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Alkyl and Alk-1-enyl Ethers of Glycerol in Lipids from Normal and Neoplastic Human Tissues[1]

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SUMMARY

The alkyl and alk-1-enyl glyceryl ether content of the neutral glyceride and phosphoglyceride fractions of 17 different human tumors and 19 normal human tissues was quantitatively determined. Neoplastic tissues generally contained much higher quantities of ether-linked neutral glycerides (primarily the alkyl type) than most normal tissues. Alkyl ethers in the phosphoglyceride fraction were also higher in most neoplasms, although the difference from normal tissues was not so pronounced as that observed for the glyceryl ethers present in the neutral glyceride fraction. The data obtained in this investigation of human tissues agree with previous observations from animal experiments, i.e., high levels of glyceryl ethers are a characteristic biochemical feature of neoplasia.

The data have also shown the relative proportions of alkyl and alk-1-enyl ethers of glycerol in a variety of healthy human tissues. The highest quantities of glyceryl ethers were found in the neutral glyceride fraction of heart and kidney and in the phosphoglyceride fraction of lung, brain, spleen, larynx, heart, colon, and testes.

INTRODUCTION

Tumor cells are rich sources of glyceryl ether diesters (GEDE). Our laboratory first described their occurrence in 4 rat and mouse tumors and in a 66-year-old man with lymphosarcoma (14). Complete chemical characterization (20) of GEDE and the widespread occurrence of GEDE in 13 transplantable rat and mouse tumors (17) have subsequently been reported. Experiments with Ehrlich ascites cells grown in tissue culture (1) and other experiments with ^{14}C-labeled precursors (17) have clearly shown that the ether bond can be synthesized in tumor cells. Other studies have also indicated that tumor cells do not contain enzymes (15) like those found in liver (7, 19) which cleave the ether linkage.

We have now made a quantitative study of the alkyl and alk-1-enyl ethers of glycerol in the neutral glyceride and phosphoglyceride fractions isolated from a variety of human neoplasms available from the National Institutes of Health Tissue Procurement Program. Nonmalignant human tissues from 2 apparently healthy persons have also been examined for their glyceryl ether content. The data obtained in this investigation reflect the conclusion previously reached in the animal experiments: most tumors can be chemically distinguished from healthy tissues by the quantity of glyceryl ether diesters in their total lipid extracts.

MATERIALS AND METHODS

All tumors used in this investigation were obtained from Roswell Park Memorial Institute in collaboration with the National Institutes of Health Tissue Procurement Program through the help of Dr. Robert H. DePue, Head of the Tissue Resources Unit, Viral Carcinogenesis Branch, National Cancer Institute. We are also extremely grateful to Dr. Donald Brown from the University of Colorado Medical Center for furnishing tissue samples from persons without neoplasms or long-standing symptoms of any other disease. These patients were a 14-year-old boy who died suddenly of bilateral lobar hemorrhagic pneumonitis and a 17-year-old boy who died instantaneously of cyanide poisoning. All tissues were maintained at dry-ice temperature until the lipids were extracted. The diagnoses listed for each tumor analyzed (Table 1) were obtained from a computerized patient-information sheet provided by Roswell Park Memorial Institute, Buffalo, New York.

Each sample was lyophilized and then extracted of total lipids by the procedure of Folch *et al.* (2). Aliquots of the lipid extracts were used to gravimetrically determine the total lipids on a Cahn electrobalance (Model M-10) and for thin-layer chromatography (TLC). The GEDE in the total lipid extracts were visualized by H_2SO_4 charring at 180–200°C after resolution of the major neutral lipid classes on thin layers of Silica Gel G in an equilibrated solvent system of benzene: hexane:diethyl ether:glacial acetic acid (45:50:5:1, v/v/v/v).

Subsequently, an aliquot of the total lipid extract was separated into neutral glyceride and phosphoglyceride fractions on silicic acid. Chloroform was used to elute the neutral glycerides from the silicic acid, and the adsorbed phospholipids were eluted with methanol. Duplicate samples of each lipid fraction were treated with $LiAlH_4$ for quantitative photodensitometric TLC analyses of the alkyl and alk-1-enyl ethers of glycerols (21), which were resolved on Silica Gel G in an equilibrated solvent system of diethyl ether:water (100:0.5, v/v).

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Table 1

Identification number[a]	Sample	Area, organ, or tissue	Total lipids (% dry wt.)	Lipid fraction	Lipid in fraction (%)	Glyceryl alk-1-enyl ether[b] (%)	Glyceryl alkyl ether[b] (%)	Alkyl/ alk-1-enyl ratio
1.	Metastatic adenocarcinoma	Liver	12.0	N	82	0.9	2.2	2.4
				P	18	2.8	2.3	0.8
2.	Neurofibrosarcoma	Retroperitoneal tissue	6.7	N	77	0.7	1.0	1.6
				P	23	25.7	11.9	0.5
3.	Squamous cell carcinoma	Larynx	5.2	N	39	1.5	1.5	1.0
				P	61	6.6	3.2	0.5
4.	Adenocarcinoma	Sigmoid colon	9.7	N	69	0.6	1.4	2.4
				P	31	7.7	4.5	0.6
5.	Adenoma	Rectum	10.9	N	36	1.1	2.6	2.4
				P	64	18.5	7.9	0.4
6.	Metastatic undifferentiated carcinoma	Liver	2.3	N	48	0.6	1.5	2.4
				P	52	5.3	4.3	0.8
7.	Metastatic embryonal carcinoma	Omentum	6.2	N	38	4.5	5.0	1.0
				P	62	3.8	1.2	0.3
8.	Metastatic liposarcoma	Peritoneal	13.3	N	75	0.4	2.8	7.7
				P	25	3.5	1.3	0.4
9.	Metastatic undifferentiated carcinoma	Omentum	22.2	N	91	0.5	0.9	1.7
				P	9	9.4	2.1	0.2
10.	Metastatic undifferentiated adenocarcinoma	Ovary	8.4	N	30	2.2	3.4	1.3
				P	70	10.0	5.7	0.6
11.	Metastatic poorly differentiated transitional cell carcinoma	Abdomen	6.5	N	31	2.8	5.1	1.9
				P	69	3.5	2.6	0.7
12.	Chronic myelocytic leukemia	Lymph	12.0	N	71	0.2	1.0	4.2
				P	29	4.4	1.4	0.3
13.	Adenocarcinoma	Breast	18.5	N	92	0.7	1.0	1.5
				P	8	4.8	2.8	0.8
14.	Astrocytoma	Brain	7.9	N	34	1.7	2.4	1.4
				P	66	7.1	3.2	0.4
15.	Metastatic squamous cell carcinoma	Lymph node	18.6	N	89	0.5	1.0	1.8
				P	11	12.8	5.5	0.4
16.	Squamous cell carcinoma	Lung	11.1	N	67	1.1	1.7	1.5
				P	33	7.8	2.6	0.3
17.	Undifferentiated carcinoma	Prostate	19.6	N	78	2.1	3.9	1.8
				P	22	7.0	2.7	0.4

Neoplastic human tissues: identification, lipid content, and glyceryl ether levels of neutral glyceride and phosphoglyceride fractions. N refers to neutral glyceride fraction; P refers to phosphoglyceride fraction.

[a] Identification numbers correspond to sample numbers used in figures.

[b] All values are expressed as the diacyl or phosphoglyceride form.

RESULTS

Our study investigated the occurrence of alkyl and alk-1-enyl glyceryl ethers in the total glyceride and total phosphoglyceride fractions of 17 different tumors (Table 1, Chart 1) and 19 different samples of normal human tissues (Table 2, Chart 2). The procedures used did not permit us to assign the contribution of individual lipid classes (e.g., triglyceride type *vs* diglyceride type) to the total ether content of the two main lipid fractions analyzed, but the contribution of all ether-linked lipids was quantitated by the $LiAlH_4$ reduction procedure (21). However, the thin-layer chromatograms in Figs. 1*A* and 1*B* show that the GEDE of human tumors are significant lipid markers for most neoplasms.

The percentage of total lipids in tumors and normal tissue is listed in Tables 1 and 2. The normal human tissues contained 10–95% of the total lipids in the glyceride fraction and 5–90% in the phosphoglyceride fraction. The relative proportion of neutral lipid (30–92%) and phospholipid (8–70%) varied with tumor type; approximately half the tumors contained greater than 50% neutral lipids. As seen previously with transplantable rat and mouse tumors, no consistent pattern was observed for the relative quantities of the glyceride and phosphoglyceride fractions among organs or tumors.

The total glyceryl ether content (alkyl plus alk-1-enyl) of the neutral glyceride fraction was higher in neoplastic cells than in most healthy cells from man with the exception of heart and kidney (Chart 1). The heart and kidney were the only normal human tissues to contain greater than 1.2% of the neutral glyceride fraction as glyceryl ethers (Chart 1), whereas all neoplastic tissues in this study contained greater than 1.2% of this glyceride fraction as ether-linked lipids. The level of ether-linked phosphoglycerides was also generally higher in neoplasms than in many normal tissues, e.g., liver and adipose tissue, but the differences were not as striking as in the neutral lipid fraction.

It is also obvious from the present study (Chart 1) that a tremendous variation in the concentration of ether-linked lipids occurred among neoplasms, although even in the borderline situations (about the 1% level) the concentration of

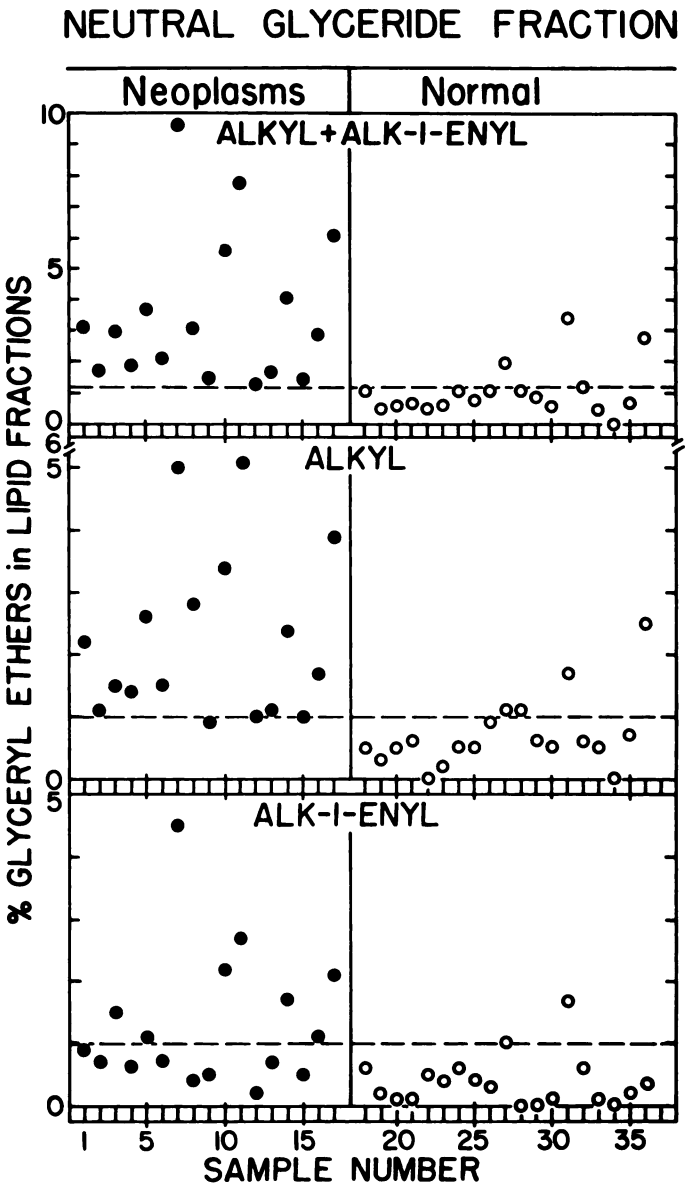

Chart 1. Percentage distribution of total glyceryl ethers, glyceryl alkyl ethers, and glyceryl alk-1-enyl ethers of the neutral glyceride fraction of normal (○) and neoplastic (●) human cells. Sample numbers along the abscissa refer to the sample description listed in Tables 1 and 2. The horizontal lines designate an arbitrary zone of demarcation between the values for neoplasms and normal samples.

glyceryl ethers, derived from the neutral glyceride fraction after $LiAlH_4$ reduction, was higher in neoplasms than in most healthy tissues. There was no correlation between the glyceryl ether percentage and the quantities of total tissue lipids or the total lipids of the neutral glyceride and phosphoglyceride fractions.

The alkyl-type ether was always higher than the alk-1-enyl ethers in the neutral glyceride fraction of both neoplastic and healthy cells, whereas the alk-1-enyl type generally predominated in the phosphoglyceride fraction (Chart 2). This statement is borne out by the alkyl/alk-1-enyl ratios for the two main lipid fractions of normal and neoplastic cells (Tables 1, 2). However, the relative prominence of alkyl-type ethers in neoplastic tissues is seen in both the neutral glyceride and phosphoglyceride fractions (Tables 1, 2; Charts 1, 2).

DISCUSSION

Glyceryl ethers (alkyl and alk-1-enyl) can occur as diacyl (triglyceride type), monoacyl (diglyceride type), free (monoglyceride type), and phosphoglyceride forms. The presence of abnormally high levels of GEDE in neoplastic cells from animals (17) prompted this quantitative survey study of ether-linked lipids in neoplastic and healthy tissues of man. Our results with human tissues confirmed our previously reported data on the generally high levels of ether-linked lipids in the neutral glyceride fraction of neoplastic tissues of 13 transplantable rat and mouse tumors (17). A separate unpublished experimental study, in which we examined the occurrence of glyceryl ethers in tumors and nonmalignant tissues from the same patients with cancer, has also shown that the neoplastic tissues contained abnormally high levels of GEDE when compared with nonmalignant tissues.

Although the widespread occurrence of plasmalogens (alk-1-enyl phosphoglycerides) in a variety of organs (5, 9) and tumors (4, 8, 17, 22) from man and animals had been previously established, our study has further demonstrated that the alkyl and alk-1-enyl glycerides and alkyl phosphoglycerides must also be considered as significant lipid constituents in many normal human tissues and especially in neoplasms.

Our investigation of the general distribution of ether-linked lipids in the normal human tissues showed the same trend as that reported earlier for normal rat tissues (8). Variations in the ether-lipid composition of identical tissues from the 2 normal persons might be a reflection of age. In the present study, the heart and kidney contained the highest proportion of neutral glyceride ethers, and the lung, brain, spleen, larynx, heart, colon, and testes contained the highest proportion of phosphoglyceride ethers. The first report of phosphorus-free alk-1-enyl ethers of glycerol occurring in mammalian cells (milk fat, beef tallow, and ox heart) was made in 1960 by Schogt *et al.* (12). Phosphorus-free alk-1-enyl ethers, as well as the alkyl ethers of glycerol, have subsequently been found in minor amounts as the diesters in a number of mammals (3, 10, 11, 21), including man (3, 10, 11). The distribution of alkyl and alk-1-enyl ethers in neutral glycerides and phosphoglycerides found in nature, and an up-to-date evaluation of biochemical studies of lipids containing ether bonds, has recently been published (13).

The significance and the biosynthesis of the ether bond in lipids are still not understood, but the occurrence of ether-linked phosphoglycerides in pure plasma membranes of rat liver isolated by zonal centrifugation (6) has suggested their possible importance in biomembrane arrangements of proteins and lipids. The distribution of ether-linked lipids in organelles of normal and neoplastic tissue remains to be investigated; preliminary studies have indicated that the glyceryl ether diesters are highest in a 15,000 × *g* × 10 min fraction (mitochondria) isolated from Ehrlich ascites cell homogenates (16). De-

Table 2

Identification number[a]	Sample	Total lipids (% dry wt.)	Lipid fraction	Lipid in fraction (%)	Glyceryl alk-1-enyl ether[b] (%)	Glyceryl alkyl ether[b] (%)	Alkyl/ alk-1-enyl ratio
18.	Lung	8.0	N	46	0.6	0.5	0.9
			P	54	6.5	2.0	0.3
19.	Adipose tissue (peritoneal)	22.3	N	88	0.2	0.3	2.0
			P	12	2.0	0.3	0.2
20.	Adipose tissue (omental)	67.6	N	83	0.1	0.5	4.3
			P	17	0.4	0.2	0.5
21.	Adipose tissue (subcutaneous)	62.7	N	87	0.1	0.6	6.7
			P	13	0.2		[c]
22.	Liver	10.3	N	40		0.5	[c]
			P	60	0.5	0.4	0.8
23.	Brain	26.5	N	35	0.4	0.2	0.5
			P	65	11.0	2.0	0.2
24.	Spleen	7.1	N	41	0.6	0.5	0.8
			P	59	7.7	3.8	0.5
25.	Lymph	56.8	N	83	0.4	0.5	1.3
			P	17	1.4	0.7	0.5
26.	Larynx	10.9	N	84	0.3	0.9	3.2
			P	16	9.0	2.1	0.2
27.	Heart	17.8	N	60	1.0	1.1	1.1
			P	40	12.1	2.2	0.2
28.	Colon	17.1	N	95		1.0	[c]
			P	5	12.8	7.6	0.6
29.	Rectum	37.7	N	10		0.9	[c]
			P	90	3.0	1.5	0.5
30.	Testes	15.7	N	49	0.1	0.5	6.0
			P	51	5.8	4.4	0.8
31.	Kidney	4.8	N	47	1.7	1.7	1.0
			P	53	4.6	0.8	0.2
32.	Prostate	15.1	N	30	0.6	0.6	1.1
			P	70	3.2	0.8	0.3
33.	Pancreas	37.3	N	85	0.1	0.5	6.0
			P	15	3.5	0.9	0.2
34.	Testes	4.0	N	50			[c]
			P	50	4.2	2.8	0.7
35.	Spleen	3.6	N	30	0.2	0.7	3.1
			P	70	4.7	2.5	0.5
36.	Heart	9.4	N	34	0.3	2.5	8.3
			P	66	16.3	5.6	0.3

Normal human tissues: identification, lipid content, and glyceryl ether levels of neutral glyceride and phosphoglyceride fractions. Samples No. 18–32 are from a 17-year-old boy who died instantaneously of cyanide poisoning, and samples No. 33–36 are from a 14-year-old boy who died suddenly of bilateral lobar hemorrhagic pneumonitis. N refers to neutral glyceride fraction; P refers to phosphoglyceride fraction.

[a] Identification numbers correspond to sample numbers used in figures.

[b] All values are expressed as the diacyl or phosphoglyceride form.

[c] Insignificant quantity of alk-1-enyl ether for calculation.

tailed studies are also required to fully understand whether the ether-linked lipids might be associated with the lipid droplets that occur in tumor cells (1). Studies on the biosynthesis of the ether bond have not yet provided any meaningful conclusions (13). However, data have demonstrated that alkyl ethers can be converted into alk-1-enyl ethers (18) and that α and β alkylglycerols can serve as acyl acceptors in an ATP-CoA-dependent reaction (15).

The single benign tumor studied showed values like those of malignant neoplasms; more data will be needed before we can determine whether this biochemical abnormality might be useful in determining the biologic potential of lesions of borderline malignancy. Work is currently in progress in our laboratory on tumors induced by a variety of agents and should contribute some clarification on this point.

At the present time we may conclude from our studies that two significant biochemical features involving lipid metabolism are characteristic of neoplasia: (*a*) an abnormally high level of glyceryl ethers in the neutral glyceride fraction and (*b*) the absence (15) of an alkyl ether-cleaving enzyme ("etherase") that is found in liver and to a lesser extent in other healthy cells (7, 19).

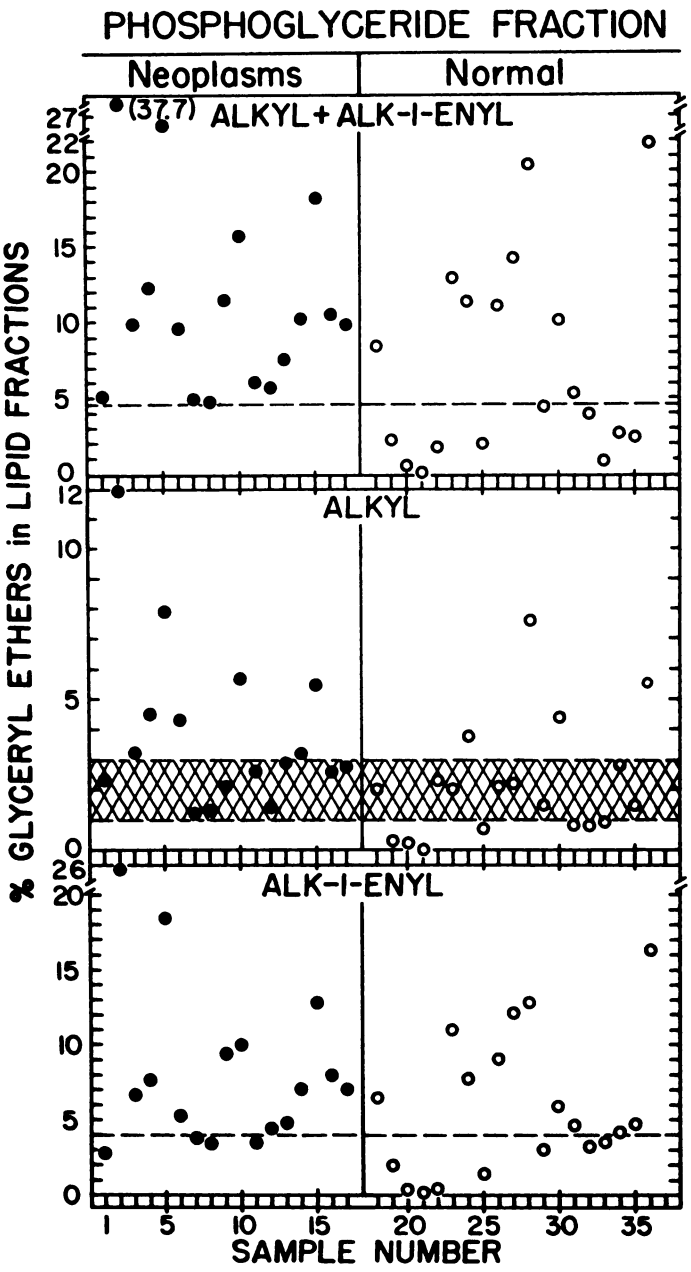

Chart 2. Percentage distribution of total glyceryl ethers, glyceryl alkyl ethers, and glyceryl alk-1-enyl ethers of the phosphoglyceride fraction of normal (○) and neoplastic (●) human cells. Sample numbers along the abscissa refer to the sample description listed in Tables 1 and 2. The horizontal lines designate an arbitrary zone of demarcation between the values for neoplasms and normal samples. The hatched area designates an arbitrary zone of overlap between the values for neoplasms and normal samples.

ACKNOWLEDGMENTS

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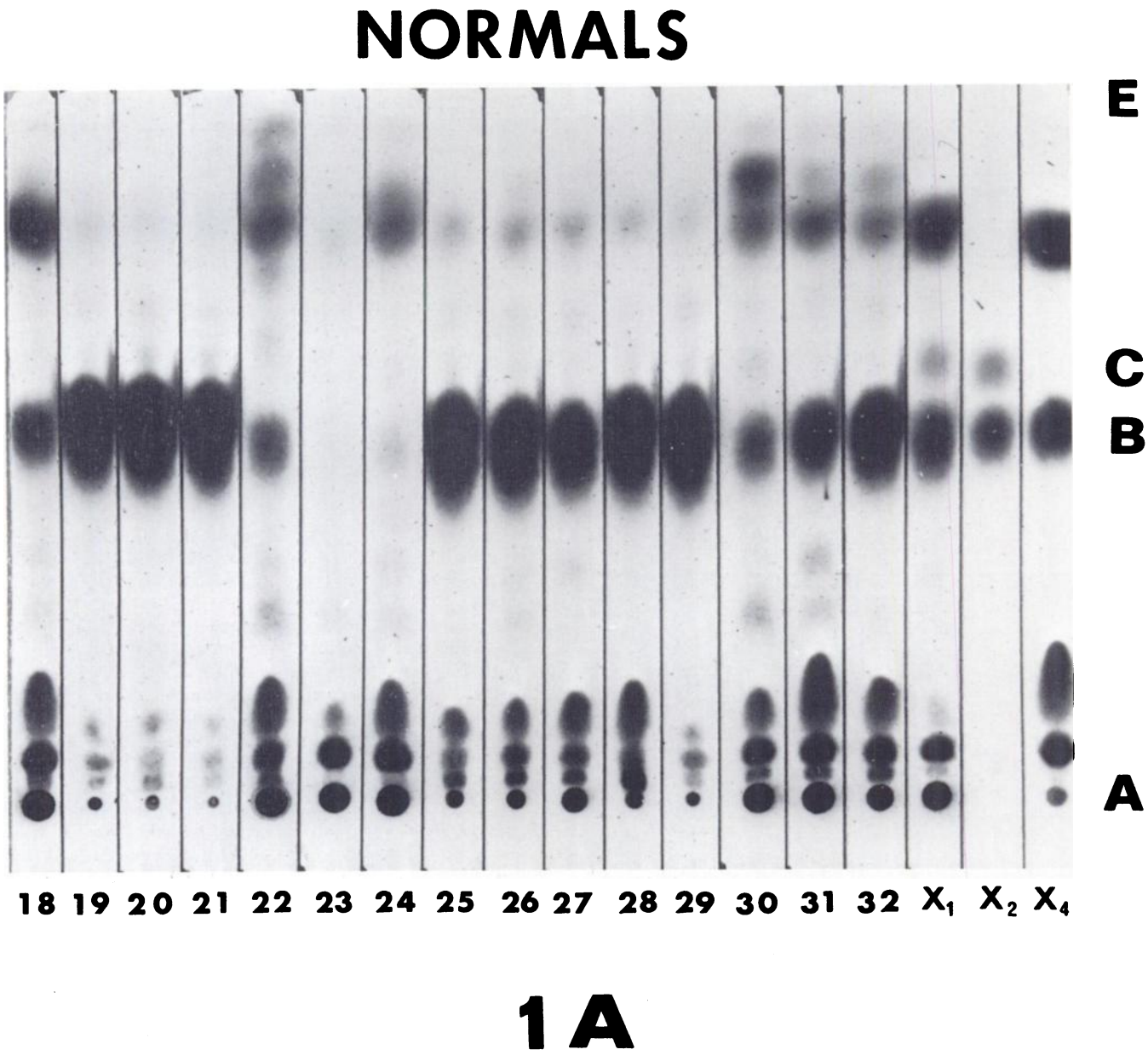
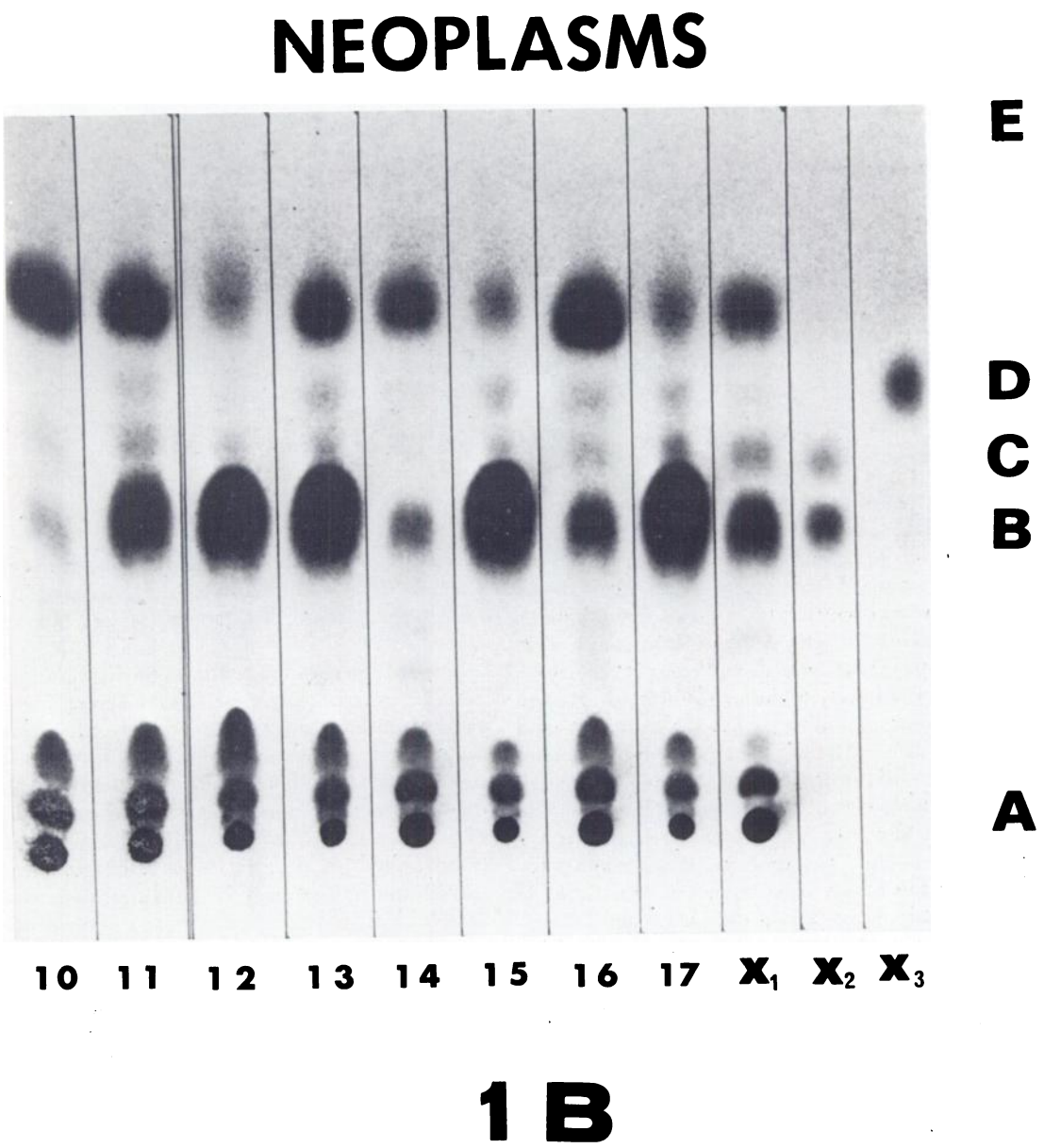


1A

Fig. 1*A*, 1*B*. Thin-layer chromatograms of total lipid extracts from normal (1*A*) and neoplastic (1*B*) tissues of man. Chromatography was carried out on Silica Gel G in a solvent system of benzene:hexane:diethyl ether:acetic acid (45:50:5:1, v/v/v/v). Letters refer to the following: *A*, origin; *B*, triglycerides; *C*, glyceryl ether diesters (alkyl type); *D*, glyceryl ether diesters (alk-1-enyl type); and *E*, solvent front. Sample numbers below each lane on chromatogram refer to sample description listed in Tables 1 and 2. Other lanes are X_1, total lipid extract of Ehrlich ascites cells; X_2, a mixture of a triglyceride (*B*) and of a glyceryl alkyl ether diester; X_3, a glyceryl alk-1-enyl ether diester; and X_4, a standard mixture (listed from origin to solvent front) of phosphatidyl choline, cholesterol, oleic acid, triolein, and cholesteryl oleate.



1 B