Biochemical Studies on Cell Fusion. II. Control of Fusion Response by Lipid Alteration

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ABSTRACT The preceding communication (Roos, D. S. and P. W. Choppin, 1985, *J. Cell Biol. 101*:1578–1590) described the lipid composition of a series of mouse fibroblast cell lines which vary in susceptibility to the fusogenic effects of polyethylene glycol (PEG). Two alterations in lipid content were found to be directly correlated with resistance to PEG-induced cell fusion: increases in fatty acyl chain saturation, and the elevation of neutral glycerides, including an unusual ether-linked compound. In this study, we have probed the association between lipid composition and cell fusion through the use of fatty acid supplements to the cellular growth medium, and show that the fusibility of cells can be controlled by altering their acyl chain composition. The parental Clone 1D cells contain moderately unsaturated fatty acids with a ratio of saturates to polyunsaturates (S/P) $\approx$ 1, and fuse virtually to completion following a standard PEG treatment. By contrast, the lipids of a highly fusion-resistant mutant cell line, F_{40}, are highly saturated (S/P $\approx$ 4). When the S/P ratio of Clone 1D cells was increased to approximate that normally found in F_{40} cells by growth in the presence of high concentrations of saturated fatty acids, they became highly resistant to PEG. Reduction of the S/P ratio of F_{40} cells by growth in *cis*-polyunsaturated fatty acids rendered them susceptible to fusion. Cell lines F_8, F_{16}, etc., which are normally intermediate between Clone 1D and F_{40} in both lipid composition and fusion response, can be altered in either direction (towards either increased or decreased susceptibility to fusion) by the addition of appropriate fatty acids to the growth medium. Although *trans*-unsaturated fatty acids have phase-transition temperatures roughly similar to saturated compounds, and might therefore be expected to affect membrane fluidity in a similar manner, *trans*-unsaturated fatty acids exerted the same effect as *cis*-unsaturates on the control of PEG-induced cell fusion. This observation suggests that the control of cell fusion by alteration of fatty acid content is not due to changes in membrane fluidity, and thus that the fatty acids are involved in some other way in the modulation of cell fusion.

The fusion of cellular plasma membranes occurs in a variety of biologically important situations and can also be experimentally induced by treatment with various chemicals, ionic conditions, electrical fields, or enveloped viruses (for review see reference 28). With the exception of certain viral systems, however, where fusion is known to be induced by specific viral surface proteins (15, 18, 25, 37), little is known of the biochemical or biophysical events involved in any cell fusion system. For any given cultured cell line, the experimental conditions required for fusion induced by polyethylene glycol (PEG)[1] are highly reproducible, but different cell lines respond very differently to this fusogen (4, 10, 43). This constancy within lines but variability between different cells, combined with the ease and rapidity of PEG-induced fusion, suggested that PEG treatment might be a convenient system in which to study the mechanisms of cell fusion. Towards this end we have isolated, from a highly fusible parental stock of mouse L-cells, a series of genetically stable, PEG-resistant derivatives which differ in their response to PEG over a fivefold range (33, 34).

As discussed in the preceding paper (32), biochemical analysis of the lipid composition of these PEG-resistant cells has

[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}, F_{40}, fusion-resistant cell lines isolated from Clone 1D by 4, 8, 16, or 40 cycles of polyethylene glycol–induced fusion; PEG, polyethylene glycol; S/P ratio, ratio of saturated to polyunsaturated fatty acids.

TABLE I. *Fusion Response and Lipid Composition of Clone 1D and Its PEG-resistant Derivatives*

Cell line	PEG-induced fusion	Ether-lipid (relative content)	S/P ratio
	%		
Clone 1D	95	1	1.0
F_{16}	46	23	3.3
F_{40}	19	36	4.0

revealed a correlation between lipid content and fusion. These results are summarized in Table I. The parental cell line Clone 1D, which is highly susceptible to PEG-induced fusion, possesses only trace amounts of alkyl-linked "ether" lipids (<0.1% total cell lipid) and contains approximately equal amounts of saturated and polyunsaturated fatty acyl chains (S/P ratio ≈ 1). Cells of line F_{16} are markedly reduced in their response to PEG; ether lipid content is substantially elevated in these cells, and their saturated fatty acid content is increased. F_{40} cells are even more highly resistant to PEG. Only 20% fusion is observed in PEG-treated F_{40} cells, and those few cells that do fuse form almost exclusively small binucleate or trinucleate cells. F_{40} cells contain high levels of ether lipid (up to 5% of whole cell lipid) and saturated fatty acids (S/P ratio ≈ 4.0).

To further investigate the relationship between lipid composition and susceptibility to PEG-induced fusion, the lipid content of these fusion-resistant cell lines has been modified by supplementation of the culture medium with different lipids. Selection of appropriate lipid supplements permitted nearly complete control of PEG-fusion in a predictable manner. Growth of the parental cell line Clone 1D in medium containing high concentrations of saturated fatty acids raises the S/P ratio of these cells to that found in untreated cultures of F_{40} and concomitantly abolishes susceptibility to PEG-induced fusion. Conversely, growth of F_{40} cells in polyunsaturated lipids causes a decrease in S/P ratio, and these normally fusion-resistant cells become highly susceptible to PEG-induced fusion.

MATERIALS AND METHODS

Cells and Media: The parental cells used in this study were a TK^- mouse fibroblast cell line, designated Clone 1D. The isolation of PEG fusion-resistant derivatives from the parental stock has previously been described (33). Media and culture conditions are described in the preceding paper (32).

Preparation of Fatty Acids: Fatty acids were obtained from Nu-chek Prep (Elysian, MN) and tested for purity by thin layer chromatography. All experiments presented here were performed by supplementation with sodium salts of appropriate fatty acids. Unless otherwise indicated, supplements were added to normal growth medium (containing 10% fetal bovine serum) to a final concentration of 10^{-4} M. Similar experiments were performed with fatty acids conjugated to bovine serum albumin (BSA) (40) or delipidated BSA (obtained from Sigma Chemical Co., St. Louis, MO) at various molar ratios. Lipid supplements have also been added to cells cultured in delipidated serum (35) or grown in entirely lipid-free medium (13). Results of these experiments were similar to those described for sodium-salt supplements.

To prepare fatty acid salts, lipids were mixed with a threefold molar excess of sodium hydroxide. Fatty acids with melting points higher than room temperature (saturated and *trans*-unsaturated compounds) were heated to allow formation of salts. Stock solutions were prepared at 5×10^{-2} M concentration (of lipid) and stored under nitrogen in the dark at −20°C prior to use. Periodic analysis by gas-liquid chromatography revealed minimal breakdown, even of highly polyunsaturated compounds, over the course of this study.

Lipid Supplementation: Cells were collected in late log phase from 75 cm^2 flasks (yielding approximately 2.5×10^7 cells per flask) and inoculated into either 60-mm petri dishes containing 5 ml of fresh culture medium or into 35-mm dishes containing 18-mm diameter #1 glass coverslips in 3 ml of medium. For most experiments, inocula were 10^6 or 3×10^6 Clone 1D cells (for the small or large dishes, respectively). In some experiments F_{40} cells were plated at slightly lower density to correct for the slightly faster growth rate of this cell line (31). Cells were plated in growth medium without added lipid and incubated at 37°C for 24 h prior to supplementation. Fatty acid salts were diluted from the stock solution into growth medium to a concentration of 10^{-3} M and aliquots from this mixture were added directly to the growing cultures without replacing the medium already present. Control cultures were supplemented with an appropriate concentration of NaOH without added lipid. Except where otherwise indicated, all fusions were performed 18 h after the addition of lipid supplements, when cells were spread as a confluent monolayer across the dish. For the experiments illustrated in Figs. 2 and 5, cells were initially set up at various densities and confluent cultures were selected at various times for fusion (see Fig. 5) or fixation without PEG treatment (see Fig. 2).

Fusion Protocol: PEG used for fusions was obtained from J. T. Baker Chemical Co. Phillipsburg, NJ, and autoclaved before use. In this study all fusions were performed using PEG 1000 (average molecular weight 950–1,050). Unless otherwise indicated (as in Fig. 3), a 50% solution (wt/vol) was prepared in growth medium without serum. For some experiments, PEG was recrystallized from ethyl ether as described by Honda et al. (16, 17), or passed over a hydrophobic column and lyophilized before use. In agreement with Smith et al. (39), no substantial differences in ability to induce fusion were observed between these purified preparations and PEG used directly as supplied from the manufacturer.

Two standard protocols for fusion were followed (10, 30). For treatment of cells in 60-mm dishes, the medium was aspirated and replaced with 3 ml of warm PEG solution. After treatment for 60 s, PEG was rapidly aspirated through a large bore pipette and the monolayer rinsed vigorously with five 10-ml washes of fresh medium without serum. Finally, 5 ml of growth medium containing serum was added and the cells were allowed to incubate in the absence of PEG for ~2 h (by which time fusion was complete). Cells grown on glass coverslips were fused by a modification of this procedure. The coverslips were removed to a fresh petri dish for treatment with PEG, and rinsed by repeated dipping through a series of containers containing large volumes of medium without serum. Fusion was then allowed to proceed in a fresh petri dish containing 3 ml of medium with serum. The entire procedure was carried out at 37°C. Control experiments were performed with and without exogenous lipid salts in the incubation medium, and it was found that fusion response was unaffected by the presence or absence of lipid supplement during the actual fusion.

Quantification of Fusion: After treatment with PEG and the subsequent incubation period, cells were rinsed in isotonic saline solution, fixed in methanol, and stained in Giemsa stain (Fisher Scientific Co., Fair Lawn, NJ). Coverslips from small petri dishes were mounted on glass slides with UVinert Aqueous Mountant (Gurr, Ltd.; distributed by Bio/medical Specialties, Santa Monica, CA). For cells grown in larger dishes, a small amount of mountant was added directly to the dish, over which was laid a 50-mm diameter #1 glass coverslip (Corning Glass Works, Corning, NY). Cells were observed with a Zeiss Photomicroscope III equipped with phase-contrast optics. Observations were recorded on Ilford Pan F film (ASA 50) by locating confluent regions at low power and photographing at higher magnification without further selection of field.

Cell fusion was scored by locating confluent regions of the monolayer (at low magnification) and moving to 500× magnification without further selection of field. All visible nuclei were scored as present in either multinucleate (fused) or mononucleate (unfused) cells. At least three fields were counted per sample, containing at least 300 nuclei. In previous reports we presented the total percentage of nuclei located in fused cells, without correcting for spontaneous cell fusion (33, 34, 43). As fusion in untreated cultures of these cells was always <5%, this approach did not complicate our overall analysis. However, in the experiments presented below, we found high rates of spontaneous fusion in cultures supplemented with saturated fatty acids (up to 30% under certain circumstances; see Fig. 2). To separate this factor from the consideration of fusion mediated by PEG, data in this paper is presented as PEG-specific fusion, defined as the percentage of total nuclei found in fused cells after PEG treatment minus the level of spontaneous fusion observed in parallel cultures not treated with PEG.

Lipid Analysis: For the analysis of lipid content in fatty acid–supplemented cells, cultures were prepared in parallel with cells set up for fusion. 10^7 cells were inoculated into 75 cm^2 T-flasks containing 25 ml of medium. 24 h later, lipid supplements were added and the cultures harvested after 18–24 h of further culture. Fatty acid analysis and other lipid chromatography was performed as described in the preceding paper (32).

RESULTS

Alteration of Lipid Content and Fusion Response by Fatty Acid Supplements

When exogenous fatty acids are available, cells incorporate these in preference to *de novo* synthesis from acetic acid (40), a metabolically more expensive route. Growth of Clone 1D cells (which normally have a 1:1 S/P ratio) (32) in medium containing high concentrations of saturated fatty acid would thus be expected to cause an increase in the acyl chain saturation of Clone 1D cell lipids. Such supplemented cells would thereby acquire a lipid composition approaching that found in untreated F_{40} cells, which have a high S/P ratio. Similarly, the S/P ratio of F_{40} cells should decrease when these cells are grown in medium supplemented with polyunsaturated fatty acids.

Table II shows the lipid composition of cells grown for 18 h in medium supplemented with 10^{-4} M polyunsaturated fatty acid (18:3; linolenic acid) or saturated fatty acid (19:0; nonadecanoic acid) as compared with unsupplemented controls.[2] Parallel cultures were treated with PEG and scored for fusion. The lipid supplements were incorporated into all cellular lipids (data not shown), altering S/P ratios in the predicted manner: when Clone 1D cells were grown in 19:0, their ratio of saturated to polyunsaturated fatty acids rose from 0.9 to 3.0, a value similar to that found in untreated F_{40} cells in these experiments. PEG treatment of these lipid-supplemented Clone 1D cells induced only 33% fusion, comparable with untreated F_{40} cells, which exhibited 24% fusion following PEG treatment. Cultures of Clone 1D grown without lipid supplement showed 90% fusion. When F_{40} cells were grown in the presence of 18:3, driving their S/P ratio toward that normally found only in the parental (fusible) cell line, 68% fusion was observed, a substantial increase over control cultures of F_{40}. These results are highly reproducible; in over twenty such experiments in which cultures of Clone 1D cells were supplemented with saturated fatty acid, the fusion response varied between 12 and 42% (average 25%, SD ± 12%). PEG-specific fusion of F_{40} cells supplemented with polyunsaturates varied between 47 and 83% (average 61%, SD ± 14%). In these same experiments, fusion of untreated Clone 1D and F_{40} cultures were 91 ± 4% and 22 ± 8%, respectively.

Fig. 1 presents micrographs of cells treated with PEG after lipid supplementation. Control cultures of Clone 1D cells fused readily after PEG treatment (*A*), while F_{40} cells were fusion resistant (*B*). When cells were grown with linolenic acid in the growth medium, however, both cell lines were extensively fused by PEG (*C* and *D*). Conversely, growth of cells in the presence of nonadecanoate (*E* and *F*) resulted in a loss of susceptibility to PEG-fusion in the parental line Clone 1D (*E*). Similar results (not shown) were obtained when Clone 1D cells were supplemented with other saturated fatty acids (e.g., palmitic, stearic, or lignoceric acids) or when F_{40} cells were supplemented with other polyunsaturates (e.g. linoleic, linolenic, or arachidonic acids). Thus control of the cellular lipid composition by supplementation of the growth medium with exogenous fatty acids allows us to control the fusion phenotype in a predictable manner.

[2] 18:1, 19:0, 18:3., etc., is a shorthand notation for fatty acid structure, in which the number preceding the colon indicates chain length, and the single digit indicates degree of unsaturation: examples given above correspond to Δ9-*cis*-octadecenoate (oleic acid; 18:1), nonadecanoic acid (19:0), and Δ9,12,15-all *cis*-octadecatrienoic acid (linolenic acid; 18:3), respectively; the stereochemistry of acyl chains is indicated by c for *cis*-unsaturated compounds (for example: 18:1c = oleic acid) and t to designate *trans*-unsaturation (example: 18:1t = *trans*-octadecenoate; elaidic acid). Unless otherwise indicated, all double bonds are in the *cis* configuration.

TABLE II. *Fatty Acid Content and PEG-induced Fusion of Lipid Supplemented Cells*

Cell line	S/P ratio			PEG-induced fusion		
	+19:0*	none	+18:3	+19:0	none	+18:3
				%	%	%
Clone 1D	3.02	0.93	0.31	33	90	91
F_{16}	ND	ND	ND	28	51	86
F_{40}	2.80	2.93	0.54	23	24	68

* 10^{-4} M lipid supplement.
ND, not done.

Cell growth and viability was unaffected by the addition of polyunsaturated fatty acids under these conditions, although higher concentrations of supplement or longer periods of culture resulted in some slowing of cell doubling time. Up to 18 h, the viability of Clone 1D cells was also unaffected by addition of saturated fatty acids such as 19:0, but these compounds were toxic to F_{40}, as indicated by the diminished cell number in Fig. 1 *F*. It appears that there is a limit to the content of saturated fatty acids compatible with viability, and that F_{40} cells (which already contain an elevated S/P ratio) cannot be supplemented with even more saturated fatty acid without pushing them beyond the limit of survival.

Spontaneous Cell Fusion

Spontaneous cell fusion is relatively infrequent in control cultures of the cell lines used in this study: ≤5% in the hundreds of fusions performed to date. In cultures treated with high concentrations of saturated fatty acids, however, substantial numbers of multinucleate cells were found, as shown in Fig. 2. Even without PEG treatment, giant cells routinely accounted for 10–15% of all Clone 1D cell nuclei in cultures grown for 18 h in the presence of 10^{-4} M 19:0, and longer incubation with saturated lipid supplements produced even more spontaneous fusion. Saturated fatty acids are toxic to F_{40} cells, as discussed above, but even so, increased levels of spontaneous fusion were also seen in F_{40} cultures (not shown). These abnormal levels of spontaneous fusion were only produced by saturated fatty acid supplements, and only at concentrations $>8 \times 10^{-5}$ M. To avoid confusion, throughout this paper PEG-specific fusion has been calculated by subtracting the number of spontaneous multinucleates in control cultures from the total fusion observed after PEG treatment.

Control of Fusion in Intermediate F-Strains

Table II also includes data on the fusion of lipid-supplemented F_{16} cells, a cell line intermediate between Clone 1D and F_{40} both in response to PEG and in lipid composition (cf., Table I). Unsupplemented cultures exhibit ~50% fusion after PEG treatment, but when F_{16} cells were grown in the presence of 18:3, 86% fusion was observed. This level is comparable with the 90% fusion seen in control cultures of Clone 1D in this series of experiments. Conversely, when F_{16} cells were grown in 10^{-4} M saturated fatty acid, driving their S/P ratio to approximate that of F_{40} cells, fusion response

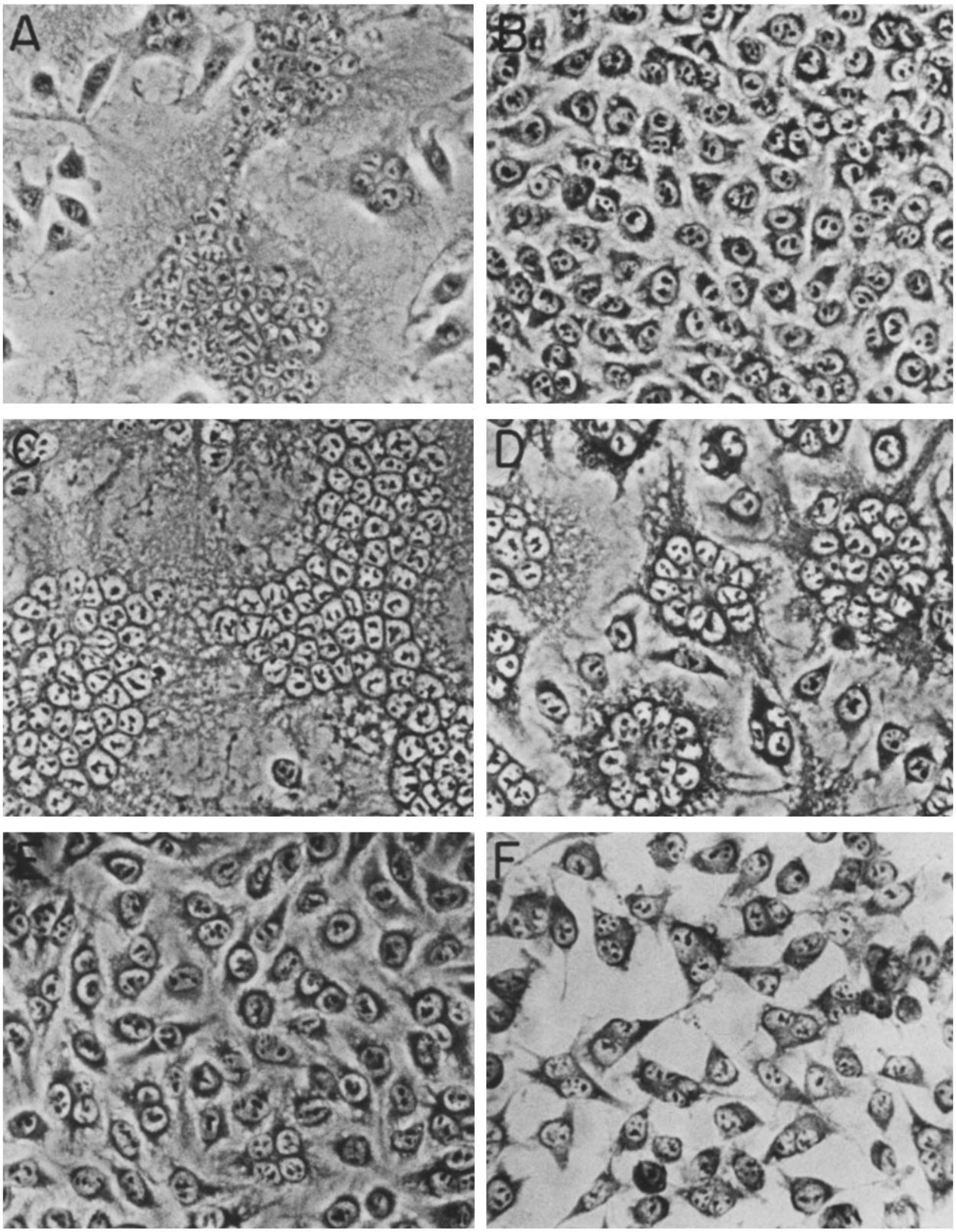

FIGURE 1 Control of PEG-induced fusion by fatty acid supplements. Clone 1D (*A*, *C*, and *E*) and F_{40} (*B*, *D*, and *F*) cells were grown to confluence in media supplemented for the final 18 h of growth with 3×10^{-4} M NaOH (*A* and *B*), 10^{-4} M linolenic acid (18:3; *C* and *D*), or nonadecanoic acid (19:0; *E* and *F*) in NaOH. Cultures were then treated with PEG, and random fields chosen for photography. Control cultures of Clone 1D cells were much more highly susceptible to PEG-induced fusion than F_{40} cells, but both cell lines fused extensively when grown in the presence of polyunsaturated fatty acid. Saturated lipids inhibited fusion in Clone 1D and caused destruction of the F_{40} monolayer.

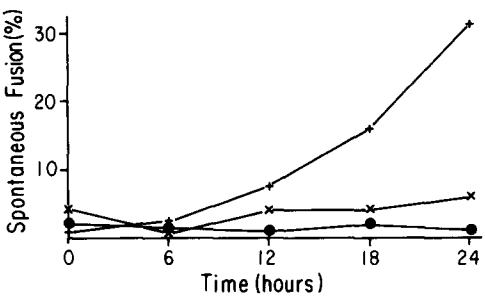

FIGURE 2 Spontaneous fusion of cells supplemented with saturated fatty acids. Cultures of Clone 1D cells were grown in normal medium (●) or medium supplemented with 10^{-4} M saturated (+) (19:0) or polyunsaturated (×) (18:3) fatty acid. At various times, confluent cultures were fixed and scored for fusion. Relatively high levels of spontaneous fusion occurred specifically in cultures incubated with saturated fatty acid.

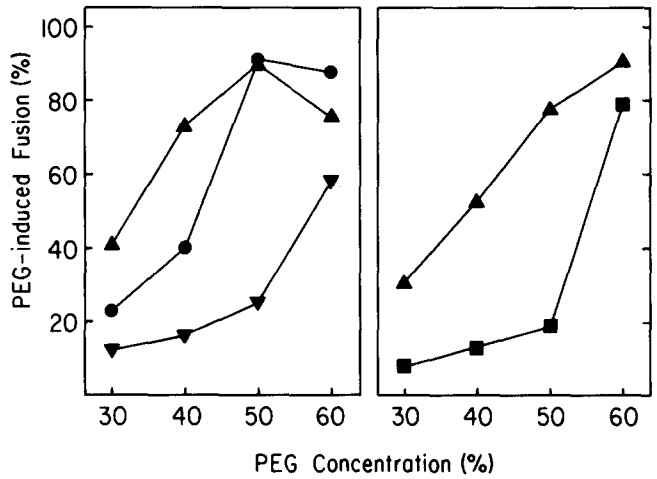

FIGURE 3 Effect of PEG concentration on the fusion of lipid-supplemented cells. Cultures of Clone 1D cells (*left*) and F_{40} cells (*right*) were treated with 30–60% PEG after an 18-h incubation in the presence of 10^{-4} M saturated (▼) (19:0) or polyunsaturated (▲) (18:3) fatty acid, and the extent of fusion was scored. Control (unsupplemented) cultures of Clone 1D (●) and F_{40} (■) cells were fused in parallel. Saturated fatty acid caused Clone 1D cells to become more resistant to all concentrations of PEG than control cultures, whereas growth of F_{40} in polyunsaturated fatty acids produced a shift towards a more PEG-sensitive phenotype. Saturated fatty acid is highly toxic to F_{40} (and therefore not shown), preventing the production of even more highly PEG-resistant cells. However, note that although Clone 1D cells were already highly fusible, growth in polyunsaturates rendered these cells even more susceptible to PEG fusion.

decreased to 23%. Similar data have been obtained for other cell lines with intermediate susceptibility to PEG-induced fusion (not shown). Thus, parallel cultures of a single cell line can be driven either towards massive fusion or extreme fusion resistance, solely by manipulation of the fatty acid content of the growth medium.

Effect of PEG Concentration on Fusion of Lipid-supplemented Cells

For most cell types, the extent of fusion is extremely sensitive to PEG concentration (4, 10, 27, 43). In unsupplemented cultures of Clone 1D cells, there is a sharp decline in susceptibility to fusion below ~45%. We have previously shown that the resistance of F_{16} cells to fusion is actually a shift in PEG sensitivity (33). F_{16} fuses readily above 55% PEG, but poorly at lower concentrations. To fully characterize the effects of lipids on cell fusion, we have treated lipid-supplemented Clone 1D and F_{40} cells with a range of PEG concentrations, as shown in Fig. 3. Although Clone 1D cells grown in the presence of saturated fatty acids are resistant to fusion with 50% PEG (Fig. 1; Table I), these cultures could be fused with 60% PEG. Thus the alteration of PEG-fusion response induced by saturated fatty acid closely mimics the selection (over a much longer time period) of F_{40} cells. (The changes induced by lipid supplements are readily reversible, however, as will be discussed below.) Similarly, the fusion response of 18:3-supplemented F_{40} cells resembled unsupplemented Clone 1D cultures at all PEG concentrations tested. Overall, despite the considerable perturbation caused by fatty acid manipulation, the general shape of the PEG response curve was maintained.

50% PEG treatment causes nearly complete fusion of unsupplemented Clone 1D cells (cf., Fig. 1*A*), and therefore it could not previously be determined whether this line can be made to fuse even better. At lower concentrations of PEG, however, where PEG fusion is less extensive, it is clear that polyunsaturated fatty acid renders Clone 1D much more susceptible to PEG fusion, just as seen in F_{40} cells. As discussed above, it was impossible to increase the PEG resistance beyond that already present in F_{40} cells, since any additional saturated fatty acid killed the cells.

Further Characterization of Fusion Alteration by Fatty Acid Supplements: Effects of Lipid Concentration and Time Course of Fusion Alteration

Fig. 4 demonstrates the dose-response relationship of lipid-modulated PEG fusion. In the presence of 10^{-5} M 19:0, no change from the highly fusible phenotype of Clone 1D was observed (*A*). At this concentration, 19:0 accounts for <20% of the available fatty acid in the medium. As the concentration of saturated fatty acid was increased, however, the specific

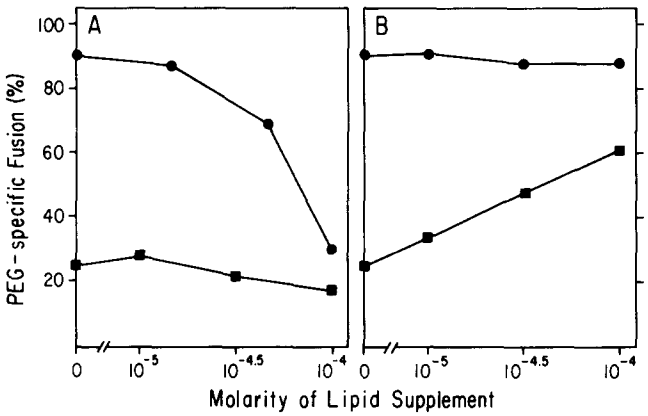

FIGURE 4 Dose dependence of lipid supplements for manipulation of the fusion phenotype. Clone 1D (●) or F_{40} (■) cells were incubated for 18 h with various concentrations of either saturated fatty acid (*A*) (19:0) or the trienoic polyunsaturate linolenic acid (*B*) (18:3), followed by treatment with 50% PEG. Saturated fatty acids inhibited, and polyunsaturates promoted, PEG-induced cell fusion in a predictable manner. At concentrations higher than 10^{-4} M, toxicity overwhelmed the effects on cell fusion.

response to PEG dropped precipitously, to 30% fusion at 10^{-4} M supplement, where the added lipid represents ~70% of the available fatty acid in the medium. The fusion-modulating effect of polyunsaturated fatty acid was similarly dose dependent, as shown in Fig. 4*B*. Unsupplemented cultures of F_{40} cells and cultures grown in 10^{-5} M 18:3 were only slightly susceptible to fusion, but increasing the concentration of lipid supplement to 3×10^{-5} or 10^{-4} M prior to PEG treatment resulted in 48% and 61% fusion, respectively.

The time required for lipid supplements to affect fusion response is illustrated in Fig. 5. Within 6 h after the addition of 10^{-4} M fatty acid, slight changes were seen in the PEG-mediated fusion response. By 12 h, fusion of Clone 1D cells supplemented with 19:0 dropped from 90 to ~50% fusion, and F_{40} cells supplemented with 10^{-4} M 18:3 rose from 20 to ~50% fusion. Saturated fatty acid supplements were maximally effective by 18 h, but polyunsaturates took slightly longer to exert their greatest effect. At 24 h, the lipid-supplemented growth medium was replaced with a large volume of fresh medium without added lipid. When this was done, fusion response rapidly reverted to nearly normal values.

Effects of Fatty Acid Saturation and Stereochemistry

For most experiments, 19:0 and 18:3 fatty acids were used as prototypical saturated and polyunsaturated fatty acids, as they are only present in small amounts in unsupplemented cells, simplifying biochemical quantitation. For the length of time that these fatty acids were present, their metabolic alteration was minimal. Those alterations that were found consisted primarily of the addition of single acetate units, preserving the degree of saturation (e.g., 18:3 was extended to form longer polyunsaturates such as 20:3, but no 18:3 was converted to saturated fatty acids such as 18:0; nonadecanoic [19:0] acid was modified to 21:0, but neither 19:1 nor 19:2 was detected).

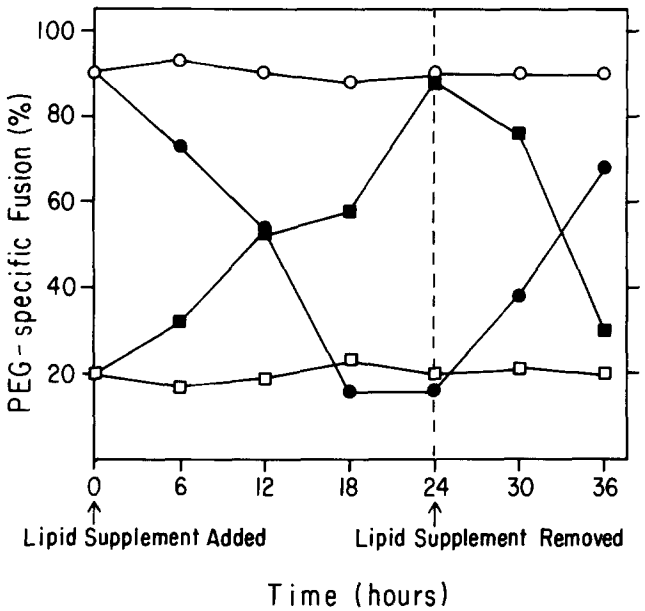

FIGURE 5 PEG-induced fusion as a function of the time exposed to exogenous fatty acid supplements. Cultures of Clone 1D (●) and F_{40} (■) cells at various densities were supplemented with 10^{-4} M supplements of either 19:0 (Clone 1D cells) or 18:3 (F_{40} cells). At 6-h intervals after the addition of lipid supplements, confluent cultures were fused with PEG. Maximal alteration of fusion response was achieved after 24 h of growth in the presence of exogenous fatty acids, at which point parallel cultures were renewed with fresh medium without added lipid. These cells rapidly reverted to their original phenotype, as shown to the right of the vertical dotted line. Open symbols indicate F_{40} (□) and Clone 1D (○) controls.

TABLE III. *PEG Fusion Response of Clone 1D and F-Strains Supplemented with Fatty Acids of Differing Saturation and Stereochemistry*

Cell line	Control	Lipid supplement 18:0 (72)*	18:1c (11)	18:1t (45)	18:2c (−5)	18:2t (57)	18:3c (−11)
Clone 1D	91‡	18	83	86	92	93	84
F_{16}	43	21	ND	ND	69	82	67
F_{40}	23	15	40	39	58	65	57

* Melting point, °C.
‡ Percent of total nuclei in multinucleate (fused) cells.
ND, not done

Other long chain fatty acids have also been used as supplements, including saturated compounds between 14 and 24 carbons in length; 16, 18, and 24 carbon monounsaturates; and various polyunsaturated lipids between 16:2 and 22:6, including both *cis* and *trans* isomers. We have not been successful in attempts to incorporate shorter chain fatty acids, such as 9:0, into cellular lipids without substantial metabolic modification. Table III presents data on the PEG-specific fusion response of cells supplemented with 18-carbon fatty acids which differ in their degree of saturation and in stereochemistry.

Supplementation with stearic acid (18:0) strongly suppressed PEG-induced cell fusion in both Clone 1D and F_{16} cells, just as seen above for the 19:0 fatty acid supplements. Every long chain saturated fatty acid tried to date has had this effect, in accord with predictions from the altered S/P ratio. *Cis*-polyunsaturates such as linoleic acid (18:2c) stimulated PEG-induced fusion, to the same extent as shown above for 18:3.

Trans-unsaturated fatty acids are not commonly found in nature, but they are useful for studying the functional biological roles of fatty acids, because of their similarity to saturated fatty acids with respect to various biophysical properties (41). For example, Δ9,12-*trans*,*trans*-octadecadienoic acid (linoelaidate; 18:2t) has a melting point of 57°C, similar to the melting point of 18:0, and actually higher than the 54°C melting point of 14:0 (which inhibits fusion as efficiently as 18:0 and 19:0). Yet as shown in Table III, this *trans*-polyunsaturate was at least as effective in enhancing PEG-induced fusion as its *cis*-counterpart, which has a melting point of −5°C. Chromatographic analysis of 18:2t-supplemented cells revealed that the *trans*-fatty acid was maintained as a *trans*-polyunsaturate over the course of this study, and was incorporated into similar lipid species as found for *cis*-unsaturated supplements (not shown). Neither oleic acid (18:1c, the most prevalent acyl chain in unsupplemented cells) nor its *trans*-isomer elaidic acid (18:1t) significantly affected PEG-induced fusion of either Clone 1D or F_{40}, although both compounds were readily incorporated into cells. These data indicate that both *cis*- and *trans*-unsaturated fatty acids affect cellular response to PEG similarly, despite different melting points. Such data pose serious difficulties for biophysical models of

cell fusion which seek to explain fusibility merely in terms of the fluidity of membrane lipids or lipid domains.

DISCUSSION

Control of Cell Fusion by Lipid Composition

The accompanying paper describes several aspects of cellular lipid composition which were directly correlated with resistance to PEG-induced fusion in a series of fibroblast cell lines. Two aspects of their lipid composition were particularly dramatic: a high S/P ratio, and increases in neutral lipid content, including an unusual alkylglycerol which can be >30-fold elevated over the parental cell line. Guided by these observations, we have altered the S/P ratios of the fusible cell line Clone 1D and various fusion-resistant derivatives by supplementing the culture medium with fatty acids of appropriate saturation. In the experiments presented here, it has been possible to predictably alter the fusion phenotype of these cell lines. Cells which are resistant to fusion can be made to fuse by decreasing their S/P ratio, while increasing the S/P ratio of fusible cells makes these cells PEG resistant.

Added fatty acids were rapidly incorporated into all cellular lipids, and acquisition of the fusion-altered phenotype paralleled the extent of incorporated supplement. Exogenous lipid was not required during the actual fusion procedure, and lipid supplements added only after PEG treatment did not significantly alter fusion (data not shown). Based on these observations, it appears that the incorporation of appropriate fatty acids into cultured cells allows us to mimic the biochemical alterations which occurred during the selection for PEG fusion–resistant cell lines. Alternatively, it is possible to manipulate the mutant cell lines so that they resemble the parental cells in both lipid composition and susceptibility to fusion across a wide range of PEG concentrations.

It is interesting that even these powerful means to control cell fusion did not allow us to produce a cell culture which was totally resistant to the fusogenic effects of PEG. This observation is consistent with our experience in the genetic selection of PEG-resistant cells, in which it has not been possible to progress beyond the fusion resistance of F_{40} cells to produce a completely unfusible cell line (34). The results presented here provide a possible explanation for this finding: higher levels of saturated lipids than already found in F_{40} cells are inconsistent with cell viability; the cells cannot be pushed beyond an S/P ratio of ~4:1.

In addition to the fatty acid alterations found in F_{40} cells, neutral lipid content is elevated in these fusion-resistant mutants (see Table I and the preceding paper [32]). In preliminary experiments (conducted according to previously published procedures of Cabot and Snyder [5] and Scott et al. [38]), we have stimulated ether lipid production in Clone 1D cells approximately eightfold by supplementing the culture medium with fatty alcohols, monoalkylglycerols, and other metabolic precursors to the mature ether lipid (not shown). In these cultures, PEG-induced fusion of Clone 1D was slightly inhibited in parallel with the increased ether lipid content. Supplementation of cells with either fatty acids or ether lipid precursors resulted in a parallel change in the other lipid, however, suggesting that the metabolism of neutral ether lipids is closely interrelated with fatty acid metabolism, perhaps by mechanisms discussed in the previous paper. Other approaches to the manipulation of S/P ratios, such as the growth of cells at different temperatures (12), also appear to affect both acyl chain and ether lipid content in parallel (preliminary data; not shown). Although we cannot, as yet, completely distinguish between the roles played by fatty acyl chains and neutral ether lipids in cell fusion, we favor a more direct role for fatty acids, based on the rapidity of action and the degree of control which can be exerted by simple media supplements (Fig.5).

Spontaneous Cell Fusion

We were surprised to discover the pronounced effect of saturated fatty acid content on spontaneous cell fusion (Fig. 2). It is possible that this is the explanation for our observation, in hundreds of fusions over the past several years, that F_{40} cells exhibit slightly higher spontaneous fusion than Clone 1D (~3% vs. 2.5%). We have also observed this effect in other cell lines supplemented with saturated fatty acids (not shown).

Spontaneous cell fusion is normally a rare event, but may be related to the types of fusion which occur as a necessary part of many cell processes, and must be subject to specific cellular control. A gene associated with spontaneous fusion in human cells has been mapped to human chromosome 10 by somatic cell genetic techniques (44). Further analyses of the role of saturated fatty acids in spontaneous fusion may be of importance in elucidating normal cellular processes.

Potential Applications to Cell Fusion Technology

The manufacture of somatic cell hybrids has many important applications, such as the generation of hybridoma cells for monoclonal antibody production, and several laboratories have detailed optimal conditions for inducing the fusion of relevant cell types (11). For many primary lymphoid cells, the toxic effects of PEG present an obstacle to efficient hybrid formation. Hence, protocols for hybridoma production usually call for concentrations of PEG where both fusion and toxicity are low. The results presented above suggest that it may be possible to grow cells for a brief period in appropriately lipid-altered medium prior to fusion to enhance susceptibility to the fusion-inducing effects of PEG. Preliminary experiments suggest that the effect of lipid composition on PEG toxicity is small compared with its effect on fusion.

Alternatively, as most cell lines are susceptible to fusion only across a rather narrow range of PEG concentrations, it may be possible to develop a panel of myeloma cell lines of differing susceptibility to PEG. Given any cell population with its characteristic response to PEG, one could then maximize the production of interspecific hybrids by choice of an appropriate immortalizing partner.

The ability of saturated fatty acids to induce cell fusion without further treatment may also find application in certain situations.

Relationship of These Experiments to the Mechanisms of Cell Fusion

Based on the different biophysical properties of saturated and *cis*-polyunsaturated fatty acids, one can speculate on possible mechanisms for the stimulation of PEG fusion by polyunsaturated acyl chains, and the inhibition of fusion by saturated fatty acids. There is some evidence from studies of synthetic membranes that lipid fluidity can play a crucial role in certain fusion systems and it has been suggested that similar events may occur in biological systems, either throughout the

plasma membrane or in membrane microdomains of differing fluidity (1, 7, 9, 22, 23, 26, 42). In fact, mechanisms have been postulated in which either high or low fluidity would be conducive to fusion. All of these models have in common the prediction that *trans* isomers of polyunsaturated fatty acids should behave more like saturated fatty acids than their *cis*-unsaturated stereoisomers; both saturates and *trans*-unsaturates have very high melting points and both stiffen membranes as a consequence of this fact. The data presented in Table III demonstrate that in the present system, both *trans*- and *cis*-polyunsaturates stimulate PEG-induced fusion, in contradiction to predictions of membrane fluidity models. *Cis*- and *trans*- fatty acids were incorporated into similar lipid species (not shown), although the possibility remains open that saturated, *cis*-unsaturated, and *trans*-unsaturated fatty acids are preferentially segregated into different lipid domains or subcellular organelles, thereby playing different roles in membrane interactions (20). Further studies on the ability of various synthetic fatty acids (e.g., mixed *cis,trans*-polyunsaturates, or *cis,cis*-Δ6,12-dienoic acids) to promote or inhibit fusion may provide insights into the structural requirements for fatty acids in the modulation of PEG-induced fusion.

The difficulty in developing suitable models for how lipids might directly regulate cell fusion leads us to consider more indirect mechanisms whereby lipids can exert biological activity. The ether-linked lipid 1-O-alkyl, 2-acetyl, phosphorylcholine is a highly potent platelet-activating factor, in part responsible for stimulating the secretion of platelet-derived growth factor for use in wound healing (for review see reference 2). In other secretory systems there is also evidence for the involvement of lipid factors (3, 6, 8, 14, 19, 21, 29). Phosphokinase-C (24) requires the presence of specific diacylglycerol molecules (synthesized by phosphatidyl inositol breakdown) for its enzymatic activity, perhaps acting to bring the kinase to the membrane from its normal location in the cytoplasm. Several similar situations are now coming to light, in which it appears that the active proteins and lipids may shuttle between different subcellular sites (36). The possibility of lipids exerting their effect through specific interactions with cellular proteins may provide a useful working approach for the further study of cell fusion.

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