] Unidentified: retention time between those of 16:1 and 18:0.
[b] Degree of unsaturation of the C_{20} and C_{22} fatty acids was not determined.
[c] PL signifies phospholipid and TL total lipid.

levels of linoleic acid (18:2) were found in the breast triglyceride fractions of animals of all five strains and this is one of the fatty acids susceptible to peroxidation (8). However, more highly unsaturated fatty acids present in low concentrations might be even more important in this regard. This point has yet to be tested by direct analytical determinations of the products of lipid peroxidation in mammary gland following administration of polycyclic hydrocarbon carcinogens, but the data above do not suggest that mammary fatty acids are an important determinant in the oncogenic response of breast tissue to these substances.

TABLE II. Results of Analysis of Variance.

Source of variance	Degrees freedom	Mean squares for major fatty acid components				
		16:0	16:1	18:0	18:1	18:2
Strain	4	3.08	1.01	3.33	2.61	0.47
Sex	1	.34	5.94[a]	1.11	.05	16.03[b]
Strain-sex	4	2.83	1.96	1.49	2.81	5.44
Error	10	1.406	.79	1.19	1.00	1.69

[a] $p < .025$.
[b] $p < .01$ by F test.

Sex differences in the proportions of some fatty acids in the various lipid fractions of rat serum and tissues have been frequently observed (9). The sex difference observed above for the 18:2 and 16:1 fatty acids is another example of this sort.

Summary. Although young female rats of

the Sprague-Dawley and Osborne-Mendel strains are quite vulnerable to the induction of mammary cancer by intragastric instillation of 7,12-dimethylbenz(*a*)anthracene and rats of Fischer, Marshall, and Long-Evans strains are relatively resistant, the fatty acid composition of mammary glands from rats of these five strains were comparable. A sex difference was noted in three strains with males having a somewhat greater proportion of 18:2 (linoleic acid) and a lower proportion 16:1 (palmitoleic acid) than females. The phospholipid levels of the breasts tissue of both male and females in all five strains were quite low relative to the total lipid.

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Effect of Mithramycin on Kidney Transamidinase of C3H Mice*† (32633)

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Mithramycin is an antibotic derived from a culture of *Streptomyces tanashiensis* (1) In the treatment of neoplastic diseases, clinical improvement has been observed in embryonal cell carcinoma of the testis, glioblastoma multiforme, and hypernephroma (2,3). These antitumor effects were associated with severe clinical toxicity including hepatocellular and renal disturbances. Hepatic injury was characterized by a marked increase in serum enzymes. Renal injury was evidenced by proteinuria, blood cells in the urine, and uremia. Acute tubular cell damage in patients dying of drug toxicity and atrophy of tubules in long surviving patients have been observed. Initial treatment studies were associated with a high mortality (2).

In the investigation of the mechanism of action of mithramycin, it was found to inhibit the synthesis of RNA in a mouse ascites tumor with little or no immediate effect on that of DNA (4). A similar inhibitory phenomenon occurred in tissue cultures of HeLa cells treated with mithramycin.[1] In a study of the differential effect of this agent on various organs of the mouse, there was a profound inhibition of the capacity for RNA synthesis in the organs studied (5). Measurement of the distribution of tritium-labeled mithramy-

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