Ehrlich ascites tumor cell surface membranes: an abnormality in ether lipid content

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Abstract. Most mammalian neoplasms have a defect in ether lipid content manifested by the presence of abnormally large quantities of *O*-alkyl glyceryl ethers, in contrast to normal tissues in which the alk-1-enyl structure predominates. These lipids are for the most part structural. The manner in which tumor cell plasma membranes differ from normal may be important, and it has been hitherto unclear whether or not the *O*-alkyl lipid abnormality of neoplasms includes the plasma membrane.

The present investigation reveals that *O*-alkyl lipids are present in the membranes of Ehrlich ascites tumor cells isolated by several different methods. The amount of *O*-alkyl lipid, on a weight basis, represents 1–3% of the total phospholipids and 1–4% of the total aliphatic lipid. These quantities are the same as or greater than the amount of *O*-alkyl lipid found in microsomes, mitochondria, and whole cell homogenate. As is generally the case for intact neoplastic tissues, the quantity of *O*-alkyl lipids of Ehrlich ascites tumor plasma membrane is greater than the amount of alk-1-enyl lipids.

Supplementary key words alk-1-enyl lipids · *O*-alkyl lipids · fatty alcohol · neoplasia

O-Alkyl glyceryl ethers have been found in abnormal amounts in a large variety of human and animal neoplasms (1–5). Only rarely have tumors been found that do not contain excessive quantities of these substances (6–8). *O*-Alkyl lipids exist as phospholipids in subcellular membrane structures such as microsomes and mitochondria and, together with the related alk-1-enyl lipids, make up a portion of the cellular structural membrane lipids (1). The presence of *O*-alkyl lipids has also been described in membranous material from Ehrlich ascites tumor cells (9). In neoplasms *O*-alkyl lipids are abundant as *O*-alkyldiacylglycerolipids (1). From the phylogenetic, and possibly the oncologic, viewpoint, it is of interest that the *O*-alkyl lipid form of phospholipids is found in protozoa to the exclusion of the alk-1-enyl structure. Both alk-1-enyl and *O*-alkyl lipids are abundant in more complex organisms such as gastropods, echinodermata, and elasmobranch fishes, whereas the alk-1-enyl structure normally predominates in mammalian species with the noteworthy exception of neoplastic tissues (1). Excessive quantities of *O*-alkyl lipids have not been identified in fetal tissues, suggesting that abundance of glyceryl ethers is not exclusively a phenomenon of rapid growth (10). Considerable investigation is currently underway to determine the manner in which the plasma membranes of tumor cells differ from those of normal cells, and to determine the relationship which these differences might have to such phenomena as lack of tumor cell adhesiveness, loss of contact inhibition, and to the occurrence of metastasis in general (11). Because the plasma membrane is made up in large part of lipids and because tumor cells synthesize abnormal quantities of *O*-alkyl lipids, it would be of interest to determine whether or not *O*-alkyl lipids are present in the plasma membranes of tumor cells. Accordingly, the current investigation was carried out to determine whether or not *O*-alkyl lipids are present in plasma membranes of Ehrlich ascites tumor cells. These cells are known to synthesize and to contain abnormal quantities of ether lipids (1).

MATERIALS AND METHODS

Materials

Precoated silica gel plates 0.25 mm thick were obtained from Analtech (Newark, DE) and washed by development in ethyl ether–methanol 95:5 followed by drying at 180°C. All reagents and solvents were analytical reagent grade and were not purified further. Vitride (sodium bis[2-methoxy-ethoxy] aluminum hydride) was obtained from Eastman (Rochester, NY).

Alk-1-enyl glycerol for use as a thin-layer standard was prepared from beef heart. The heart was homogenized in 2 M potassium chloride, and the lipids were extracted by the method of Bligh and Dyer (12). The extract was treated with Vitride to reduce the alk-1-enyl lipids to alk-1-enyl glycerol (13). The alk-1-enyl glycerol was then isolated and purified by preparative thin-layer chromatography on 2 mm thick precoated silica gel G plates (Brinkmann, Westbury, NY). The developing solvent was ethyl ether saturated with water.

Selachyl alcohol (1-octadec-9-enyl glyceryl ether)

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and boron trifluoride–methanol were obtained from Applied Science laboratories (State College, PA). Hexadecyl aldehyde sodium sulfite compound was obtained from Aldrich (Milwaukee, WI).

Preparation of membranes and other cellular fractions

Ehrlich ascites tumor cells were grown in male HA/ICR Swiss white mice (13–20 g). The cells were harvested from the peritoneal cavity 7–9 days after inoculation and washed three times with 0.9% sodium chloride at room temperature. The plasma membranes were obtained by the method of Forte, Forte, and Heinz (14) except that on occasion further purification of the harvested membranes was achieved by resuspending the membranes in cold 15 mM Tris buffer, pH 8.0, containing 37 mM sodium chloride and 30% sucrose. The suspension was applied to a second 60–50–45–40% discontinuous sucrose gradient and centrifuged for 45 min at 26,000 rpm in a Spinco SW 27 rotor. Separations were monitored by phase light microscopy and electron microscopy.

Ehrlich ascites tumor cell microsomes and mitochondria were obtained as previously described (15). Plasma membranes were also obtained by the method of Warren, Glick, and Nass (16), using fluorescein mercuric acetate, and by the method of Brunette and Till (17).

Chemical procedures

Lipids were extracted by the method of Bligh and Dyer (12). Phospholipids were measured by the method of Bartlett (18). Total lipids were reduced to *O*-alkyl glycerol, alk-1-enyl glycerol, free cholesterol, and fatty alcohols with Vitride by the method of Snyder, Blank, and Wykle (13).

Lipids were separated by thin-layer chromatography on 0.25-mm silica gel G layers. For separation of ether lipids, development was carried out in ethyl ether saturated with water. Cholesterol and total fatty alcohol, a measure of total aliphatic lipid, were separated on plates developed in hexane–ethyl ether–acetic acid 50:50:1 (v/v).

Quantitative photodensitometry of lipids (19, 20) separated by thin-layer chromatography was carried out with Quick Scan Flur-vis (Helena, Beaumont, TX). Charring was usually done with sulfuryl chloride (21). Quantitation of unknowns was performed by thin-layer chromatography of an aliquot of the unknown solution and increasing multiples of these aliquots. Increasing multiples of cochromatographed quantitative standards were simultaneously analyzed. Protein was measured by the method of Lowry et al. (22).

Gas–liquid chromatography

For gas–liquid chromatography (GLC), *O*-alkyl glycerols isolated by thin-layer chromatography were converted to their isopropylidene derivatives (23).

Alk-1-enyl glycerols were separated by GLC as the dimethyl acetals of the aliphatic moieties. The dimethyl acetals were obtained by treatment of the alk-1-enyl glycerols with 1.5 ml of boron trifluoride methanol–benzene–methanol 1:1:1 (v/v) by the method of Morrison and Smith (24). When necessary, the dimethyl acetals were purified by thin-layer chromatography on silica gel G. The developing solvent was hexane–ethyl ether–acetic acid 90:10:1 (v/v).

GLC was carried out in a Packard Model 7400 apparatus using a flame ionization detector and a 6-ft coiled column of internal diameter 2 mm containing 15% ethylene glycol succinate on ANACHROM AB (Analabs, North Haven, CT). Dimethyl acetals of alk-1-enyl glycerols were separated at 170°C while isopropylidene derivatives of *O*-alkyl-glycerols were separated at 190°C. Nitrogen was the carrier gas. Peaks were identified by means of appropriate standards.

Enzymatic procedures

Sodium-potassium-dependent ATPase was assayed by the method of Brunette and Till (17). NADH diaphorase was measured by the method of Kamat and Wallach (25).

RESULTS

Plasma membranes

Plasma membranes, prepared by the method of Forte et al. (14), were examined by electron microscopy (**Fig. 1**) and appeared as vesicles identical to those reported by other workers. Sodium-potassium-dependent ATPase activity in these membranes showed a 12-fold increase in specific activity as compared with whole homogenate. The activity measured was the sodium-potassium-dependent ATPase activity increment above the magnesium-dependent ATPase activity. Following a second purification, as described in the preceding section, a purification ratio of up to 28-fold was obtained. There was considerable loss of material following repurification and the fraction containing greatest ATPase activity was found in the 30–40% sucrose interface rather than in the 40–45% fraction. There was also a considerable loss of protein. NADH-diaphorase specific activity, a putative measure of smooth endoplasmic reticulum, was equal to or up to twice that of homogenate in the first sucrose gradient

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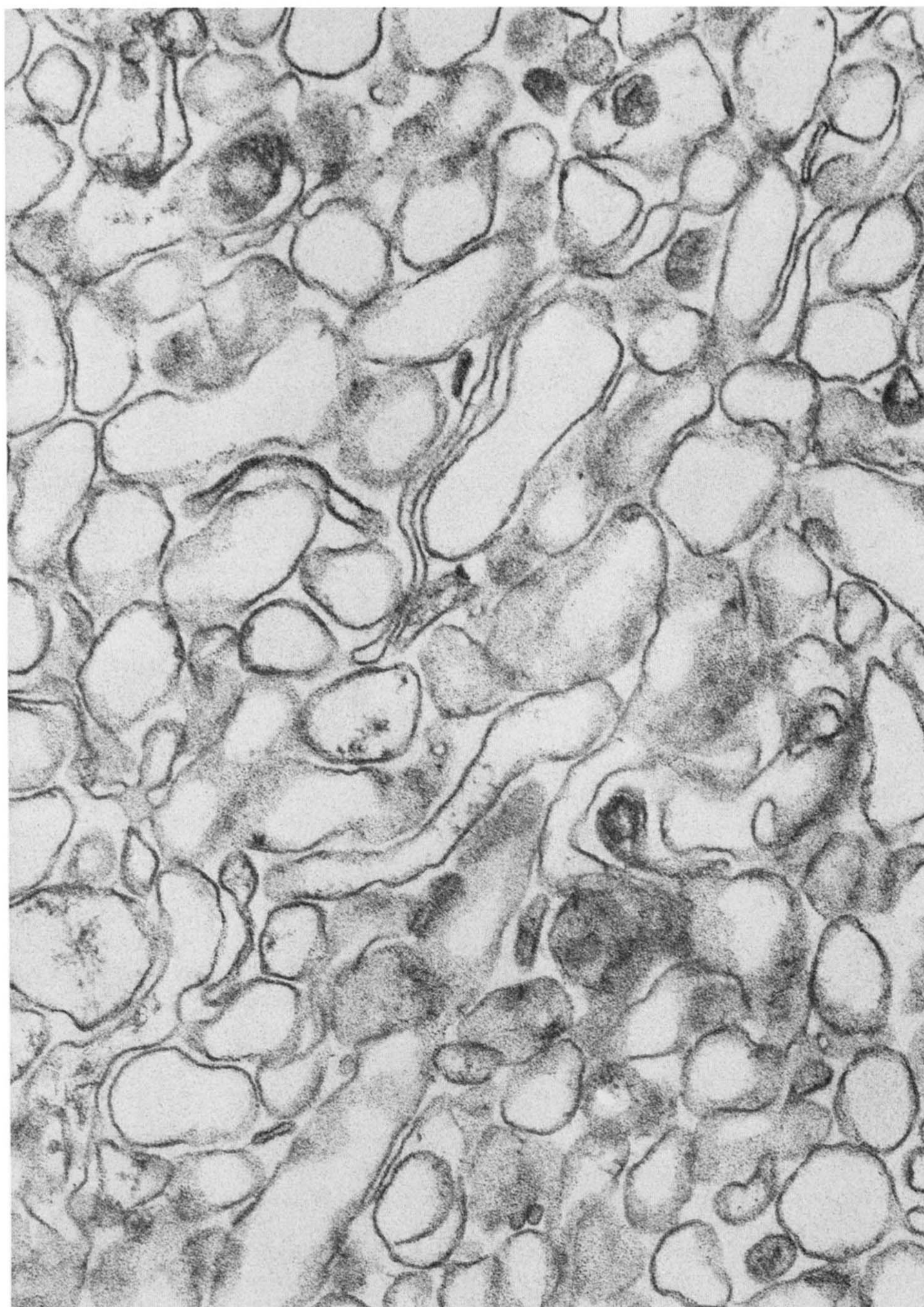

Fig. 1. Electron micrograph of thin section of plasma membrane pellet. Plasma membranes are present as irregular vesicles. The pellet was fixed with cacodylate-buffered glutaraldehyde and OsO_4 and stained with lead citrate and uranyl acetate. Magnification ×56,000.

and increased up to fivefold in repurified membranes. This increase in activity represented activity that could not be dissociated from the membrane since the relative increase was proportional to the relative increase in ATPase activity obtained by repurification. In a typical experiment, diaphorase specific activities for the unmodified procedure were: homogenate, 0.207; membranes, 0.226; mitochondria, 0.604; microsomes, 0.143 (μmol/mg per min).

Quantitative lipid analyses

Plasma membrane and mitochondrial, microsomal and whole homogenate fractions were analyzed for their protein and phospholipid contents. The lipid ex-

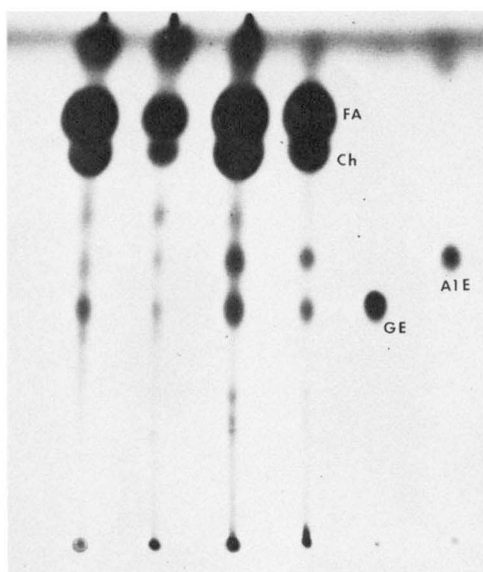

Fig. 2. Thin-layer chromatogram of Vitride-treated lipids of plasma membranes and of other cell fractions. Lipids were developed in ether saturated with water. From left to right: mitochondrial, microsomal, plasma membrane, and homogenate lipids. *GE*, 1-*O*-octadecenyl glycerol (selachyl alcohol); *A-1-E*, mixed beef heart alk-1-enyl glycerols; *Ch*, cholesterol; *FA*, fatty alcohol.

tracts of the several cellular fractions were reduced by Vitride. This procedure converts *1*) all *O*-alkyl and alk-1-enyl lipids to 1-*O*-alkyl glycerol and to alk-1-enyl glycerol; *2*) all fatty acids, free or esterified, to long chain fatty alcohols; and *3*) cholesteryl esters to free cholesterol. **Fig. 2** illustrates a thin-layer chromatogram of plasma membrane lipids after reduction by Vitride. This reveals that *O*-alkyl lipids are present in plasma membranes. *O*-Alkyl glycerol is present in amounts slightly greater than the amount of alk-1-enyl glycerol. This was true in all fractions examined. The major lipid component present is fatty alcohol (formerly acyl groups). Cholesterol is the next most abundant lipid. One or more unidentified lipid components of intermediate abundance were also noted.

The quantitative results of these lipid studies are presented in **Table 1** and are expressed in μg lipid/mg protein. The data in this table are the means and standard deviations from 3 to 16 individual lipid analyses performed on lipid extracts of various membrane preparations.

Table 2 presents the *O*-alkyl and alk-1-enyl data from Table 1, including several other parameters, expressed in relation to the phospholipid and total fatty alcohol of the various lipid fractions. The data indicate that the plasma membrane fraction contains the highest percent of both *O*-alkyl and alk-1-enyl lipids. The plasma membrane fraction contained 4.29% *O*-alkyl glycerol in relation to total aliphatic lipid (fatty alcohol) and 2.75% in relation to phospholipid. These figures were higher than the corresponding values for microsomes, mitochondria, and homogenate. For the microsomal fraction, these values were 2.70% and 1.65%, respectively, and for the mitochondrial fraction 1.78% and 1.37%, respectively. The plasma membranes also contained larger quantities of alk-1-enyl lipids. Table 2 also contains calculations showing the percent of phospholipids present as *O*-alkyl acyl and alk-1-enyl acyl phospholipids. A conservative interpretation of these results is that the plasma membrane fraction contains *O*-alkyl lipids in at least the same proportion as other cell membranes and perhaps in a significantly greater proportion. Table 2 also provides data showing that the ratios of Vitride-generated fatty alcohols to phospholipids in the various membrane fractions vary from 0.609 for microsomes to 0.770 for mitochondria. The plasma membrane fraction yielded a much higher cholesterol to fatty alcohol ratio than any other subcellular fraction (Table 2).

Gas–liquid chromatography

The identity of membrane *O*-alkyl and alk-1-enyl glycerol isolated by thin-layer chromatography was established by GLC. **Fig. 3** illustrates a gas–liquid

TABLE 1. Lipid content of plasma membranes and other subcellular fractions

	Total Phospholipid	*O*-Alkyl Glycerol[a]	Alk-1-enyl Glycerol[a]	Fatty Alcohol[a]	Cholesterol[a]
Homogenate	70.6, 66.5	0.558 ± 0.094[b] (5)[c]	0.442 ± 0.030 (4)	51.8 ± 9.6 (7)	9.06 ± 0 (3)
Plasma membranes	282.0 ± 14.0 (3)	7.77 ± 0.883 (6)	5.57 ± 0.699 (6)	181.1 ± 34.2 (6)	73.0 ± 7.8 (7)
Microsomes	205.0 ± 5.3 (4)	3.38 ± 0.28 (4)	2.47 ± 0.47 (4)	125.0 ± 11.0 (4)	18.95 ± 0.90 (4)
Mitochondria	80.1 ± 3.6 (3)	1.10 ± 0.17 (7)	0.645 ± 0.12 (4)	61.7 ± 6.7 (6)	15.0 ± 2.17 (6)

[a] Obtained by Vitride hydrogenolysis of total lipids.
[b] Data expressed in μg lipid/mg protein ±SD.
[c] Numbers in parentheses indicate number of analyses performed on pooled extracts from 10–20 mice.

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TABLE 2. Relative lipid content of plasma membranes and of other cell fractions[a]

	O-Alkyl Glycerol		Alk-1-enyl Glycerol			
	As % Fatty Alcohol[b]	As % Total Phospholipid	As % Fatty Alcohol[b]	As % Total Phospholipid	Ratio Cholesterol to Fatty Alcohol[b]	Ratio Fatty Alcohol[b] to Phospholipid
Homogenate	1.07	0.81 (1.7)[c]	0.85	0.64 (1.4)[c]	0.175	0.755
Plasma membranes	4.29	2.75 (5.9)[c]	3.07	1.98 (4.3)[c]	0.403	0.642
Microsomes	2.70	1.65 (3.5)[c]	1.97	1.20 (2.6)[c]	0.152	0.609
Mitochondria	1.78	1.37 (2.9)[c]	1.04	0.81 (1.7)[c]	0.243	0.770

[a] Data expressed as percent by weight or ratio by weight.
[b] Fatty alcohol represents total aliphatic lipid obtained by hydrogenolysis.
[c] Numbers in parentheses represent calculated O-alkyl and alk-1-enyl phospholipid values expressed as a percent of the total phospholipids. These values were obtained by taking the average mol wt of O-alkyl glycerol and alk-1-enyl glycerol as 360 and the average mol wt of the corresponding phospholipids as 775.

chromatogram of the isopropylidene derivatives of the O-alkyl glycerols and **Fig. 4** the dimethyl acetals derived from the alk-1-enyl glycerols. The membrane alk-1-enyl and O-alkyl glycerols were compared qualitatively with these species obtained from the whole homogenate. The major aliphatic chains present are 16:0, 18:0, and 18:1 for both species of lipids. There are no striking or highly significant qualitative differences between the membrane and total homogenate lipids or between the O-alkyl and alk-1-enyl lipids. In addition, the results of GLC presented here are in agreement with results obtained by others on whole cell ether lipids from Ehrlich ascites tumor cells (26). Thus, the chain length distributions of the alk-1-enyl and alkyl lipids of the plasma membranes examined in this investigation are not different from the total cellular ether lipids.

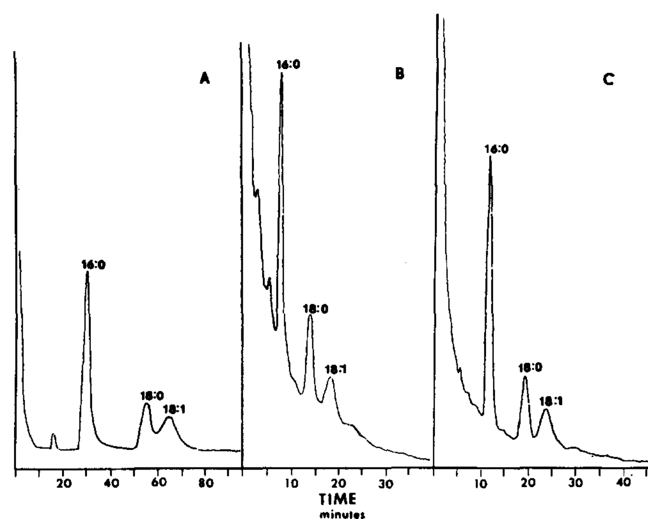

Fig. 3. Gas–liquid chromatogram of isopropylidene derivatives of O-alkyl glycerol isolated by thin-layer chromatography. Peaks were identified by cochromatography with standards. Conditions are described in the text. *A*, Homogenate; *B*, plasma membranes; *C*, fluorescein mercuric acetate membranes.

Ether lipid content of fluorescein mercuric acetate-stabilized plasma membranes and of repurified plasma membranes

It was considered useful to compare the results described above with the results obtained by an entirely different method for preparing plasma membranes. To do this we selected the procedure described by Warren, Glick, and Nass (16) which utilizes fluorescein mercuric acetate to stabilize plasma membranes. This yields large structures which, by phase contrast light microscopy, are clearly identifiable as plasma membranes. The results of lipid analyses of these mem-

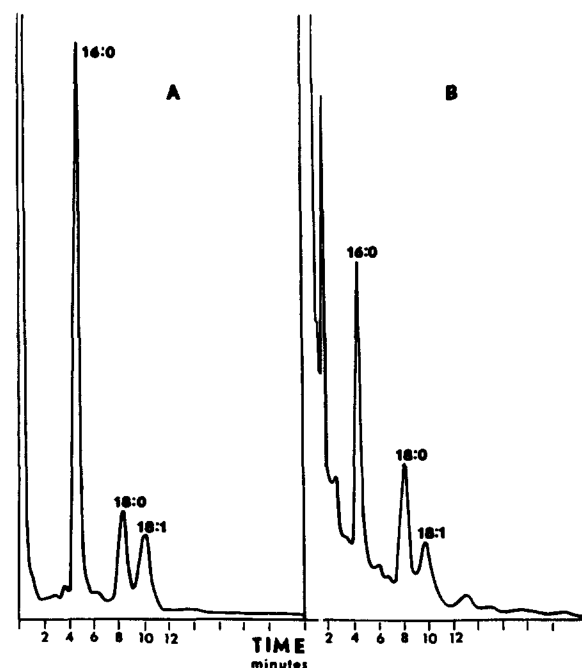

Fig. 4. Gas–liquid chromatogram of dimethyl acetals of long chain aldehydes derived from alk-1-enyl lipids isolated by thin-layer chromatography. Peaks were identified by cochromatography with standards. Conditions are described in the text. *A*, Homogenate; *B*, plasma membranes.

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TABLE 3. Lipid content and relative lipid content of plasma membranes isolated by repurification and with fluorescein mercuric acetate

	Repurified Plasma Membranes	Plasma Membranes (Fluorescein Mercuric Acetate Method)
	μg lipid/mg protein	
Phospholipid	569, 555	136.5, 137.5
O-Alkyl glycerol[b]	5.44 ± 0.61 (10)[a]	1.84 ± 0.23 (4)
Alk-1-enyl glycerol[b]	3.87 ± 0.18 (9)	1.51 ± 0.36 (3)
Fatty alcohol[b]	444.0 ± 28.4 (8)	120.0 ± 12.7 (6)
Cholesterol[b]	120.0 ± 19.0 (16)	34.1, 35.2
O-Alkyl glycerol[b]		
% of fatty alcohol	1.23%	1.53%
% of phospholipid	0.97% (2.1%)[c]	1.34% (2.9%)[c]
Alk-1-enyl Glycerol[b]		
% of fatty alcohol	0.87%	0.85%
% of phospholipid	0.69% (1.5%)[c]	0.64% (1.4%)[c]
Ratio cholesterol to fatty alcohol	0.270	0.288
Ratio fatty alcohol to phospholipids	0.790	0.875

[a] Numbers in parentheses indicate total number of analyses performed on one or more pooled extracts from 10–20 mice (± one SD).
[b] Obtained by Vitride hydrogenolysis of total lipids.
[c] Numbers in parentheses represent calculated *O*-alkyl and alk-1-enyl phospholipid values expressed as a percent of the total phospholipids. These values were obtained by taking the average mol wt of *O*-alkyl glycerol and alk-1-enyl glycerol as 360 and the average mol wt of the corresponding phospholipids as 775.

branes are presented in **Table 3.** The relative *O*-alkyl lipid content is lower than that reported above for the method of Forte et al. (14) and is more nearly the same as for the *O*-alkyl lipid content of mitochondria and microsomes. Electron microscopy of these membranes revealed a considerable amount of particulate material not present in other preparations.

Table 3 also provides results on the *O*-alkyl lipid content of membranes repurified as described in Methods. It can be seen that the *O*-alkyl content of these plasma membranes is lower than the *O*-alkyl lipid content of nonrepurified membranes. As indicated above, the specific activity of sodium-potassium-activated ATPase was very high in this fraction. However, the significance of these data in terms of *O*-alkyl lipid content is not clear since the repurification process resulted in the appearance of an excessive amount of material in the form of large discrete bands at all of the other density interfaces of the discontinuous gradients. Thus considerable fragmentation of the plasma membrane material may occur in this procedure.

In addition to the two procedures described above for the isolation of plasma membranes, namely the procedure of Forte, Forte, and Heinz (14) and the fluorescein mercuric acetate method of Warren, Glick, and Nass (16), we have also chosen to evaluate the ether lipid content of membranes isolated by the procedures of Brunette and Till (17). This procedure yields large intact structures or membrane ghosts after cell homogenization. In the purified preparation the plasma membranes appear in sheets by light microscopy. In our hands, diaphorase specific activity was 53% of homogenate activity and 43% of mitochondrial activity. Sodium and potassium-dependent ATPase specific activity in these preparations was increased twenty-fold. **Table 4** details the data obtained by this method. **Table 5** compares the amount of *O*-alkyl and alk-1-enyl lipids present in plasma membranes obtained by four different procedures. The data are expressed as percent of Vitride-generated fatty alcohol. Data on the percent of cholesterol relative to the Vitride-liberated fatty alcohols are also provided. These results will be discussed below.

DISCUSSION

The results of these studies establish *1*) that *O*-alkyl lipids are present in plasma membranes of Ehrlich ascites tumor cells, *2*) that the amount present is slightly greater than the amount of alk-1-enyl lipid, *3*) that the amount present in relation to other lipids is at least the same or up to two times greater than the amount in microsomes, mitochondria, and whole cell homogenate. The amount of *O*-alkyl glycerol in these plasma membranes represents, on a weight basis, approximately 1–3% of the total phospholipid and approximately 1–4% of the total aliphatic lipid. Our findings with respect to total homogenate *O*-alkyl glyc-

TABLE 4. Ether lipid content of Ehrlich ascites tumor cell membranes isolated by the method of Brunette and Till (Ref. 17)

O-Alkyl glycerol	(5)[e]	3.9 ± 0.61 (SD)[a]	2.7%[d]
Alk-1-enyl glycerol	(4)[e]	2.9 ± 0.56 (SD)[a]	2.0%[d]
Total cholesterol	(4)[e]	73 ± 11 (SD)[a]	51 %[d]
Total aliphatic lipids as fatty alcohol	(3)	143 ± 32 (SD)[a]	
ATPase (K^+ dependent) (relative sp act vs. homogenate sp act)		21.5[b]	
Diaphorase activity			
a. Plasma membranes		0.18[c]	
b. Homogenate		0.33[c]	
c. Mitochondria		0.40[c]	
d. Microsomes		0.05[c]	

[a] Micrograms per mg of protein.
[b] Ratio of specific activity to homogenate specific activity.
[c] Micromoles per mg protein per min.
[d] Expressed as percent of Vitride-generated fatty alcohol.
[e] Number of determinations.

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erol content agree with those of Snyder and Wood (27). To obtain these data we have measured the relative quantity of O-alkyl and alk-1-enyl lipids in plasma membranes of Ehrlich ascites tumor cells by four procedures. The method of Forte et al. (14) yields membranes with a cholesterol:protein ratio that is severalfold higher than that of other subcellular fractions. The procedure of Brunette and Till yields almost identical results. The method of Forte et al. (14), however, gives the highest ether lipid content. The concentration of ether lipid obtained by this method is higher than that found in other subcellular fractions or that obtained by the other three isolation procedures. Further manipulations of the membranes, as in the repurified preparation of Forte et al. (14), resulted in substantial losses of O-alkyl lipids and protein. Because the concentrations of both cholesterol and ether lipid are high by the procedure of Forte et al. (14), we speculate that this procedure may be the most reliable. The fact that the relative amount of O-alkyl lipids present in plasma membranes is the same or greater than that in other subcellular fractions militates against the possibility that the O-alkyl lipid found in the plasma membrane fraction represents contamination from other subcellular fractions, in particular microsomes or mitochondria. Furthermore, the nature of the material analyzed was confirmed by electron microscopy.

With respect to the problem of purity of the membrane preparation isolated by the method of Forte et al., we have noted higher diaphorase specific activities than those reported by others (14). This activity increased in proportion of the increase in Na^+, K^+ ATPase activity upon repurification of our plasma membrane fraction on a second gradient. The validity of the use of diaphorase specific activity as a criterion for lack of contamination by smooth endoplasmic reticulum is moot (28). By the current method, mitochondria contain the highest diaphorase activity (14). Thus, our membrane preparation could contain as much as 30% mitochondrial contamination. However, the data presented above show clearly that cholesterol specific activity is a good criterion for plasma membrane purity since plasma membrane cholesterol specific activity greatly exceeds that of any other fraction (Table 1).

In an investigation, also pertaining to Ehrlich ascites tumor cells, Wood, Anderson, and Swartzendruber (9) found only a trace amount (≃1%) of O-alkyl lipids in what they describe as a "membranous fraction." Alk-1-enyl lipids were not found in their preparation. The reason for this is not clear. The material analyzed was not characterized enzymatically as plasma membranes. Van Hoeven and Emmelot (29) found alk-1-enyl lipids in several plasma membrane preparations from normal mouse and rat livers and hepatomas ranging from 1.8 to 3.1% of total phospholipid. These results compare to our value of 2.9% (Table 1).

TABLE 5. Comparison of lipid content of Ehrlich ascites plasma membranes isolated by different methods

	Forte et al. (14)	Forte at al. (14) Repurified	FMA[d]	Brunette and Till (17)
Fatty alcohol[a]	181	444	120	143
Cholesterol[a]	73	120	34	73
O-Alkyl lipids[a]	7.8	5.4	1.84	3.9
Alk-1-enyl lipids[a]	5.6	3.9	1.5	2.9
C:FA[b]	40%	27%	28%	51%
GE:FA[c]	4.3%	1.2%	1.5%	2.7%

[a] Obtained by Vitride hydrogenolysis (μg/mg protein).
[b] C:FA is ratio of cholesterol to fatty alcohol expressed as percent.
[c] GE:FA is ratio of O-alkyl lipid to fatty alcohol expressed as percent.
[d] FMA is fluorescein mercuric acetate method.

With respect to lipid quantitation by thin-layer chromatography and densitometry, which was found to be valuable for measuring small amounts of lipids, the procedure was accurate within ±20% or better, especially if varying quantities of unknowns and standards are cochromatographed.

O-Alkyl lipids are synthesized in Ehrlich ascites cell microsomes (1). Since the membrane O-alkyl composition is almost the same as that of microsomes, mitochondria, and whole homogenate, one interpretation of the present result is that the plasma membrane lipids are acquired indiscriminately from whatever phospholipids are synthesized by other cellular lipid synthetic systems. If lipid synthesis is abnormal, then the lipid compositions of the plasma membranes may also be abnormal. It is known that membrane composition can be altered by manipulation of the lipid environment and that abnormal lipids can be deposited in plasma membranes (30). With respect to the cause of O-alkyl lipid overabundance in tumor cells, there is evidence for marked reduction in O-alkyl lipid cleavage enzyme (31). The effect of excess quantities of O-alkyl lipids on plasma membrane function is not known and at present remains perhaps a fruitful direction for further investigation. Furthermore, the biologic role of ether lipids in general, both the O-alkyl and the alk-1-enyl variety, also remains currently unknown. Their abundance in certain tissues such as the heart and central nervous system, however, points to some critical function.

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