Biochemical Studies on Cell Fusion. I. Lipid Composition of Fusion-resistant Cells

DAVID S. ROOS and PURNELL W. CHOPPIN
The Rockefeller University, New York 10021. Dr. Roos's current address is Department of Biology, Stanford University, Stanford, California 94305.

ABSTRACT A series of stable cell mutants of mouse fibroblasts were previously isolated (Roos, D. S. and R. L. Davidson, 1980, *Somatic Cell Genet.*, 6:381–390) that exhibit varying degrees of resistance to the fusion-inducing effect of polyethylene glycol (PEG), but are morphologically similar to the parental cells from which they were derived. Biochemical analysis of these mutant cell lines has revealed differences in whole cell lipid composition which are directly correlated with their susceptibility to fusion. Fusion-resistant cells contain elevated levels of neutral lipids, particularly triglycerides and an unusual ether-linked lipid, O-alkyl, diacylglycerol. This ether lipid is increased ~35-fold over parental cells in the most highly PEG-resistant cell line. Fusion-resistant cells also contain more highly saturated fatty acyl chains (ratio of saturated to polyunsaturated fatty acids [S/P ratio] ≈ 4:1) than the parental line (S/P ratio ≈ 1:1). Cells which are intermediate in their resistance to PEG have ether lipid and fatty acid composition which is intermediate between the parental cells and the most fusion-resistant mutants. In a related communication (Roos, D. S. and P. W. Choppin, 1985, *J. Cell. Biol.*, 100:1591–1598) evidence is presented that alteration of lipid content can predictably control the fusion response of these cells.

One of the most dramatic forms of membrane–membrane interaction is the fusion of cell membranes (for review see references 42 and 52), an event which occurs naturally both within cells, as required for cell division, and between cells (e.g., during myogenesis). Cell fusion is also a characteristic feature of certain tumors (23, 79) and viral infections (17, 29, 55). Membrane fusion occurs at the subcellular level during such events as endocytosis via the coated pit pathway, receptor recycling, and the specific delivery of endogenously synthesized proteins to appropriate subcellular destinations (6, 22, 74). Despite the biological importance of membrane fusion, little is known about either the precise biochemical and biophysical events in fusion or the way in which cells control fusion of their membranes.

Cell fusion has been studied in vitro in systems often more amenable to experimental analysis than naturally occurring fusion. Many treatments, including viral infections, electrical fields, ionic manipulations, lysolecithin, and other membrane-active chemicals, have been reported to cause fusion under certain conditions (1, 7, 30, 38, 45, 46, 53, 64, 65, 76, 80, 85). The polyene polyethylene glycol (PEG)[1] is a potent fusogenic compound that has found wide acceptance as a tool for studying mechanisms of cell fusion (7, 39, 54, 77, 81), and as a convenient technique for many applications of membrane fusion (14, 34, 50). PEG is routinely used for the production of hybridomas (16), delivery of various compounds (chemotherapeutic agents, nucleic acids for transfection, etc.) to cells (2, 21, 41, 44, 47, 51), and a wide range of experiments in somatic cell genetics (13).

To investigate cellular factors involved in the control of fusion, we isolated a series of genetically stable, PEG fusion–resistant mutants from a highly fusible line of mouse L-cell fibroblasts (59). The least fusible of these cells exhibit <20% fusion under conditions of PEG treatment in which the entire monolayer of parental cells fuses into a giant syncytium. The selection procedure yielded a spectrum of intermediate fusion–resistant phenotypes spanning these two extremes which have proved very useful in detailed study of the morphology of cell fusion (60). We present here data on the direct correlation between lipid composition and sensitivity to fusion by

[1] *Abbreviations used in this paper:* Clone 1D, 5-bromodeoxyuridine–resistant mouse fibroblast cell line LM (TK$^-$) Clone 1D; F_4, F_8, F_{16}, and F_{40}, fusion-resistant cell lines isolated from Clone 1D by 4, 8, 16, or 40 cycles of polyethylene glycol–induced fusion; FAME, methyl ester of fatty acid; PEG, polyethylene glycol; S/P ratio, ratio of saturated to polyunsaturated fatty acids.

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PEG. The accompanying paper (58) further examines this relationship, using defined alterations of cellular lipids to completely control PEG-induced fusion.

MATERIALS AND METHODS

Cells and Media

The parental cell line was the 5-bromodeoxyuridine–resistant mouse fibroblast cell line LM (TK^-) Clone 1D. PEG-resistant derivatives of Clone 1D were isolated by repeated cycles of treatment with PEG followed by passage of those cells which remained unfused (58). PEG-resistant cell lines F_4, F_8, F_{16}, and F_{40} (selected by 4, 8, 16, and 40 cycles of treatment, respectively) exhibit ~85, 70, 50, and 20% fusion after exposure to PEG under conditions in which >95% of Clone 1D cells fuse (60). Cultures were monitored for mycoplasma contamination by staining with Hoechst 33258 and microscopic examination for fluorescence (12). Cells were grown in plasticware (Falcon Labware, Oxnard, CA) in reinforced Eagle's medium containing 4.5 g of glucose per liter and supplemented with 10% fetal bovine serum (Flow Laboratories, Inc., McLean, VA). In most experiments, antibiotics were added at concentrations of 500 U/ml penicillin + 0.1 mg/ml streptomycin, or 50 μg/ml gentamycin sulfate (Schering Corp., Kenilworth, NJ). Cultures were incubated at 37°C in a humid atmosphere containing 7% CO_2.

PEG Treatment and Analysis of Fused Cells

Confluent monolayers of cells in 60-mm diameter petri dishes were treated for 60 s with a 50% solution of PEG 1000 (J. T. Baker Chemical Co., Phillipsburg, NJ) as previously described (59). After a 2-h incubation at 37°C in fresh medium, cells were fixed, stained, and mounted under large glass coverslips. The extent of cell fusion in randomly selected fields was quantitated by scoring the percentage of all nuclei which were present in fused cells.

Preparation of Cell Stocks for Chromatography

Unless otherwise indicated, cells were harvested from late log-phase cultures grown in T-flasks or roller bottles by brief trypsinization or incubation in 10 mM EDTA. No differences in lipid content were seen between cells harvested with or without trypsin. Typically, ~2.5×10^7 cells were obtained per 75 cm^2 T-flasks. Cells were pelleted, washed twice in phosphate-buffered saline, repelleted, and either extracted directly for chromatography or frozen until needed. No significant alteration of lipid content was observed in cells stored for up to 6 mo at −70°C. Stocks were usually prepared as pellets of 5×10^7 cells; 0.2 ml containing ~10 mg protein. Radiolabeled lipids were prepared from subconfluent cultures of cells grown in normal medium containing 25 μCi/ml ^{14}C-acetic acid (sodium salt; New England Nuclear, Boston, MA). Cells were labeled for 24 h to insure the labeling of all cellular lipids without substantial entry of ^{14}C into other pathways; >85% of all counts were incorporated as lipid components. Extraction was carried out in combination with unlabeled "carrier" cells to improve recovery, which was usually >80%.

Lipid Extraction

Lipid analysis was carried out in acid-washed glassware fitted with either ground glass stoppers or teflon-lined screw-caps (Kontes Co., Vineland, NJ). Solvents were all reagent grade (Fisher Scientific Co., Fairlawn, NJ) and were redistilled before use. Samples were kept under nitrogen gas and in the dark wherever practical. Phospholipid standards were obtained from Avanti Polar Lipids, Inc., Birmingham, AL; fatty acids and methyl esters of fatty acids (FAMEs) from Nu-chek Prep, Inc., Elysian, MN, and other neutral lipids from Supelco, Inc., Bellefonte, PA, or Sigma Chemical Co., St. Louis, MO. General chromatographic procedures were adapted from Kates (36).

Cells were extracted by a modification of the standard procedure of Folch et al. (18). Cell pellets were suspended in 2 ml of methanol with a pasteur pipette and allowed to stand for ~10 min with occasional mixing. 2 ml of chloroform was added, the tube vortexed, and allowed to sit for a further 10 min. A second aliquot of 2 ml $CHCl_3$ was then added, bringing the final solution to a ratio of 2:1 $CHCl_3$/MeOH. Cellular debris was pelleted by centrifugation; the solvent containing extracted lipids was transferred to a fresh tube and brought to a total volume of 6 ml with $CHCl_3$/MeOH (2:1). To this mixture we added 1.2 ml of 0.1 M KCl in H_2O, and the tube was vortexed vigorously several times. After a brief centrifugation, the aqueous phase was removed and polar glycolipids (gangliosides) prepared by dialysis against three changes of 3,000 ml H_2O over the course of 24 h, followed by lyophilization. The organic phase from the Folch extraction, containing phospholipids, neutral glycolipids, and neutral lipids, was dried under a stream of nitrogen gas in a 37°C water bath. Lipids were stored under N_2 in tightly sealed tubes or applied directly to thin layer chromatographic plates or to columns for liquid chromatography.

Thin Layer Chromatography

Thin layer chromatography plates were obtained precoated (0.25-mm silica gel 60 without fluorescence indicator, on 20 × 20 cm glass plates; E. Merck, Cincinatti, OH). Plates were prewashed in solvent system A (see below) in one or two directions (depending on the experimental protocol) and activated at 120°C for 1 h immediately prior to use. Samples were dissolved in a minimal volume of 2:1 $CHCl_3$/MeOH and spotted (under a gentle flow of nitrogen gas) across a horizontal band at a distance of 1.5 cm from the bottom of the plate. Chromatography was carried out at room temperature in tanks lined with filter paper and equilibrated for at least 2 h prior to development. Solvent schedules used were as follows and are referenced by letter in the text.

ONE-DIMENSIONAL SEPARATIONS: (*A*) Hexanes/ethyl ether/acetic acid; 80:20:2; run for 18 cm. (*B*) Hexanes/ethyl ether; 95:5; run for 18 cm, dried under N_2 and rerun in the same direction. (*C*) $CHCl_3$/MeOH/H_2O; 100:42:6; run for 11 cm, dried under N_2, and redeveloped (in same direction for 18 cm) according to solvent schedule A. (*D*) $CHCl_3$/MeOH/0.2% $CaCl_2$ in H_2O; 60:40:9; run to 18 cm.

TWO-DIMENSIONAL SEPARATIONS: Plates were run 17 cm in first solvent system, then dried under N_2, turned 90° counterclockwise, and run 17 cm in second solvent system. (*E*) (First dimension) $CHCl_3$/MeOH/30% NH_3OH; 65:25:5. (Second dimension) $CHCl_3$/MeOH/H_2O/CH_3COCH_3/CH_3COOH; 30:10:5:40:10. (*F*) (First dimension) $CHCl_3$/MeOH/30% NH_3OH; 95:5:0.5. (Second dimension) $CHCl_3$/MeOH/H_2O/CH_3COCH_3/CH_3COOH; 80:2.5:2:15:2.5. (*G*) (First dimension) Hexanes/ethyl ether/acetic acid; 80:20:2. (Second dimension) $CHCl_3$/MeOH/30% NH_3OH; 95:5:0.5.

Plates were air dried and stained by exposure to iodine vapor or sprayed with various specific detection reagents as described in the text. Some plates were also sprayed with a strong oxidizing solution, charred by brief heating at 120°C and analyzed by scanning densitometry. All chromatograms in this communication were stained with iodine vapor (except for autoradiograms). Permanent records were made on 4 × 5 inch negatives or as direct photocopies. Radiolabeled chromatography plates were autoradiographed directly or sprayed with a 20% solution of 2,5-diphenyloxazole in ethyl ether or EN^{3}HANCE spray (New England Nuclear). Autoradiography was performed using Fuji RX or Kodak X-Omat AR X-ray film.

For preparative thin layer chromatography, compounds were run in parallel with reference lanes containing known cellular lipids or lipid standards. Plates were dried under N_2, scored and broken, and the reference lanes stained to determine the location of specific compounds. Adjacent areas on the unstained plate were lightly moistened with water, scraped, dried under N_2, and the lipid eluted from the sorbent with several washes of 1:1 $CHCl_3$/MeOH. Aliquots of the eluted lipid were always checked for purity by thin layer chromatography.

Liquid Column Chromatography

For separation of whole cell lipid extracts into phospholipid, glycolipid, and neutral lipid fractions, columns were prepared as follows. 1 g of 325 mesh silicic acid (Bio-Sil HA; Bio-Rad Laboratories, Richmond, CA) was washed in two changes of methanol, dried, and activated for several hours at 120°C. The silicic acid was slurried in $CHCl_3$ and packed on top of glass wool in a 4-mm (inside diameter) thick-walled glass chromatographic column equipped with a solvent reservoir and pressure couple (Kontes Co.). The column was washed with 50 ml $CHCl_3$ and the sample applied in a minimal volume of $CHCl_3$. Three fractions were eluted: neutral lipids (including monoglyceride, diglyceride, triglyceride, cholesterol, free fatty acid, alkyldiacylglycerol, and cholesteryl ester of fatty acid) were removed by three successive 5-ml aliquots of chloroform; neutral glycolipids were eluted in three 15-ml washes of acetone; and phospholipids were eluted with three 5-ml aliquots of methanol. Solvents were forced through the fine mesh silica under nitrogen supplied at 30 psi. Each fraction was found to be >98% pure by two-dimensional thin layer chromatography.

Hydrolysis and Detection of Plasmalogen (Alk-1-enyl) Linkages

A modification of previously published procedures (31, 62) was used to detect neutral and polar plasmalogen (alk-1-enyl lipids) on a single thin layer plate. Each sample was spotted on a separate plate in a single 1-cm lane spotted 1 cm from the left edge of the plate and separated according to the two-step

chromatography schedule C, as described above. This lane was then exposed to 12 N HCl at 40°C for 1–4 min at a distance of 4 cm while the remainder of the plate (all but the left-most 3.5 cm) was masked to prevent exposure to acid. Acid was removed by extensive evaporation under N_2, and the plate rotated counterclockwise for development in the second dimension according to chromatography schedule A. Aliquots of unhydrolyzed lipid and synthetic octadecenal were run in parallel to allow identification of the aldehyde products released by hydrolysis of the alk-1-enyl double bond.

Methanolysis and Fatty Acid Analysis

Samples were mixed in a 15-ml round bottom tube with 1.5 ml of a 9:1 mixture of methanol/acetyl chloride (prepared on ice) and 0.5 ml of dry toluene to aid in solubilization of neutral lipids (43). Tubes were gassed with nitrogen, tightly sealed using teflon-lined screw-caps and heated overnight at 80°C in a block heater. The next day, samples were cooled and the reaction quenched with 1 ml of H_2O. Lipids were extracted with two washes of 3 ml hexane each, and the aqueous phase discarded. The hexane fraction was dried completely by addition of a small amount of 4:1 $NaSO_4/NaHCO_3$, vortexed, filtered through glass wool and dried under nitrogen. Saponified lipids were examined by thin layer chromatography (Fig. 6 only) or resuspended in a small volume of CS_2 for gas–liquid chromatography.

Gas–liquid chromatography was carried out on a Hewlett-Packard HP7620A gas chromatograph with a model 3380S integrator. The FAME products of methanolysis were separated on a 6-ft × 2-mm glass column packed with 10% SP-2330 on 100/120 mesh Chromosorb W-AW (Supelco, Inc.). Nitrogen carrier flow was maintained at 1.8 psi, and the injection port and flame ionization detector set at 250°C. Oven temperature was programmed to hold 150°C for 2 min following injection, rise at the rate of 4°C/min to 200°C and hold that temperature for 30 min until all FAMEs were detected. Identical results were obtained when FAMEs were prepared by methanolysis of unextracted cells or of $CHCl_3$/MeOH extracts; no change was observed when FAME products of the saponification reaction were purified by preparative thin layer chromatography before injection.

RESULTS

Fusion-resistant Cell Lines

When the parental cell line, Clone 1D, is treated with a standard PEG–fusion protocol, <5% of the cells remain unfused (54, 59; Fig. 1 *A*). Most cells are fused into giant syncytia, containing hundreds or even thousands of nuclei. Repeated rounds of PEG treatment resulted in progressively more highly PEG-resistant cells, as shown in Fig. 1 for cell lines Clone 1D, F_4, F_8, F_{16}, and F_{40}. Treatment of F_{40} cells with PEG produced ~20% fusion (Fig. 1 *E*), and those cells which did fuse formed smaller multinucleates than found in fused monolayers of Clone 1D. The F_4, F_8, and F_{16} cell lines are intermediate in their response to PEG, as shown in Fig. 1, *B–D*. We have previously analyzed several subclones from cell lines with varying degrees of fusion resistance, and found that these cell lines are homogeneous with respect to PEG sensitivity (reference 59, and similar unpublished experiments). The fusion-resistant phenotype is stable at each level of selection (59). These results indicate that the PEG-resistant phenotype is gradually acquired by the population as a whole, rather than being produced by enrichment of the population with descendants from a mutant, PEG-resistant cell which was present in the original culture. Since isolation, the highly fusion-resistant cell line F_{40} has been maintained for over 250 generations in continuous culture with no effect on its sensitivity to PEG-induced fusion. For the biochemical studies presented below, we have primarily used cell lines Clone 1D, F_{16}, and F_{40}, which exhibit ~100, 50, and 20% fusion, respectively, following PEG treatment.

Analysis of Lipid Class Composition

To investigate specific cellular properties correlated with differences in response to PEG, we analyzed the genetic and biochemical characteristics of these cells. Fig. 2 illustrates the lipid class composition observed when Clone 1D, F_{16}, and F_{40} cells were extracted with organic solvents and separated by one-dimensional thin layer chromatography according to solvent schedule C. In Fig. 2 *A*, extracts of Clone 1D (lane *2*) and F_{40} (lane *5*) were run in parallel with lipid standards. The direction of solvent flow was from bottom to top, with the more polar lipids nearer the bottom. Major cellular lipids in the lower third of the plate correspond, in order of increasing mobility, to various phospholipids: sphingomyelin (doublet), phosphatidyl choline/phosphatidyl serine/phosphatidyl inositol/phosphatidic acid (not completely resolved from each other), cardiolipin, and phosphatidylethanolamine. Most of the minor bands visible in the lower two-thirds of the chromatogram (particularly between cardiolipin and phosphatidylethanolamine, and above the latter) are neutral glycolipids, confirmed by reaction with an α-naphthol spray reagent (not shown).

In the upper third of the chromatogram various neutral lipids are separated, producing a dense band of cholesterol followed by more hydrophobic compounds, including free fatty acids, diglycerides, triglycerides, alkyldiacylglycerols, and cholesteryl esters, in order of decreasing polarity. Most neutral lipids were considerably more pronounced in F_{40} cells than in Clone 1D, particularly the triglycerides (single arrows) and alkyldiacylglycerols (double arrows). Further characterization of alkyldiacylglycerol, an unusual ether-linked glyceride, is provided below. Quantitative densitometry of charred chromatograms indicated that triglyceride content is elevated approximately sixfold in F_{40} over Clone 1D, and that the ether lipid content is increased ~36-fold in F_{40} (average of three experiments). In these PEG-resistant cells, GE may represent as much as 20% of the neutral lipid, second only to cholesterol in prominence. Substantial levels of fatty alcohols, a metabolic precursor of the ether-linked lipids (24, 84), were also found in F_{40} cells (not clearly visible in this chromatogram). Monoglyceride and, to a lesser extent, diglyceride content was usually slightly increased in F_{40} relative to Clone 1D. The content of free fatty acids found in these cells was somewhat variable, with free fatty acid content usually slightly elevated in Clone 1D over F_{40}. Cholesteryl esters are less common in most preparations of Clone 1D than in F_{40}, but differences in cholesterol level were not consistently observed.

One of the advantages of a graded series of cell lines is the ability to examine not only fusible and fusion-resistant cells, but also a wide range of intermediate cell lines. Fig. 2 *B* demonstrates that the differences in neutral lipid composition observed between Clone 1D and F_{40} were acquired gradually throughout the selection procedure. Both triglyceride and ether lipid content increase between Clone 1D and the moderately fusion-resistant cell line F_{16} (compare lanes *1* and *2* with lane *3*), and rise further in F_{40} (lanes *4* and *5*). As in Fig. 2 *A*, no differences in polar lipid composition were seen between cell lines. This figure also illustrates the reproducibility of these results and the stability of the cell lines: the samples shown in lanes *1* and *5* were separated from those used in lanes *2–4* by ~1 yr of continuous culture, and the earlier and later cell stocks were grown in medium supplemented with different serum lots. Nevertheless, the two extracts of Clone 1D cells (lanes *1* and *2*) are virtually indistinguishable from each other, and both contain low levels of triglyceride and alkyldiacylglycerol, while both F_{40} cell extracts (lanes *4* and

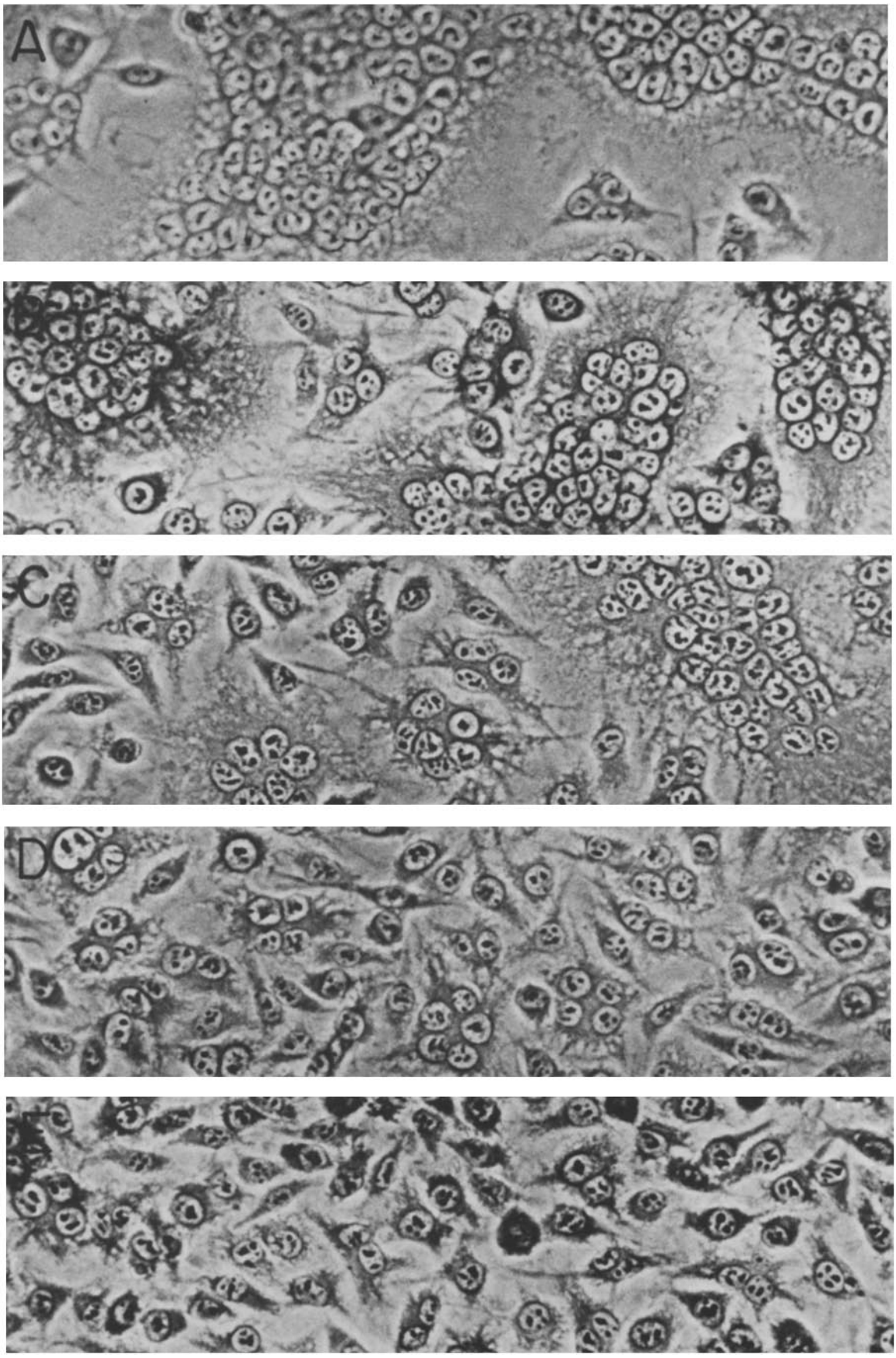

FIGURE 1 Response of Clone 1D and fusion-resistant cell lines to PEG. Confluent cultures of Clone 1D and cell lines selected from Clone 1D by 4, 8, 16, or 40 cycles of PEG treatment (designated F_4, F_8, F_{16}, and F_{40}, respectively) were fused with 50% PEG 1000. Fields shown were selected at random. (*A*) Clone 1D; (*B*) F_4; (C) F_8; (*D*) F_{16}; and (*E*) F_{40}.

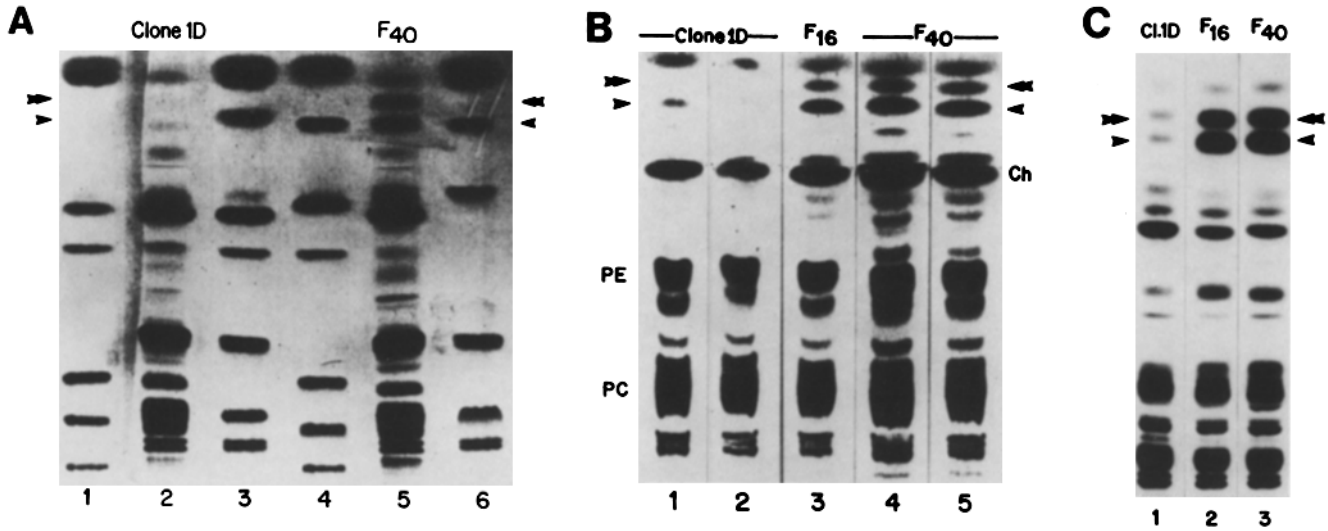

FIGURE 2 Lipid class composition of Clone 1D, F_{16}, and F_{40} cells. Each lane contains lipids extracted from 10^7 cells (*A* and *B*) or 25,000 cpm ^{14}C derived from labeled acetate (C). All thin layer chromatograms were run according to solvent schedule C, and exposed to iodine vapor (*A* and *B*) or autoradiography film (C). Direction of solvent flow is from bottom to top. (*A*) Lipid extracts of Clone 1D and F_{40} cells are shown in lanes *2* and *5*, respectively. Other lanes contain mixtures of standards at 15 μg/compound (30 μg for cholesterol [Ch]); these are as follows: (lane *1*) (from bottom to top) lysophosphatidyl choline (LPC); phosphatidyl inositol (PI); cardiolipin (CL); monoglyceride (MG); fatty alcohol (FAOH); cholesteryl ester of fatty acid (CE); and long chain hydrocarbon (HC; merged with CE). (Lane *3*) Sphingomyelin (SM; doublet); phosphatidyl choline (PC); phosphatidylethanolamine (PE); MG; Ch; FAME; CE; and HC. (Lane *4*) LPC; phosphatidyl serine (PS); CL; MG; free fatty acid; triglyceride (TG); and HC. (Lane *6*) SM; phosphatidic acid (PA); PE; diglyceride (DG); TG; CE; and HC. TG is indicated by single arrows; double arrows denote the unusual ether-linked lipid discussed in the text (alkyldiacylglycerol [GE]). (*B*) Correlation of ether lipid with fusion-resistant cells. (Lanes *1* and *2*) Clone 1D. (Lane *3*) F_{16}. (Lanes *4* and *5*) F_{40}. Cells extracted for lanes *1* and *5* were grown in one serum lot, and were separated by nearly 1 yr of continuous culture from cells in lanes *2–4*, which were grown in a different serum lot. The presence of GE (double arrows) was correlated with fusion resistance, but not with age in culture or growth medium used. The ether lipid and triglyceride content of F_{16} is intermediate between Clone 1D and F_{40}. (C) Metabolic labeling of Clone 1D, F_{16}, and F_{40} lipids. Cells were incubated for 24 h in the presence of ^{14}C-labeled acetate, followed by extraction, thin layer chromatography, and autoradiography. Single and double arrows indicate the position of TG and GE, respectively. (Lane *1*) Clone 1D. (Lane *2*) F_{16}. (Lane *3*) F_{40}.

5) contain elevated levels of triglyceride and alkyldiacylglycerol.

Fig. 2*C* illustrates the metabolic labeling of Clone 1D, F_{16}, and F_{40} cells with ^{14}C-acetate. Labeling of polar lipids was similar in Clone 1D, F_{16}, and F_{40} (lower portion of lanes *1*, *2*, and *3*, respectively), but differences in neutral lipid composition that were observed in unlabeled cells were also seen with the radioactive lipids. Indeed, the enhancement of triglyceride and alkyldiacylglycerol production is somewhat more pronounced in the metabolically labeled cells.

To examine lipid class composition in greater detail, a series of two-dimensional thin layer chromatographic separations was used. Fig. 3 shows the analysis of Clone 1D (*A–C*) and F_{40} (*D–F*) using three different solvent schedules designed to separate different groups of compounds. For each solvent schedule used, a composite drawing has been provided (*G–I*), with the lipid species numbered as described in the legend. In the left panels are shown separations of phospholipids and neutral glycolipids. Despite clear separation of a wide variety of phospholipid and neutral glycolipid species (Fig. 3*G*), neither qualitative nor quantitative differences have been detected between Clone 1D and F_{40} (compare *A* and *D*). Phospholipids are numbered *1–8*; glycolipids are separated along an irregular concave-up curve of doublets and singlets which have not been numbered (identity determined by staining with specific spray reagents and pattern of elution from a silicic acid column). The chromatograms reproduced in the center panels (Fig. 3, *B* and *E*) separated lipids of intermediate polarity, including monoglycerides, cholesterol, and free fatty acids, as indicated in Fig. 3*H*. Again, no substantive differences were detected between Clone 1D and F_{40} cells (the increase in monoglyceride in F_{40} cells is not always as apparent as here; cf. Fig. 2*A*). Neutral lipid separations are shown in the right panels (Fig. 3, *C*, *F*, and *I*). As already seen in the one-dimensional separations, triglyceride (single arrows) and alkyldiacylglycerol (double arrows) were greatly elevated in F_{40} (Fig. 3*F*) over Clone 1D (Fig. 3*C*).

The highly polar gangliosides partition into the aqueous phase during Folch extraction, and hence are not visible in the chromatograms shown above. Because glycolipids are primarily found in plasma membranes and have been implicated in various membrane interactions (27), ganglioside composition was examined in several of the fusion-resistant mutants. Fig. 4 shows the separation of the polar gangliosides purified (as described in Materials and Methods) from radiolabeled Clone 1D, F_{16}, and F_{40} extracts (lanes *1–3*) and an unlabeled mixture of Clone 1D and F_{40} gangliosides (lane *4*). This chromatogram suggests that increased resistance to PEG-induced fusion is correlated with decreased ganglioside content. The significance of these results is unclear, however, as so little ganglioside is present in any of the cell lines under study. Polar glycolipids account for <0.5% of the ^{14}C-acetate label in Clone 1D, and even less in F_{16} and F_{40} cells (0.25% and 0.2%, respectively). Neutral glycolipid composition varied considerably from experiment to experiment, and appears to be strongly affected by cell density (not shown). No repro-

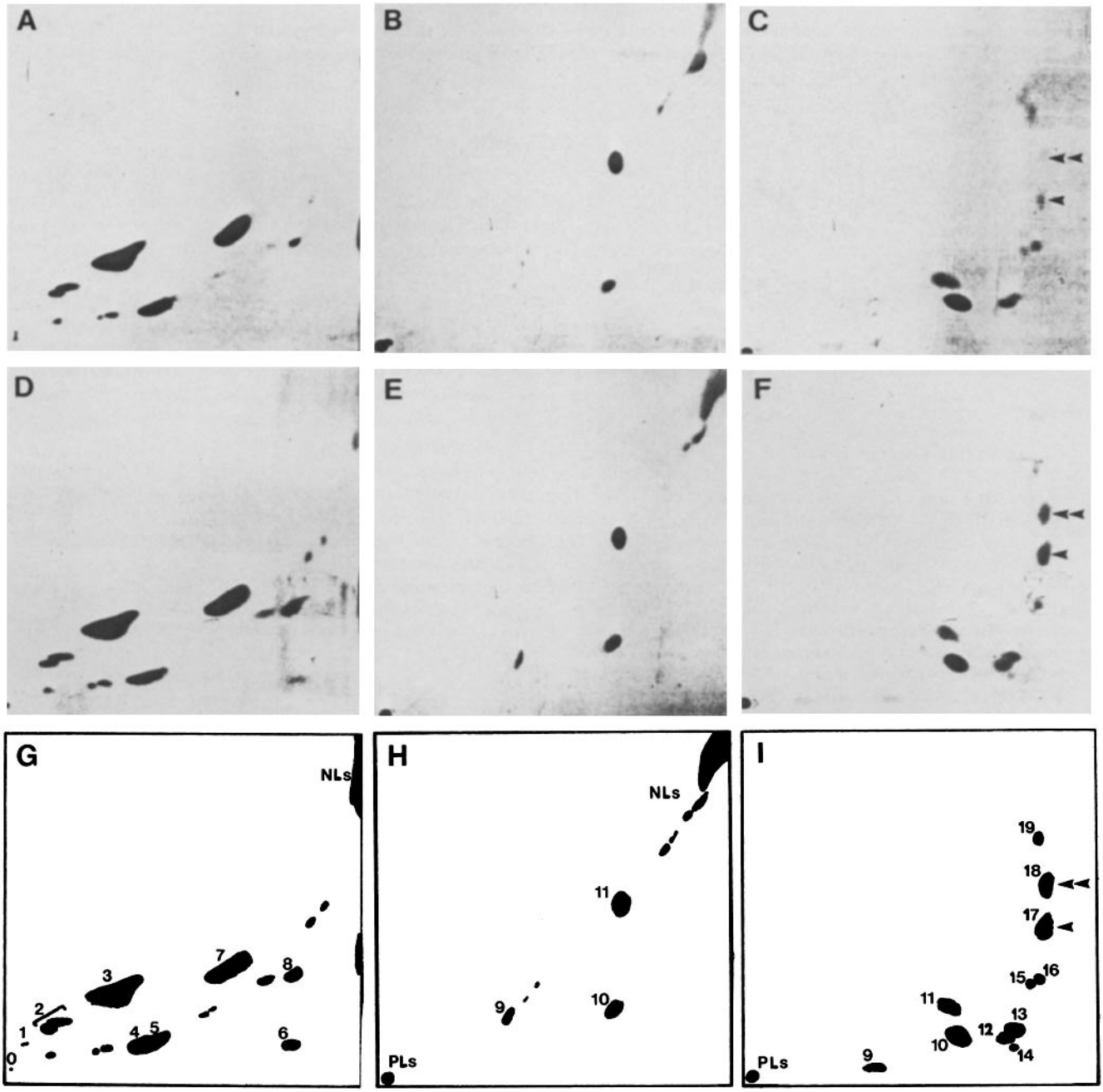

FIGURE 3 Two-dimensional analysis of lipid composition. Lipid extracts of Clone 1D cells (*A–C*) or F_{40} cells (*D–F*) were subjected to two-dimensional thin layer chromatography according to solvent schedule E (*A* and *D*) to separate polar lipids, schedule F (*B* and *E*) to separate lipids of intermediate polarity, or schedule G (*C* and *F*) to separate neutral lipid classes. Lipids from 3×10^7 cells were spotted at the lower left corner of each plate, and in all panels the direction of solvent flow was from bottom to top in the first solvent mixture and from left to right in the second dimension. *G–I* are composite drawings representing spots detected in the corresponding chromatograms. Numbered spots have been identified as follows: *0*, origin; *1*, LPC; *2*, SM; *3*, PC; *4*, PS; *5*, PI; *6*, PA; *7*, PE; *8*, CL; *9*, MG; *10*, Ch; *11*, free fatty acid; *12*, 1,2-DG; *13*, 1,3-DG; *14*, unknown; *15*, 1-alkyl,2-acylglycerol?; *16*, 1-alkyl,3-acylglycerol?; *17*, TG; *18*, GE; *19*, CE. Most of the unlabeled spots which are separated along a gentle convex-up curve in panels *A* and *D* are neutral glycolipids. Clone 1D and F_{40} lipids are similar in all respects save GE (double arrows) and TG (single arrows). Abbreviations are as in Fig. 2.

ducible correlation could be established between neutral glycolipids and fusion response, however, suggesting that these compounds do not play a direct role in PEG-induced fusion.

Characterization of the Ether Lipid That Is Enriched in Fusion-resistant Cells

Alkyldiacylglycerol, the neutral lipid compound whose presence is most highly correlated with the PEG fusion-resistant phenotype, is an unusual one, intermediate in polarity between triglyceride and cholesteryl ester in the solvent systems thus far illustrated (double arrows in Figs. 2 and 3). We were first alerted to the presence of this lipid by chance, due to the development of a thin layer chromatography plate in a solvent in which the polarity had changed due to absorption of excessive moisture from the air. The then-unidentified

lipid was characterized by peculiar mobility, moving differently with respect to other neutral lipids depending on the solvent system used, a phenomenon illustrated in Fig. 5.

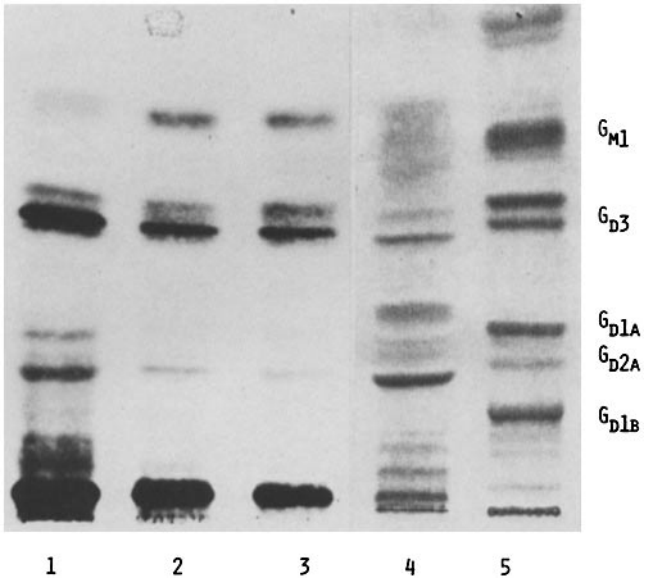

FIGURE 4 Gangliosides of Clone 1D, F_{16}, and F_{40} cells. The aqueous (upper) phases from Folch extractions of [^{14}C]acetate-labeled (lanes *1–3*) or unlabeled (lane *4*) cells were extensively dialyzed against water, lyophilized, and chromatographed according to solvent schedule D. Extracts from 3×10^7 labeled cells were spotted in lanes *1–3* (Clone 1D, F_{16}, and F_{40} cells, respectively) and fluorographed for 61 d. Lane *4* contains a mixture of 2×10^8 Clone 1D and 2×10^8 F_{40} cells, and was developed by exposure to iodine vapor. Lane *5* contains bovine brain gangliosides.

Panels *A* and *B* show reproductions of identically spotted thin layer plates which were developed in different solvents. Lipids extracted from Clone 1D are shown in lanes *2*, while F_{40} lipids are in lanes *3*. The ether lipid (indicated by a star) was clearly present at high concentration in F_{40}, but was undetectable in Clone 1D cells. Lanes *1* and *4* contain mixtures of neutral lipid standards. The only relevant difference between these standard mixtures is the presence of FAME in lanes *1* (methylolein; indicated by single arrows) and a synthetic ether lipid (1-O-hexadecyl, 2,3 dipalmityl-glycerol; double arrows) in lanes *4*. In Fig. 5*A*, the ether lipid ran very rapidly, migrating ahead of FAME, and considerably faster than triglyceride. In a different solvent system, however, the synthetic ether lipid ran far more slowly than FAME and only slightly faster than triglyceride (Fig. 5*B*). This pattern of migration in different solvents is characteristic of alkyldiacylglycerols (69), an interesting minor lipid species synthesized by the replacement of the acyl chain of acyl-dihydroxyacetone phosphate with an alkyl group derived from free fatty alcohols (24, 84). In each solvent system, the unusual lipid associated with F_{40} cells comigrates with the synthetic alkyldiacylglycerol, and is clearly distinct from triglyceride, FAME, cholesteryl ester of fatty acid, and other neutral lipids.

To prove the identity of the unusual F_{40} lipid, this compound and the putative triglyceride band was isolated by preparative thin layer chromatography and subjected to methanolysis. Extensive acid hydrolysis in the presence of methanol would be expected to release any esterified fatty acid (acyl) chains as FAME while leaving ether-linked (alkyl) fatty acids attached to the glycerol backbone. Fig. 6*A* shows products from the methanolysis of synthetic standards. Lipid standards in lanes *1*, *4*, and *5* demonstrate the mobility of various

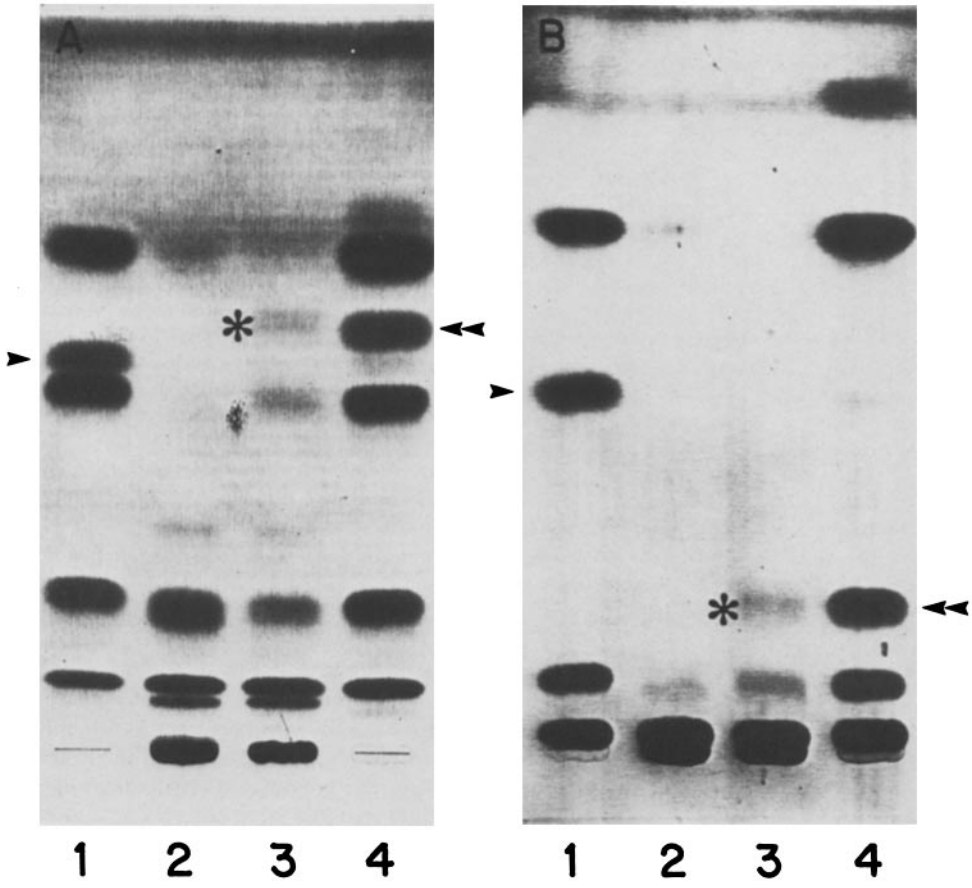

FIGURE 5 Thin layer chromatographic mobility of the unusual lipid found in F_{40} cells. Lipid extracts from Clone 1D and F_{40} cells were chromatographed in parallel with authentic standards. *A* and *B* are identical except for being run in different solvent systems (*A*, solvent schedule A; *B*, solvent schedule B). (Lanes *1*) Ch; free fatty acid; TG; FAME; and CE (in order of decreasing polarity, from top to bottom). (Lanes *2*) Lipid extract from 1.5×10^7 Clone 1D cells. (Lanes *3*) Lipid extract from 1.5×10^7 F_{40} cells. (Lanes *4*) Ch; free fatty acid; TG; GE; CE; and HC. The synthetic ether lipid (1-O-hexadecyl, 2,3 dipalmityl glycerol, indicated by double arrowheads) migrates particularly rapidly in *A* and particularly slowly in *B* relative to other standards (compare especially with FAME, indicated by single arrows). In both panels, however, the unusual lipid found in F_{40} (*) co-migrates with GE. Abbreviations are as in Fig. 2.

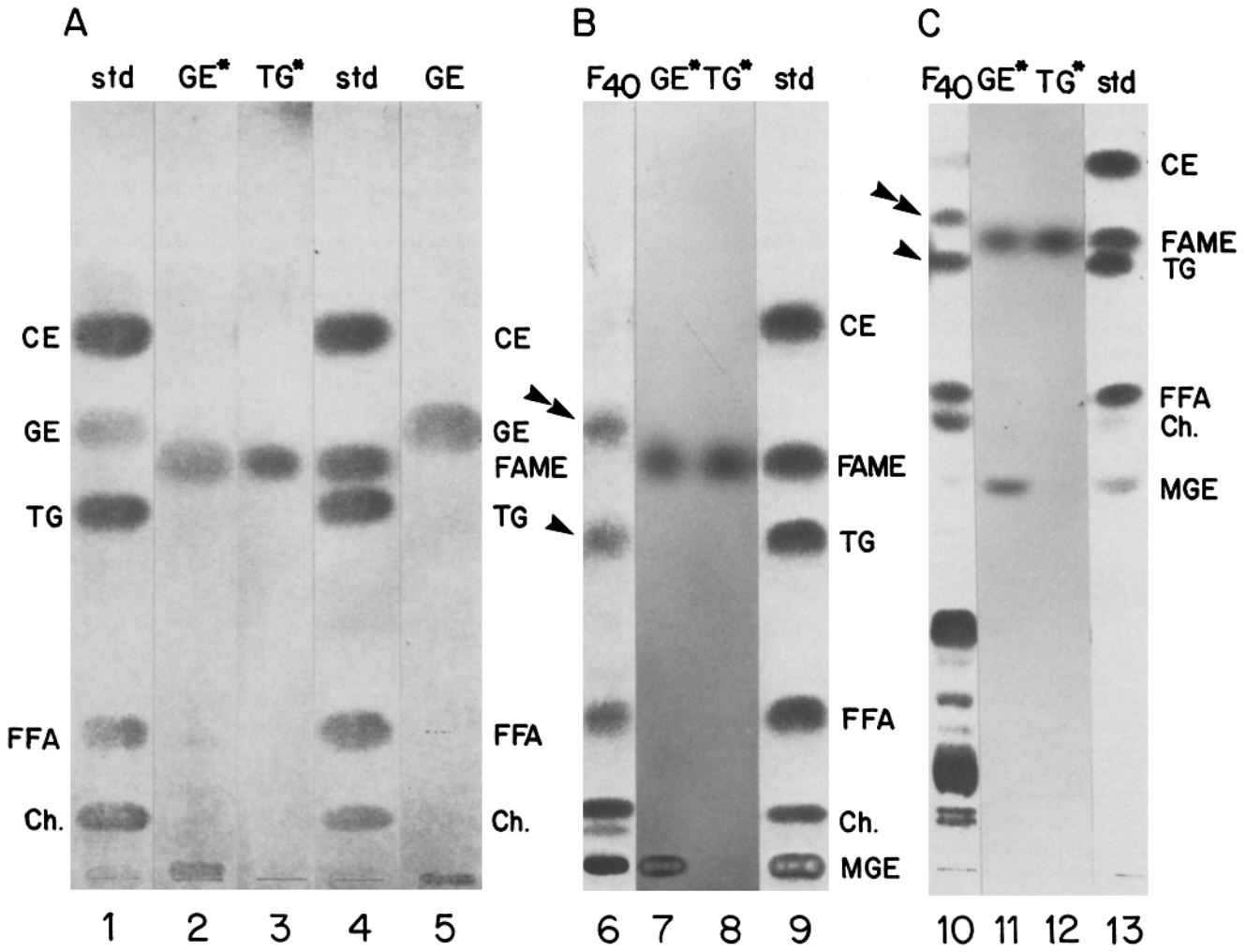

FIGURE 6 Methanolysis of the ether lipid. Synthetic lipid standards or experimental samples prepared by preparative thin layer chromatography of F_{40} cell lipid extracts were refluxed in methanolic HCl and saponified products extracted in hexane for chromatography in parallel with unreacted lipid standards. Comparison of the methanolysis products of known compounds in *A* (lanes *2* and *3*) with F_{40}-derived lipids run in the same solvent system (*B*, lanes *7* and *8*) confirms the identity of these F_{40} lipids as GE and TG. (*A*) Methanolysis of synthetic tripalmitylglycerol (TG) and 1-O-hexadecyl, 2,3-dipalmitylglycerol (GE). Thin layer chromatography was run according to solvent schedule A. (Lane *1*) Unreacted mixture of Ch, free fatty acid, TG, GE, and CE (from bottom to top in order of decreasing polarity). (Lane *2*) Methanolyzed GE, producing FAME (upper spot) and monoalkylglycerol (MGE) (near the origin). (Lane *3*) Methanolyzed TG, producing FAME only. The glycerol backbone is not soluble in hexanes, and hence was not extracted from the saponification mixture. (Lane *4*) Untreated Ch, free fatty acid, TG, FAME, and CE. This lane differs from lane *1* only in the substitution of FAME for GE. (Lane *5*) Unsaponified GE; when juxtaposed with FAME in lane *4*, this compound is clearly distinct. (*B* and *C*) Methanolysis of putative GE and TG bands isolated from ^{14}C-acetate-labeled F_{40} cells. *B* and *C* differ only in the solvent system for separation: *B* was run in system *A*, and *C* according to schedule C. (Lanes *6* and *10*) Unreacted lipid extracts from F_{40} cells. Double and single arrowheads indicate, respectively, the putative GE and TG bands which were eluted for saponification. (Lanes *7* and *11*) Methanolysis products of the putative GE band purified from F_{40} cells. Two spots are visible, corresponding to FAME (upper spot; compare lane *11* with the FAME standard in lane *13*) and MGE (lower spot; compare lane *7* with the synthetic standard in lane *9*). Relative activity of these compounds was approximately 2:1, as expected for GE. (Lanes *8* and *12*) Methanolysis of the putative TG band from F_{40}. Only FAME was found in this sample, indicating that the original sample contained acyl, but not alkyl linkages. Abbreviations are as in Fig. 2.

compounds in this system; alkyldiacylglycerol was clearly distinguishable from other neutral lipids (including FAME) as shown by comparison of lanes *4* and *5*. Lipids produced by the methanolysis of alkyldiacylglycerol and triglyceride are shown in lanes *2* and *3*, respectively. The only product of the triglyceride reaction was FAME (the glycerol backbone does not extract from the reaction mixture into organic solvent). Methanolysis of alkyldiacylglycerol, however, produced both acyl chains and 1-O-alkylglycerol, visible slightly above the origin in lane *2*.

Fig. 6, *B* and *C*, illustrates the same procedure applied to lipids extracted from F_{40}. These two chromatograms were spotted with identical samples but run in different solvent systems. In lanes *6* and *10*, unfractionated F_{40} lipids are shown, indicating the mobility of the putative ether lipid (double arrows) and triglyceride (single arrows) in these solvent systems. These bands were extracted, methanolyzed, and run in lanes *7* and *11* (alkyldiacylglycerol) or *8* and *12* (triglyceride). Lipid standards were run in lanes *9* and *13*. In lanes *8* and *12*, only FAME was found, confirming the

compound indicated by single arrows as triglyceride. When the ether lipid was saponified, however, both monoalkylglycerol and FAME were released (most clearly seen in lanes *7* and *11*, respectively). Removal of these spots and quantitation in a scintillation counter demonstrated a 2:1 ratio of fatty acid chains between the ether lipid–derived FAME and monoalkylglycerol, confirming this unusual lipid from F_{40} cells as alkyldiacylglycerol. The ratio of radioactivity released as FAME by saponification of triglyceride and alkyldiacylglycerol was ~3:2, as predicted. Similar analyses of other neutral lipid bands (not shown) demonstrated the presence of alkyldiacylglycerol and small amounts of monoalkylglycerol in F_{40}. No di- or trialkylglycerols were detected.

The neutral ether lipid found in F_{40} cells is distinct from plasmalogens (lipids which contain an alk-1-enyl–linked hydrocarbon chain), based both on thin layer chromatographic mobility and susceptibility to acid hydrolysis. As the plasmalogens are the most prominent ether-linked lipid in most animal tissues (32), it was of interest to examine plasmalogen content in Clone 1D and F_{40} cells. Because of the proximity of the double bond to the ether-oxygen in plasmalogens, these compounds are very sensitive to acid hydrolysis, liberating the hydrocarbon chain as an aldehyde (3, 62). Taking advantage of this fact, a single-plate chromatographic procedure was used to simultaneously allow distinction of alkyl and alk-1-enyl linkages in both neutral and phospholipids.

In Fig. 7, lipids extracted from Clone 1D (*A*) or F_{40} (*B*) were separated in the vertical dimension, briefly exposed to acid fumes, and the partial hydrolysis products separated from left to right in parallel with a fresh aliquot of unhydrolyzed lipid (at the top of the plate). The migration of an aldehyde standard is indicated at the bottom of the chromatograms by large arrows. Substantial amounts of ethanolamine plasmalogen were cleaved by the action of HCl fumes, as indicated

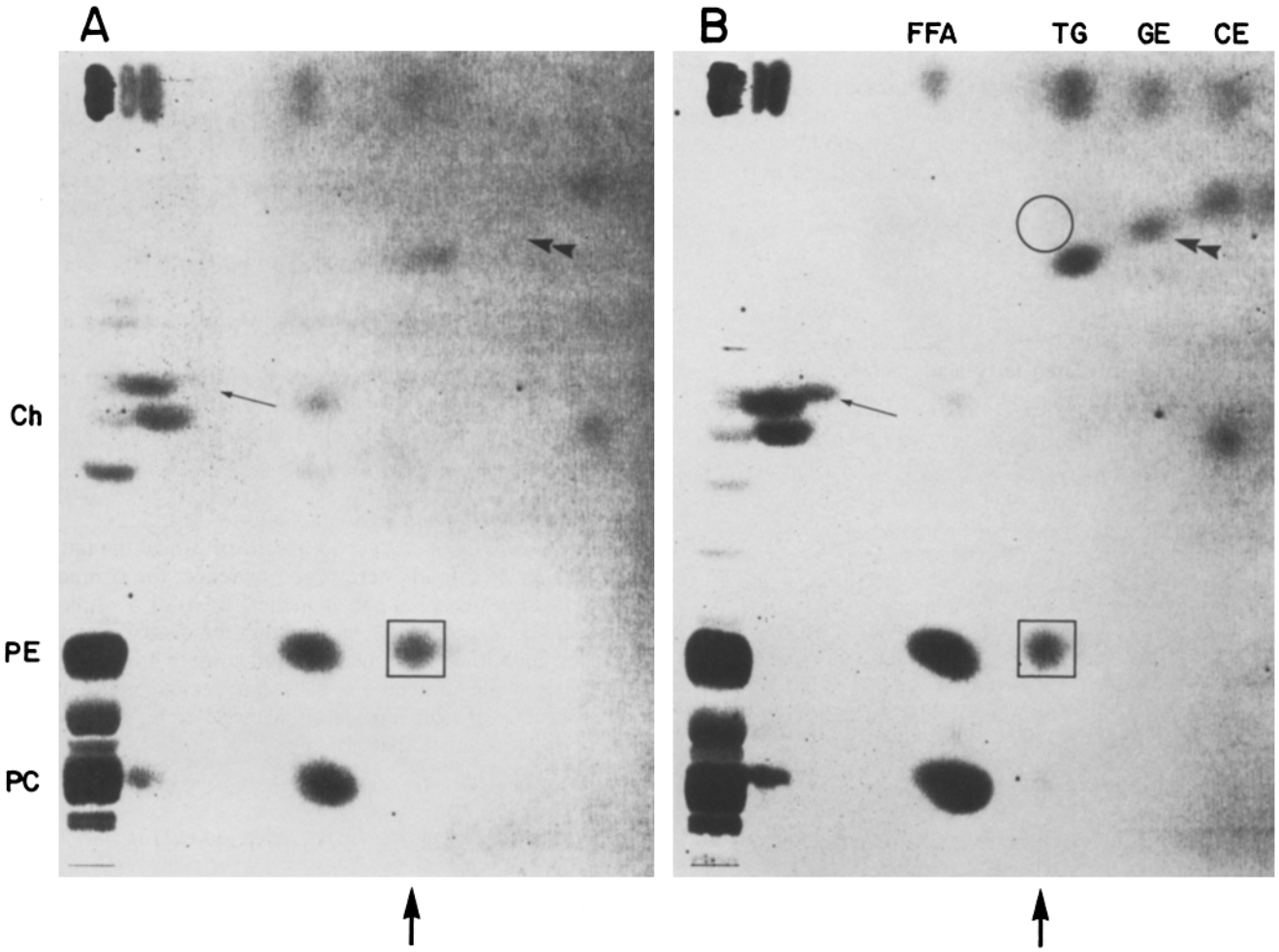

FIGURE 7 Plasmalogen content of Clone 1D and F_{40} cells. Lipid extracts from 5×10^7 Clone 1D or F_{40} cells (*A* and *B*, respectively) were separated according to solvent schedule C, exposed to HCl fumes briefly to hydrolyze the alk-1-enyl bonds of plasmalogens, and separated in the second dimension (from left to right) in solvent system A, in parallel with unhydrolyzed sample at the top of the plate. Hydrolysis of the plasmalogen alk-1-enyl linkages released aldehydes from PE (marked by the square) but not from the neutral ether lipid (double arrowhead), demonstrating that the ether-linked lipid in F_{40} is not a neutral plasmalogen. The arrow at the bottom of the figure indicates the migration of an aldehyde standard; the expected position for aldehydes released from neutral plasmalogens is marked with a circle. In this particular experiment, lipids separated in the first dimension were exposed to acid fumes for approximately twice as long as necessary to detect aldehyde release to ensure that plasmalogen hydrolysis was complete in the neutral lipids, which are somewhat less accessible due to their tendency to form lipid droplets within the chromatogram sorbent. As a result of this prolonged hydrolysis, some fatty acids were also released from PE and PC. Several compounds are identified at left and in the unhydrolyzed sample at top. Increased FAOH content can be seen in F_{40} cells compared with Clone 1D (small arrows). Abbreviations are as in Fig. 2.

by the boxes in this figure, but no differences in plasmalogen content were detected between Clone 1D and F_{40} lipids. Low levels of choline plasmalogens were also detected in both cell lines. No aldehydes were released from the ether lipid found in F_{40} cells (open circle), confirming this compound as an alkyl, rather than alk-1-enyl, lipid. These chromatograms also allow clear visualization of fatty alcohols (small arrows), demonstrating considerably higher concentrations of this important ether lipid precursor in F_{40} cells than were found in Clone 1D.

Composition of Fatty Acid Chains

Biologically relevant fatty acids are found with diverse chain length and degree of saturation. As a major component of cell membranes, they may exert considerable influence on membrane interactions (35, 73, 75). Quantitative analysis of the fatty acid composition of Clone 1D and several of the fusion-resistant cell lines is shown in Table I. Individual fatty acids are compiled into saturated, monounsaturated, or polyunsaturated classes at the top of the table. The most striking variation observed was the gradual increase of saturated fatty acids in the more fusion-resistant cells, and the concomitant loss of polyunsaturated fatty acids. The ratio of saturated to polyunsaturated fatty acids (S/P ratio) increased from 1:1 in the parental cells to 4:1 in the F_{40} mutants. Cell lines of intermediate resistance to PEG treatment exhibited intermediate S/P ratios: 2.4 for F_4, 2.9 for F_8, and 3.4 for F_{16}.

In the course of the selection of our most highly fusion-resistant cell line (F_{40}), 55% of the arachidonic acid (20:4) found in Clone 1D was lost. In parallel, the content of the most common saturated fatty acid, stearic acid (18:0), was increased by 59%. There did not seem to be any strong preference for loss of specific polyunsaturates or acquisition of any particular species of saturated fatty acid. The most striking change was the prominence of a rare saturated fatty acid (lignoceric acid, 24:0) in F_{40}. This compound was barely detectable in Clone 1D, but increased fivefold in F_{40} cells, accounting for 5% of the whole cell saturated fatty acid (by weight). In general, however, across the transition from Clone 1D to F_{40} cells, individual acyl chain species varied in parallel with other fatty acids of similar saturation.

TABLE I. *Fatty Acid Composition of Clone 1D Cells and Cell Lines with Increased Resistance to Polyethylene Glycol–induced Fusion**

	Clone 1D	F_4	F_8	F_{16}	F_{40}
Class‡					
Saturated	29	38	41	42	43
Monoenoic	39	45	44	43	44
Polyenoic	29	15	14	12	11
S/P RATIO	1.00	2.43	2.88	3.35	4.04
Individual Acids					
14:0	0.3	0.5	0.6	0.7	1.0
14:1	0.8	1.1	1.3	1.0	1.2
16:0	14.4	17.0	18.1	18.2	19.4
16:1	5.8	4.2	4.3	4.2	4.4
18:0	12.4	17.4	19.1	19.7	19.7
18:1	31.3	38.3	37.2	36.9	37.6
18:2	4.5	3.5	2.5	2.6	2.5
20:0	0.3	0.4	0.4	0.5	0.4
20:1	0.7	1.1	1.0	1.0	1.1
20:2	0.5	0.4	0.1	0.1	0.1
20:4	10.1	4.8	5.4	4.9	4.5
22:0	0.9	0.6	0.7	1.0	0.8
22:4	4.3	2.2	2.7	2.0	1.8
22:5	3.2	1.1	0.7	1.0	0.5
22:6	4.6	1.9	2.1	1.9	1.3
24:0	0.4	1.5	1.7	1.8	1.9
Others§	5.4	4.2	2.1	2.6	1.9

* Results are presented as the percentage of total fatty acid chains. Average of five experiments.
‡ Compilations by class do not total 100% because of unidentified lipids.
§ "Others" includes both unidentified and minor identified species.

TABLE II. *Fatty Acid Composition of Lipid Fractions**

	Whole cells			Phospholipids			Neutral lipids		
Cell line	S	M	P	S	M	P	S	M	P
Clone 1D	32	42	24	32	36	31	52	33	14
F_{16}	38	44	16	33	38	24	45	41	12
F_{40}	40	44	12	35	43	20	54	34	12

* Percent of fatty acyl chains present as saturated (*S*), monounsaturated (*M*), or polyunsaturated (*P*) in whole cells or phospholipid or neutral lipid column fractions from liquid chromatography on silicic acid.

To examine the relationship between the elevated neutral lipid content and increased saturation of fatty acids, both of which correlate with increased resistance to PEG-induced fusion of these cells, phospholipid and neutral lipid fractions were separated from Clone 1D, F_{16}, and F_{40} cells, and these fractions were analyzed for fatty acid content. Data from these experiments are shown in Table II. As already shown in Table I, the whole cell lipids of PEG-resistant cell lines exhibit increased saturation and a decreased content of polyunsaturated fatty acids. However, separated fractions of neutral lipids or phospholipids showed relatively little difference in fatty acid composition between cell lines. The S/P ratio in neutral lipids changed from 3.7 to 4.5 and the S/P ratio of phospholipids changed from 1.0 to 1.8. Data not presented here indicated nearly constant S/P ratios in glycolipid fatty acid chains as well. When individual species of lipids (e.g., phosphatidyl choline or triglyceride) were examined the fatty acid chains of these lipids were found to reflect the composition of the class (phospholipid or neutral lipid) as a whole (data not shown). These results suggest that the increased fatty acid saturation and elevated neutral lipid content seen in F_{40} cells are related phenomena: F_{40} cells derive a large part of their high S/P ratio from the elevated levels of highly saturated neutral lipids which they contain.

DISCUSSION

Lipid Composition of PEG-resistant Cells

To explore the biochemical mechanisms by which cells control membrane fusion, we have taken advantage of a series of cell lines that were selected for resistance to the chemical fusogen polyethylene glycol. The studies described above demonstrate a direct correlation between resistance to PEG and two aspects of cellular lipid composition: elevated levels of alkyldiacylglycerols, and increased saturation of fatty acyl chains. The content of these lipid components is correlated with fusibility not only at the extremes of highly fusible and highly fusion-resistant cells, but also across a wide range of intermediate phenotypes. Cells that are partially resistant to PEG exhibit an intermediate lipid composition (cf., Fig. 2*B* and Table I). These differences in lipid composition are highly reproducible and independent of cell density.

Ether-linked lipids are common constituents in the tissues of certain marine organisms and are found at low concentration in many other animals, but they are only rarely prominent in mammalian cells (for review see reference 68). Some ionic ether lipids appear to have potent biological activities, including platelet-activating factor (5), a choline ether lipid with platelet-activating and anti-hypertensive activities (28, 49). Several investigators have demonstrated a correlation between neutral ether lipids and a wide variety of human and animal tumors of different tissues (4, 8, 11, 40, 67, 68, 82). Tumors induced by the injection of cultured cells also contain high levels of alkyldiacylglycerol (3, 33, 70). In experiments presented elsewhere, the series of cell lines from Clone 1D through F_{40}, which were selected without any direct reference to tumorigenicity but which possess gradually increasing amounts of alkyldiacylglycerol, have provided direct evidence for a correlation between ether-linked lipids in vitro and tumorigenicity in vivo (57).

The first committed step in the biosynthesis of ether lipids is formation of the alkyl bond, which occurs by substitution of a fatty alcohol for the esterified fatty acid of acyl-dihydroxyacetone phosphate (24, 84). F_{40} cells contain increased fatty alcohol levels in addition to elevated ether lipid content (Fig. 7). Recent research has elucidated further molecular details of the primary synthetic mechanism (10, 20), although little is known of the complex metabolic pathways which may act to regulate the biosynthesis of ether lipid (26). Ether lipid content has been linked to the activities of many enzymes, including a specific etherase (71), glycerol-3-phosphate dehydrogenase (24, 33), and other glycolytic enzymes (66).

In addition to increased ether lipid content, PEG-resistant cells contain dramatically elevated fatty acid saturation. Differences in acyl chain composition, particularly chain saturation, have been studied in many systems (73, 75). There is a surprising degree of flexibility in the bulk fatty acid composition of cells, however, and cells in culture will generally incorporate any fatty acid supplied, with minimal change in physiology (72). Nevertheless, under certain conditions, altered fatty acid composition has been associated with several biological phenomena, including the activation of membrane receptors, membrane-bound enzymes, and transport channels (for review see reference 61). The fluidity of membranes is largely a function of fatty acid composition, and there has been considerable speculation that cellular lipids may segregate into microdomains within membranes (35, 37, 78). Experiments not presented here have shown similar fatty acid saturation to that presented in Table I in Clone 1D and F_{40} cells grown in delipidated medium, suggesting that these cells maintain substantial control over their membrane fluidity even when grown in serum-containing medium.

Although the increases in ether lipid content and fatty acid saturation in fusion-resistant cells were discovered separately, the data from Table II is compiled in Fig. 8 to suggest that these phenomena may be related: F_{40} cells derive their saturated fatty acids primarily from the large amounts of neutral lipids they contain. Neutral lipids usually possess highly saturated fatty acids (72), and the PEG-resistant cells have very high neutral lipid content as compared with the parental line. Thus the high S/P ratio of neutral lipids makes a major contribution to F_{40} cells, while S/P measurements of Clone 1D are largey determined by the relatively unsaturated phospholipids.

It is important to note that the analyses presented above reflect the lipid composition of whole cells. The majority of neutral lipid fatty acids are attached to triglycerides in intracellular lipid droplets, but substantial amounts of neutral lipid fatty acid in F_{40} cells are also present in ether-linked glycerides. The enzymes involved in the biosynthesis of ether lipid are microsomal (9, 15), and the alkyl-dihydroxyacetone phosphate synthase is found in particularly large quantities in peroxisomes (25). Cell fractionation studies from several laboratories have previously demonstrated neutral ether lipids in membranous material derived from tumors (70, 83) and in the plasma membranes of cultured cells (19, 48, 63). Analysis of the membranes of enveloped viruses grown in F_{40} cells, and preliminary analysis of plasma membranes prepared by classical cell fractionation techniques has confirmed this observation (56).

Other biochemical associations with PEG resistance may also play a role in altered fusion response. In Fig. 4, progressive changes in ganglioside content are visible across this series of cell lines. Several arguments lead us to suspect only a minor role for these compounds in fusion, however. Very small amounts of ganglioside were found in any of the cell lines studied, and the progression from Clone 1D to F_{40} was not always seen as clearly as shown in Fig. 4. Variation in ganglioside content from experiment to experiment is considerable, and does not correlate with any detectable change in response to PEG. We have also examined the effects of a wide variety of lectins on cell fusion, and although differences in

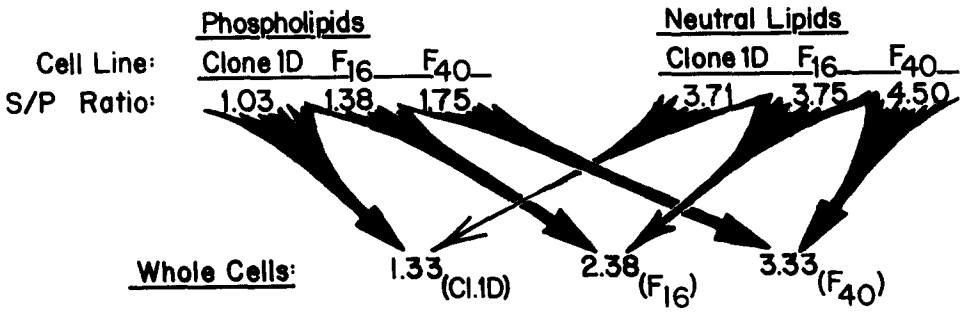

FIGURE 8 Relative contributions of phospholipids and neutral lipids to Clone 1D, F_{16}, and F_{40} fatty acyl chain composition. In the upper part of the figure, the S/P ratios are given for phospholipids and neutral lipids of three cell lines (data compiled from Table II). S/P ratios of total cell lipids are shown on the lower line. The relative contributions of phospholipids and neutral lipids to the total cell fatty acid content is indicated by the thickness of the arrows. The observed progression toward increased saturation in increasingly PEG-resistant cells is primarily due to increasing amounts of neutral lipid, which is highly saturated. Neutral lipid only makes a minor contribution to the acyl chain composition of Clone 1D cells.

lectin binding are seen between Clone 1D and F_{40}, the PEG fusion response remains relatively unchanged by agglutination of surface sugars (data not shown). The possible role of cell proteins in the differential fusion of these cell lines has also been examined. Although description of these experiments is beyond the scope of this report, any differences that have been observed are relatively minor and usually depend more on cell density than on which cell line is examined. By contrast, both the increasing levels of ether lipid and the elevated S/P ratios observed between Clone 1D and F_{40} are highly reproducible.

Implications of Lipid Composition for Membrane Fusion

The multistep procedure required for the isolation of PEG-resistant cells suggests the involvement of several factors in cell fusion, and there are likely to be other biochemical changes that play some role in the modulation of cellular response to PEG. A strong argument in favor of a major role for the neutral lipid and fatty acid alterations described above is provided by studies involving lipid supplementation, presented in the accompanying paper. By specific alteration of cellular lipid composition we have been able to alter the fusion response of cells in a predictable manner. The lipid modifications which alter fusion correlate directly with alkyldiacylglycerol and fatty acid content, but not with the other factors discussed above.

From the experiments presented in this paper, it is not possible to determine whether either or both of the observed lipid differences are responsible for the induction of fusion resistance in F_{40} cells. At least two models can be considered. In the first model, the ether lipid component could be of fundamental importance: a cell in the process of becoming PEG resistant turns on the synthesis of neutral lipids, increasing triglyceride and alkyldiacylglycerol production especially. These lipids are synthesized with the highly saturated acyl chain composition typical of neutral lipids, so that an elevated S/P ratio results as the byproduct of having more ether lipid. Alternatively, it may be that increased saturation of acyl chains is responsible for the PEG-resistant phenotype. According to this second model, the ether linkage could be a "red herring" with no active role in the fusion process. Rather, the synthesis of large amounts of neutral lipid is a convenient way for the cell to incorporate more saturated fatty acids. The alkyl group of alkyldiacylglycerol may serve as some sort of intracellular signal, perhaps encouraging the incorporation of this lipid into membranes in specific microdomains which have been postulated as sites of fusion. Ether lipids are unusual among glycerides in that they are synthesized by membrane-bound enzymes and may be incorporated into membranes, as discussed above.

Lipid molecules may directly modulate the fusion response, perhaps by changing biophysical parameters at the cell membrane. It is tempting to imagine that the increased S/P ratio in F_{40} cells results in much stiffer membranes which are less prone to disruption and PEG-induced fusion. Results presented in the following paper cast doubt on this interpretation, however. It is also possible that the lipids of PEG-resistant cells exert a more distant regulatory effect on fusion. Many examples are known of lipid activation of enzymes and association with other proteins, and it may be that the ether lipid and/or elevated S/P ratios act in this fashion. In such a model, even lipids which are not present at the plasma membrane could play a role in modulating the fusion response.

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