Lipid composition of rat mammary carcinomas, mammary glands, and related tissues: endocrine influences

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ABSTRACT The lipids of mammary glands and mammary carcinomas from rats in various hormonal states were studied and compared with each other, with adipose tissue, and with a new transplantable sarcoma derived from cultured mammary carcinoma cells. When large doses of estradiol-17β were administered to the host, cells of a few carcinomas became engorged with triglyceride containing an increased proportion of C_{10}–C_{14} fatty acids—a characteristic of milk fat. Cancers capable of fatty transformation must retain in latent form the enzyme system for fatty acid synthesis possessed by mammary epithelium; estradiol-17β apparently activates this system.

The lipid composition of retroperitoneal adipose tissue resembled that of the mammary tissue of virgin rats; this indicates similarity between retroperitoneal fat and the adipose component of mammary gland. Relative to the dry nonfat material present, the phospholipid content of adipose tissue was greater than that of the other tissues. Generally, differences in lipid composition between tissues were in amounts of triglyceride present and proportions of fatty acids in the triglyceride fraction. The ratios of cholesterol and cholesterol ester to phospholipid were similar in normal and neoplastic tissues. The amounts of free fatty acid, monoglyceride, and diglyceride were roughly proportional to the amount of triglyceride present.

KEY WORDS mammary carcinoma · fatty development · 3-methylcholanthrene · 7,12-dimethylbenz(a)anthracene · estradiol-17β · C_{10}–C_{14} fatty acids · triglycerides · deposition · enzymic synthesis · milk · rat · adipose tissue · phospholipid

RAT MAMMARY GLAND is composed of three tissue components: epithelial, adipose, and connective. The proportions of these tissues vary greatly with the hormonal state of the host (1, 2). Connective and adipose components predominate in the breasts of virgin rats whereas epithelium predominates in lactating rats. During lactation the epithelial cells produce milk fat.

Like mammary gland, the morphologically and biochemically well-differentiated mammary adenocarcinomas induced in rats by oral or intravenous administration of certain polycyclic aromatic hydrocarbons respond to changes in the hormonal state of the host. These carcinomas presumably originate from mammary epithelial cells. Changes in proportions of carcinoma cells and connective tissue with the hormone state of the host are, however, quite small compared to the changes observed in mammary gland (3). The cells of some carcinomas become enormously engorged with fat when large doses of estradiol-17β are administered (4). The alterations in lipid content of an endocrine target organ, mammary gland, and its related neoplasm are striking examples of hormone responsiveness.

Although changes in the total lipid content of mammary gland and mammary carcinoma have been well established, changes in lipid composition accompanying hormonal changes have not been well defined. In the present study the composition of lipid from mammary carcinomas and mammary glands of hosts in various hormonal states was determined.

METHODS

Source of Tissue

Female rats of the Holtzman strain were used. The animals were not fasted; tissues were obtained immediately after decapitation. The diet consisted of standard chow pellets (Purina Chow Mill, Davenport, Iowa) plus

Abbreviations: 3-MC, 3-methylcholanthrene; DMBA, 7,12-dimethylbenz(a)anthracene; TC sarcoma, designation of new sarcoma derived from tissue culture.

lettuce twice a week and pork liver once a week. The abdominal and inguinal mammary glands were used for breast tissue analyses. Adipose tissue was obtained from the retroperitoneal region. The analyses reported for mammary glands, fatty carcinomas, and adipose tissue were obtained from single animals. In the case of non-fatty neoplasms, 3–5 tumors (totaling 50–80 g) were obtained from several rats and pooled for analysis.

The mammary carcinomas were induced by oral administration (5, 6) to 50-day old rats of either 3-methylcholanthrene (3-MC), 10 mg on 5 days/week for 5 weeks or a single 20 mg dose of 7,12-dimethylbenz-(a)anthracene (DMBA). Fatty carcinomas were produced by starting injections of estradiol-17β, 50 μg daily, 3 weeks after ovariectomy of hosts bearing cancers; however, only a few carcinomas on the hosts accumulated lipid. After 3 weeks, some of the animals that had not developed a fatty carcinoma were given progesterone, 4 mg daily, along with estradiol-17β, and a few more fatty tumors developed. The estradiol-17β and progesterone were given intramuscularly in 0.2 ml of sesame oil–ethanol 9:1. Animals listed in Tables 3 and 4 as having received progesterone only were obtained from Dr. Katherine Sydnor; this group of animals was unique in that the animals received testosterone propionate daily from the age of 4 through 25 days and as a result were in "constant estrus" when DMBA was given at 50 days. Administration of progesterone, 4 mg daily, was also started at 50 days and was continued until the cancers were harvested about 2 months later. A new sarcoma (derived from cells from a mammary cancer that were grown in tissue culture for 15 months, then inoculated subcutaneously into a rat with subsequent growth of a sarcoma which has been repeatedly transplanted from one animal to another) was also studied. This sarcoma was designated TC sarcoma. Rat milk was obtained manually or by the method of McBurney, Meier, and Hoag (7) after injection of 2 units of oxytocin intraperitoneally into lactating rats anesthetized with diethyl ether.

Lipid Chromatography

Lipid was extracted from tissue by the method of Bligh and Dyer (8); an amount of tissue containing 0.5–1 g of total lipid was used and adjustments for the amount of tissue and its water content were made. Of the lipid, 100–250 mg was placed on a chromatographic column containing 30 g of Florisil 60–100 mesh (Fisher Scientific Company, St. Louis, Mo.). The lipids were eluted with the solvent system described by Carroll (9) and collected in 20-ml fractions for quantitative estimations. Water at 18°C from a temperature controlled bath was circulated through the jacketed column described by Hirsch and Ahrens (10). Reagent grade *n*-hexane (redistilled), chloroform (redistilled), anhydrous diethyl ether, methanol, and acetic acid were used to prepare the elution mixtures. Column fractions were evaporated in vacuo or under a stream of dry nitrogen in tared collection tubes for gravimetric determination to 0.1 mg. The SD (from empty weights) for weights of individual tubes containing no lipid after solvent evaporation was ± 0.2 mg. The mean recovery of neutral lipid from the Florisil columns was 100% (SD $\pm 6.7\%$). Thin-layer chromatography of samples from all collected fractions was performed to establish purity and confirm identity: the major lipid classes were separated on Silica Gel G (activated at 100°C) with *n*-hexane–diethyl ether–acetic acid 90:10:1. The identity of sterol and sterol esters in the eluted fractions was verified in several experiments by cholesterol analysis (11).

Phospholipids could not be satisfactorily eluted from Florisil columns (9), so phospholipids were separated on a small column of silicic acid (7.5 g): neutral lipids were eluted with 150 ml of chloroform and phospholipids with 75 ml of methanol–chloroform 9:1 (12). The mean recovery of 100–200 mg of total lipid placed on silicic acid columns was 98% (SD $\pm 2.3\%$). Retroperitoneal fat and mammary gland (virgin and pregnant) contained a very small proportion of phospholipid, which was determined by analysis of phosphorus (13). A conversion factor of 840 mg of phospholipid per mmole of lipid phosphorus was used.

Gas–Liquid Chromatography

Methyl esters of fatty acids were prepared from triglyceride fractions for gas–liquid chromatography by methanolysis (14). An Aerograph-600 (Wilkens Instrument and Research, Inc., Walnut Creek, Calif.) gas chromatograph with a hydrogen flame ionization detector was used; the area under each peak was determined with a disc integrator. Preliminary screening of samples was performed on a 5 ft $\times$ $^1/_8$ inch stainless steel column containing 5% silicone grease DC-11 (Wilkens Instrument and Research, Inc., Walnut Creek, Calif.) on 60–80 mesh Chromosorb W. For separation and quantitative determination of constituents (including the unsaturated fatty acids) a 10 ft $\times$ $^1/_8$ inch stainless steel column of 12% diethylene glycol succinate polyester on 60–70 mesh Anakrom A (Analytical Engineering Laboratories, Inc., Hamden, Conn.) was used. Samples were eluted from the columns for 75 min or longer. Quantitative results with National Heart Institute reference mixtures B, C, and D agreed with the stated compositions with a relative error less than $\pm 2\%$ for major components ($> 10\%$ of total mixture) and less than $\pm 5\%$ for minor components ($< 10\%$ of total mixture). Unsaturated fatty acids were hydrogenated with sodium borohydride (15) and chromatograms of samples before and after hydrogenation were compared.

Several (two to five) chromatograms were obtained for each experimental sample and the means for the proportions (in per cent) of each fatty acid are listed in the tables; standard deviations from the means were less than ±1%. Chromatographic peaks were identified as follows: *a*) by comparison of retention times with those of standards, *b*) from graphs (constructed from the individual sample data obtained on the polar polyester column) relating log of retention time to carbon number and degree of unsaturation, and *c*) separation of the sample on the nonpolar silicone column. The degree of unsaturation of C_{20} and C_{22} fatty acids was not determined.

Total Tissue Lipid Determination

Specimens of some tissues were weighed and dried at 100°C for 18 hr, and the lipid was extracted from the dry tissue by refluxing with carbon tetrachloride in a Nolan fat extractor (Macalaster Bicknell Co., New Haven, Conn.) overnight. The data were used to ascertain the proportions of water, lipid, and residual material. Specimens from all tissue used in this study were prepared for microscopic examination.

RESULTS

Total Lipid Content of Tissues

There was a wide range in the lipid content of the tissues examined (Table 1). The lipid content of breast tissue from pregnant rats covered a range of values between those found for virgin and for lactating rats. For most tissues about 7 mg of dry nonfat material yielded 1.0 mg of tissue nitrogen.

TABLE 1 MEAN COMPOSITION OF TISSUES FROM FEMALE HOLTZMAN RATS

	Water	Lipid
	mg/mg dry nonfat component	
Retroperitoneal fat (20)	6.24 ± 0.20	46.3 ± 1.7
Mammary gland, virgin rats (10)	6.24 ± 0.25	6.9 ± 0.7
" " pregnant (17)	5.34 ± 0.15	*
" " lactating (16)	4.54 ± 0.26	0.74 ± 0.09
Mammary carcinoma (9)	5.55 ± 0.20	0.12 ± 0.03
TC Sarcoma† (9)	9.30 ± 0.26	0.045 ± 0.008

Values are given as means ± SEM. The numbers in parentheses indicate the number of animals used and the number of individual analyses from which the means were obtained.

Wet wt = 1.0 mg (dry nonfat material) + mg water + mg lipid. Generally, 6.9–7.3 mg of dry nonfat tissue = 1.0 mg total tissue nitrogen. The entire wet breast tissue of virgin rats weighs 3–4 g; of lactating rats, 16–19 g.

* Lipid contents covered a range of values intermediate between those found for virgin and for lactating rats.

† Designation of a new transplantable sarcoma derived from cultured cells obtained from a mammary carcinoma.

Lipid Fractions of Normal Tissues and Neoplasms

The major lipid fractions of both normal tissues (Table 2) and neoplasms (Table 3) were well separated on Florisil columns. The lipid composition of mammary gland from virgin rats closely corresponded to that of retroperitoneal adipose tissue. During pregnancy, and especially at the onset of lactation, the lipid content of the mammary gland decreased in relation to the dry nonfat material and the decrease was predominantly in the triglyceride fraction. The fatty acid composition (Table 4) of the triglyceride fraction of virgin rat mammary gland and retroperitoneal fat also corresponded.

The lipid composition of mammary carcinomas (Table 3) differed substantially from that of mammary gland. However, this difference was due primarily to the large differences in triglyceride content. When the cholesterol esters, cholesterol, diglyceride, monoglyceride, and free fatty acid fractions were expressed in terms of the phospholipid content, the differences between mammary cancer and normal tissue (adipose and mammary gland) were small: mean values (± SEM) for cholesterol esters (mg/mg phospholipid) were 0.15 ± 0.05 and 0.2 ± 0.1, respectively, for mammary cancers and normal tissues, cholesterol 0.27 ± 0.03 and 0.5 ± 0.2. The amounts of diglyceride, monoglyceride, and free fatty acid fractions were roughly proportional to the amount of triglyceride present in both the neoplastic and normal tissues.

Triglyceride Fatty Acids

The fatty acid compositions of the triglyceride fraction of mammary glands from virgin and pregnant rats were quite similar to that of retroperitoneal fat (Table 4). In lactating mammary glands the C_{10}–C_{14} fatty acids were moderately increased; rat milk had even larger proportions of these fatty acids. From the proportions of 10:0, 12:0, and 14:0 fatty acids in milk and in mammary glands from virgin and pregnant rats, it was estimated that about 15% of lipid found in the lactating mammary glands was from retained milk.

There was no evidence for the presence of C_{24} fatty acids (in amounts of 0.5% or more) in mammary glands, TC sarcoma, fatty mammary carcinomas, and several of the mammary cancers. However, the presence of C_{24} fatty acids in samples from necrotic TC sarcoma and from several mammary carcinomas could not be excluded since some samples (hydrogenated and nonhydrogenated) were not eluted from the silicone column for a sufficiently long time.

The most striking observations noted in the triglyceride fatty acids of cancers (Table 5) were: *a*) a more marked variation in proportion of C_{20} and C_{22} fatty acids of triglycerides from mammary carcinomas than from mammary glands, *b*) a high proportion of C_{22} fatty acids in

TABLE 2 LIPID COMPOSITION OF RAT MAMMARY GLANDS, ADIPOSE TISSUE, AND MILK

Host	Hydrocarbon	Sterol Ester	Triglyc.	Sterol	Diglyc.	Monoglyc.	Free Fatty Acids	Phospholipids
			% of total lipids					
Retroperitoneal fat — Virgin		0.3	95.9	1.0	0.6		1.7	0.5
" " "			97.6				1.8	0.6
Mammary gland "		0.7	95.3	1.0	1.7		0.5	1.0
" " "	0.6		93.8	0.8	1.0	0.3	2.0	1.5
" " Pregnant 20 days	0.5	1.0	90.9	1.3	1.2	0.6	1.0	3.5
" " " 19 "			93.2		0.6		2.2	4.0
" " Lactating 7 "			86.7	1.0	0.5		2.5	9.3
" " " 7 "		0.7	88.6	0.9	1.4		1.6	6.6
Rat milk " 10 "	1.3	1.5	83.3	2.5	3.5	1.9	6.2	<0.5

TABLE 3 LIPID COMPOSITION OF MAMMARY CARCINOMAS AND OF A NEW SARCOMA

Host	Total Lipid	Hydrocarbon	Sterol Ester	Triglyc.	Sterol	Diglyc.	Monoglyc.	Free Fatty Acids	Phospholipids
	% dry wt				*% of total lipid*				
Mammary carcinoma — Virgin	8	0.9	4.4	25.0	11.6	0.2		1.3	56.1
" " "	8		2.4	14.2	11.5	1.4	2.7	0.1	67.8
" " "	11	1.1	5.6	22.2	13.8	1.5	2.2	5.7	47.1
" " "	10	2.0	6.1	14.9	13.3	2.0	0.6	9.2	52.0
" " (necrotic) Virgin	12		6.6	26.4	13.1	0.5	2.2	0.8	50.6
" " (nonfatty) Ovarex* + 50 μg estradiol	4	0.3	4.1	22.3	19.5	0.9		2.1	50.9
" " " " " " "	12		8.1	18.0	19.3	0.9		6.4	47.2
" " (fatty) " " " "	56		3.1	76.0	2.8	2.5	1.8	6.8	6.9
" " " " " " "	41		1.9	79.1	2.3	1.4		3.2	12.1
" " " Ovarex + 50 μg estradiol + 4 mg progesterone	46		0.7	76.6	2.4	1.1		2.3	16.9
" " " " "	12	1.6	3.9	25.0	14.0	1.2	0.4	4.2	49.8
" " 4 mg progesterone	13	3.5	2.3	47.9	5.2	2.5	1.0	4.3	33.4
" " " "	12	0.7	2.2	32.0	10.4	1.4	1.0	18.3	34.0
TC sarcoma — Virgin	9	0.9	1.9	4.9	10.0	0.9	0.6	1.6	79.2
" " (necrotic) "	8	1.3	2.7	10.5	8.2	0.4	0.7	7.3	69.0

* Ovarex = ovariectomized.

TABLE 4 FATTY ACID COMPOSITION OF TRIGLYCERIDE FRACTION OF MAMMARY GLANDS, ADIPOSE TISSUE, AND MILK

Host	8:0	10:0	12:0	14:0	14:1	16:0	16:1	18:0	18:1	18:2	20un	22un
					% of total triglyceride fatty acid							
Retroperitoneal — Virgin				1.4		23.0	5.9	5.0	39.7	25.4	0.5	
" "			0.3	2.5	0.7	18.8	5.5	6.9	34.8	26.4	3.3	0.9
Mammary gland "				1.5		23.0	5.3	5.5	40.0	25.2		
" " "		0.2	0.8	3.0	0.9	18.1	6.4	6.8	32.2	24.5	4.0	2.1
" " Pregnant 19 days		0.4	0.4	2.0	0.3	25.1	6.8	4.0	38.9	21.5	0.5	
" " " 20 "	0.7	0.9	0.7	3.6	2.0	24.1	6.4	5.9	34.0	19.0	2.7	
" " Lactating 7 "		1.5	3.1	4.6		25.3		4.9	36.9	23.1		
" " " 7 "		1.6	1.4	2.5	0.4	25.5	4.6	6.2	36.8	17.0	1.6	2.4
Rat milk " 13 "		6.1	7.6	10.9	0.5	22.5	3.8	6.6	21.0	18.1	1.8	0.8
" " " 10 "		3.1	7.5	8.5	0.6	22.9	4.8	6.2	25.6	17.7	1.6	1.1
" " " 14 "		7.1	6.4	5.5	0.4	19.9	3.8	6.4	28.2	19.3	1.8	1.1
" " " 11 "		5.4	5.1	6.1	0.4	21.6	4.4	5.8	30.7	17.4	2.5	
" " " 13 "	0.2	5.0	7.7	10.5	0.5	25.5	2.2	5.6	26.0	16.5		
Chow pellets		0.7	0.2	2.4	0.2	22.4	3.7	9.8	32.5	25.8	2.3	

the two samples from necrotic neoplasms (mammary carcinomas and TC sarcoma), *c*) a marked increase of C_8, C_{10}, C_{12}, and C_{14} and decrease of 18:1 and 18:2 fatty acids in the triglyceride from fatty mammary carcinomas as compared to nonfatty carcinomas, and *d*) a fairly constant proportion of 16:0, 16:1, and 18:0 fatty acids in all of the neoplasms. Carcinomas from hosts receiving only progesterone were unusual in that the total lipid content (12–13%) was within normal limits but the composition deviated from normal, with an increased

TABLE 5 FATTY ACID COMPOSITION OF TRIGLYCERIDES OF CARCINOMAS AND SARCOMAS

Tissue		8:0	10:0	*	12:0	†	14:0	14:1	16:0	16:1	18:0	18:1	18:2	18:3	20un	22un
							% of total triglyceride fatty acid									
Mammary carcinoma	a		0.6		5.9	0.3	9.6	0.4	24.1	3.8	10.9	27.4	9.9		1.3	6.4
" "	b		0.2		0.5	0.8	4.0	1.0	24.2	4.6	7.1	30.7	20.4			5.5
" "	b		0.2		0.4		1.5		23.1	4.7	7.0	39.3	22.7		0.3	0.6
" "	c		2.6	0.3	4.3	0.3	7.5	0.4	24.9	4.2	7.5	30.0	11.2		0.8	6.2
" "	d		2.5		2.5		6.0	0.5	19.8	5.0	7.5	30.4	19.4		2.3	3.8
" "	d		1.0		1.1		5.0	0.8	21.8	5.8	8.7	31.5	20.0		1.5	2.9
" "	e		0.5		1.2		3.6	0.5	24.5	5.9	6.7	34.9	19.1		1.9	1.0
" "	f		0.5		1.0		3.7	0.6	21.7	4.5	11.5	29.7	14.0		2.0	11.4
" " (necrotic)	b	3.0	5.7		6.7	0.6	5.6	0.7	21.1	4.3	7.9	22.2	9.7	1.0	1.6	8.5
" " (fatty)	g	2.8	7.1	1.1	16.6	1.1	18.6	1.2	25.9	3.6	5.0	11.6	4.2		0.8	
" " "	g	0.4	12.2	0.7	24.2	1.4	22.4	1.4	23.4	2.8	2.4	7.5			0.8	0.5
" " "	h		7.7	1.0	11.4	0.8	11.3	0.7	21.1	4.6	3.8	19.2	9.3	1.0	1.6	6.2
" " (less fatty)	h		4.3	0.9	9.2	1.1	9.9	1.1	21.7	4.5	7.3	24.4	7.4		0.9	6.8
TC sarcoma					1.2		3.8	0.5	22.3	3.3	8.9	37.2	22.3		0.9	
" " (necrotic)			0.3		1.2		6.3	0.3	21.9	3.5	12.5	26.3	17.6		0.7	9.5

a, cancers induced with 3-MC. *b*, some cancers induced with 3-MC, others DMBA. *c*, cancers induced with DMBA. *d*, cancers induced with DMBA, animals received progesterone 4 mg daily. *e*, nonfatty 3-MC cancers from ovariectomized hosts receiving estradiol-17β, 50 μg daily. *f*, nonfatty DMBA cancers from ovariectomized hosts receiving estradiol-17β, 50 μg daily. *g*, fatty 3-MC cancer from ovariectomized host receiving estradiol-17β, 50 μg daily. *h*, cancers from ovariectomized hosts receiving daily 50 μg of estradiol-17β and 4 mg of progesterone, for 3 weeks.

* Unidentified: retention time between those of 10:0 and 12:0.

† Unidentified: retention time between those of 12:0 and 14:0.

proportion of triglycerides and decreased phospholipid, cholesterol, and cholesterol esters. Differences in fatty acid composition between cancers could not be related to 3-MC or DMBA induction.

Phospholipid Content

Table 6 indicates the amount of phospholipid in various tissues relative to the amount of dry nonfat material. Adipose tissue has a high ratio of phospholipid; and since about 10% of the weight of dry nonfat constituents in breast tissue from virgin rats is derived from the adipose component (1), the increased phospholipid in mammary glands from virgin rats must simply reflect the relative abundance of phospholipid in adipose tissue.

DISCUSSION

The administration of large doses of estradiol-17β (50 μg daily) has a pronounced effect on the lipid composition of some of the induced experimental mammary carcinomas (4). In these experiments, not only did the total lipids increase because of the deposition of triglyceride but the fatty acid composition was markedly altered, with increased proportions of C_{10}–C_{14} fatty acids and decreased linoleic acid. However, most carcinomas did not become fatty and they had a triglyceride fatty acid composition similar to that of cancers from hormonally unmodified hosts. Some hosts given estradiol-17β did not develop fatty carcinomas until progesterone (4 mg/day) was administered; increased proportions of C_{10}–C_{14} fatty acids were also found in neoplasms from these animals. The ability of some carcinomas to synthesize large amounts of triglycerides containing increased proportions of C_{10}–C_{14} fatty acids may be a vestige of the origin of the cancers from mammary epithelium. The presence of these fatty acids implies the presence of enzyme systems which are latent in these tumors on normal hosts but which can be brought to great activity by hormonal modification of the host. These neoplasms are of interest for the study of enzyme systems that synthesize fatty acids, since unlike mammary glands the carcinomas have no adipose tissue to complicate the study. Since there is no organized system of excretory ducts in the cancers, the lipid accumulates in situ.

It is important to keep the structure of mammary gland in mind in the analysis of the structural and metabolic changes occurring in mammary glands during preg-

TABLE 6 PHOSPHOLIPID LEVELS IN NORMAL AND NEOPLASTIC TISSUES

	Host	Phospholipid
		mg/100 mg dry nonfat residue
Retroperitoneal fat	Virgin	23
" "	"	32
Mammary gland	"	12 ± 2 (n = 4)
" "	Lactating 6 days	5
" "	" 10 "	8
" "	" 7 "	5
Liver	"	5
Mammary cancer	Virgin	6 ± 0.5 (n = 5)
" " (necrotic)	"	7
TC sarcoma	"	8
" " (necrotic)	"	6

nancy, lactation, and involution. For example, adipose tissue contributes approximately 10%, connective tissue about 90%, and epithelium less than 5% of the dry nonfat substance in mammary glands of virgin rats (1). In contrast, in lactating rats the adipose tissue contributes 1–4%, connective tissue 22–31%, and epithelium 77–65% (1). However, even in lactating mammary gland, the lipid associated with the adipose component (in 1 mg of dry nonfat material from the mammary gland of a lactating rat) amounts to 0.5–2 mg and thus accounts for most of the lipid present. This predominance of adipose tissue lipid occurs even though the great bulk of the dry nonfat material present in lactating breast is from the epithelium and very little is from the adipose tissue. On the other hand, if fatty acid synthesis (or some other metabolic characteristic) is studied and if the rates of fatty acid synthesis per milligram of dry nonfat material are similar in the epithelial and adipose components, then the synthesis of fatty acids by epithelium would be quantitatively more important (16–77 times) than synthesis by the adipose component of the lactating mammary gland. In the virgin rat mammary gland the adipose tissue would be more important (by twofold or more).

The fatty acid composition of the white fat of male albino rats (strain not specified) has been described by Chalvardjian (16) and the results correspond closely to those obtained in the present study on female Holtzman rats, though our 16:0 and 18:0 values were slightly higher. In human adipose tissue, Heffernan (17) recently described a slight sex difference in fatty acid compositions; males had a somewhat greater proportion of 14:0, 16:0, and 18:0 fatty acids and a lower 18:1 component. The fatty acid composition of human adipose tissue obtained from various sites is similar to that of breast tissue, except that the latter has a somewhat lower proportion of 18:1 (18). The fatty acid compositions of human and rat adipose tissue differ primarily in their proportion of linoleic acid. In rats, 20–25% of the fatty acids present in adipose lipid is linoleic acid, whereas human adipose tissue contains only 5–10% (17, 18). Mammary and axillary fat from normal patients and from patients with breast neoplasms have similar fatty acid composition (19). Comparison of analyses of nonlactating human breast tissue with analyses of human milk (20) indicates a higher proportion of C_{10}, C_{12}, and C_{14} fatty acids in the milk. Similarly, in our study, rat milk had a higher percentage of C_{10}, C_{12}, and C_{14} fatty acids than did mammary tissue.

In 1956 Haven and Bloor presented an extensive review of lipids in cancer (21). Methods of lipid analysis have greatly improved since then, and this has stimulated a large amount of work on the lipid compositions of normal tissues. Among the relatively few studies performed with neoplastic tissue is one by Sueyoshi and Nagao (22), who found decreased linoleic acid in a rat sarcoma. In our study the linoleic acid contents of induced mammary cancers and the TC sarcoma were similar to that of mammary gland. Veerkamp, Mulder, and van Deenen (23) noted that the phospholipid content was lower in a rat hepatoma than in rat liver and that the stearic–oleic proportion in liver was also reversed in the hepatoma. The fatty acid analysis of mouse glial tumors has been reported by Stein, Opalka, and Rosenblum (24). Geyer, Bennett, and Rohr (25) studied the lipid composition of HeLa cells grown in tissue culture. However, at present, it does not seem possible to make a statement of general validity about the lipid compositions of cancers; but the different responses of experimental mammary carcinomas to hormonal modification of the host are of interest with regard to the nature and pathogenesis of this neoplasm. The structure and metabolism of mammary gland vary with the hormonal state of the host, and short-chain fatty acids are characteristic of the milk in lactating mammary glands. Some of the mammary tumors induced by polycyclic hydrocarbon have these same characteristics and this may be considered an indication of *a*) the highly differentiated nature of the induced mammary carcinomas and *b*) the origination of the neoplasm in mammary epithelium. The morphology and metabolism (4) of mammary carcinomas from different hosts as well as from the same host are quite similar, provided the hosts have not been hormonally modified. A single host may bear several cancers, of which some respond to hormonal modification while others do not. Two types of hormone responsiveness have been demonstrated thus far (4). In one type the tiers of large, plump neoplastic cells surrounding the acini are transformed (following ovariectomy or hypophysectomy) into a rim of thin, flattened cells and there is a loss of neoplastic cells from the connective tissue substratum between acini. The other type of hormone responsiveness is manifested by massive intracellular accumulation of lipid in some carcinomas present on ovariectomized hosts receiving large doses of estradiol-17β. This indicates that although these carcinomas on normal hosts are morphologically similar they differ in subtle but demonstrable ways. The differences may be analogous to those found in the aberrant plasma cells of multiple myeloma, which can produce abnormal serum and urinary proteins uniquely characteristic (in one subtle respect or another) of an individual patient.

The high content of phospholipid in adipose tissue (relative to the amount of dry nonfat substance) noted in the present study is of interest, since phospholipid is an important component in all membranous structures of cells. Within fat cells there are many small lipid-containing bodies surrounded by a membrane (26). The addi-

tion of these membranes to the usual membranous structures in cells may account for the greater amount of phospholipid (relative to dry nonfat material) in adipose tissue.

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