Alteration of Fatty Acid Composition of LM Cells by Lipid Supplementation and Temperature†

Karen A. Ferguson,‡ Michael Glaser,§ William H. Bayer, and P. Roy Vagelos*

ABSTRACT: Alteration of the fatty acid composition of monolayer cultures of LM cells grown in chemically defined medium was achieved by supplementation with fatty acids complexed to bovine serum albumin. Phospholipids containing up to 40% linoleate were found in cells grown in medium containing 20 μg of linoleate/ml. Incorporation of linoleate into phospholipids reached a plateau after 12–24 hr, and cells remained viable for at least 3–4 days. Although linoleic, linolenic, and arachidonic acids were incorporated into LM cells equally well, only the latter was elongated by these cells under these experimental conditions. Nonadecanoic acid was incorporated to a lesser extent than the polyunsaturated fatty acids. Phosphatidylcholine and phosphatidylethanolamine of LM cells had different fatty acid compositions; phosphatidylethanolamine contained more longer chain and unsaturated fatty acids. Cells were also grown in the absence of choline and presence of choline analogs such as *N,N*-dimethylethanolamine, *N*-methylethanolamine, 3-amino-1-propanol, and *l*-2-amino-1-butanol. The analog phospholipids in these cells had fatty acid compositions which were intermediate between those of phosphatidylethanolamine and phosphatidylcholine of control cells grown in the presence of choline. Linoleate was found in both phosphatidylcholine and phosphatidylethanolamine of cells supplemented with linoleate. The sphingolipid fraction of these cells, however, did not contain significant amounts of linoleate. When linoleate was present in the phospholipids, compensatory decreases in the oleate and palmitoleate content of phospholipids were observed. Lowering of the growth temperature to 28° produced an increase in unsaturated fatty acid content of the phospholipids. When linoleate was supplied to cells grown at 28°, there was no further increase in the unsaturated fatty acid composition of the phospholipids. Using both fatty acid supplementation and lowered growth temperature, LM cell membranes can be produced which have phospholipids with vastly different fatty acid compositions.

Many experiments have been reported recently which have attempted to probe the relationship between the lipid composition of a biological membrane and the physical properties and enzymatic activities of that membrane. It is known that the lipid bilayer of a membrane is in a gel-like state at low temperatures and in a liquid crystalline state at higher temperatures (reviewed by Oldfield and Chapman, 1972) and that the temperature-linked transitions from gel to liquid crystalline state affect the growth of some organisms (Overath *et al.*, 1970; McElhaney, 1974) and some membranous enzyme functions (Schairer and Overath, 1969; Mavis and Vagelos, 1972). Although many efforts have been made to elucidate the effect of fatty acid composition on lipid and membrane properties of microorganisms (reviewed by Machtiger and Fox, 1973, and Silbert *et al.*, 1974), only recently have studies been undertaken to explore these effects in mammalian cells (Wisnieski *et al.*, 1973; Williams *et al.*, 1974). The results of the studies with mammalian cells suggested that the alteration of phospholipid fatty acid composition required the inhibition of endogenous fatty acid biosynthesis by the addition of desthiobiotin and supplementation with fatty acids as the Tween esters because of the toxicity of free fatty acids (Williams *et al.*, 1974). We have found, however, that phospholipid fatty acid composition can be altered by growing LM cells in a lipid-free medium and supplying fatty acids complexed to bovine serum albumin. A preliminary account of this work has appeared (Glaser *et al.*, 1974a). With this carefully defined system, experiments are now possible to define the effects of fatty acid supplementation and altered growth temperature on properties of LM cell membranes.

Materials and Methods

General. Organic solvents were distilled before use. Unisil (100–200 mesh, Clarkson Chemical Co., Williamsport, Pa.) was washed with methanol and activated at 100° overnight. All fatty acids were obtained from Sigma Chemical Co. Fatty acids were complexed to fatty acid-free bovine serum albumin (Pentex Biochemicals) by the method of Spector and Hoak (1969), and fatty acid content of the complex was determined by the titration method of Dole (1956). Solutions routinely contained 8.4–9.2 μmol fatty acid/μmol of bovine serum albumin. The fatty acid–bovine serum albumin solution was sterilized by filtration and stored frozen at −20°.

Cell Culture. Mouse LM cells were obtained from the American Type Culture Collection and grown as monolayers in 75-cm^2 Falcon tissue culture flasks. The lipid-free, chemically defined culture medium described by Higuchi (1970) was modified to contain 20 mM Hepes[1] buffer (pH

† From the Department of Biological Chemistry, Division of Biology and Biomedical Sciences, Washington University, St. Louis, Missouri 63110. *Received August 16, 1974.* Supported in part by National Science Foundation Grant GB-38676X and National Institutes of Health Grant HL-10406.

‡ Supported by a Postdoctoral Fellowship (AM-54171) from the National Institutes of Health. Present address: Department of Chemistry, Eastern Illinois University, Charleston, Illinois 61920.

§ Present address: Department of Biochemistry, University of Illinois, Urbana, Illinois 61801.

[1] Abbreviations used are: Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; DMEA, dimethylethanolamine; MEA, monomethylethanolamine; PA, 3-amino-1-propanol; BA, *l*-2-amino-1-butanol; PBS, phosphate-buffered saline; PE, phosphatidylethanolamine; PC, phosphatidylcholine.

7.4). Cells to be transferred were detached by swabbing with sterile perforated cellophane after the addition of fresh medium. The suspension was repeatedly pipetted to break up cell clumps, and aliquots were transferred to new flasks containing a final volume of 10 ml of medium. Sufficient volume of the fatty acid–bovine serum albumin complex to produce the desired final concentration of fatty acid was added directly to the medium. Culture of cells in medium containing choline analogs (dimethylethanolamine, DMEA; monomethylethanolamine, MEA; 3-amino-1-propanol, PA; and *l*-2-amino-1-butanol, BA) was described by Glaser *et al.* (1974b).

To harvest the cells, the monolayers were washed three times with 5 ml of phosphate-buffered saline (PBS) (Dulbecco and Vogt, 1954) made up without calcium and magnesium, and then suspended as described above in 5 ml of PBS and pelleted by low-speed centrifugation. The pellet was resuspended in 1.0 ml of PBS and 0.2 ml was removed for protein determination. This aliquot was diluted to 1.0 ml with 0.5 N KOH and heated in a boiling water bath for 15 min to reduce the viscosity of the solution for accurate pipetting. Protein concentrations were determined by a microbiuret procedure (Munkres and Richards, 1965).

Growth curves were obtained by inoculation of identical aliquots of cells into 25-cm^2 Falcon tissue culture flasks. At each time point, a flask was washed with PBS and the cell monolayer was dissolved in 1 ml of 0.5 N KOH. Aliquots of this solution were assayed for protein as described above.

Lipid Determinations. The cell suspension was extracted by the method of Bligh and Dyer (1959) as described by Ames (1968), and the phase containing the lipids was dried under a stream of nitrogen. Neutral lipids and phospholipids were separated by chromatography on short Unisil columns (0.3 g of Unisil in a disposable pipet plugged with glass wool). The neutral lipids and phospholipids were eluted with 5 ml of chloroform and 4 ml of methanol, respectively.

Fatty acid methyl esters were prepared from neutral lipid or phospholipid fractions by adding 1 ml of HCl–CH_3OH (1:20) to samples in screw-capped tubes which were then flushed with nitrogen, sealed, and heated overnight at 75°. After the tubes had cooled, 1 ml of water and 2 ml of ether were added, and the aqueous phase was removed. The ether phase was washed once with 1 ml of water and dried over anhydrous Na_2SO_4, and then the ether was evaporated under a nitrogen stream. The samples were dissolved in CS_2 and analyzed by gas–liquid chromatography (glc) at 165° on a 6-ft column of 10% DEGS on Anakrom SD, 60–70 mesh (Analabs). Fatty acid methyl esters were identified by comparison of their retention times with those of authentic standards (NHI-D and PUFA-2, Applied Science Laboratories) and also comparison of their retention times relative to methyl stearate with the comparable values calculated from the data of Ackman and Burgher (1965).

Sphingolipid and glycerophospholipid fatty acid compositions were determined separately by a procedure (Ferguson *et al.*, 1972) in which glycerophospholipids were first subjected to methanolysis with 0.5 N NaOH in CH_3OH (Applied Science Laboratories). The fatty acid methyl esters derived from the glycerophospholipids were separated from sphingolipids by column chromatography. Methyl esters from sphingolipid fatty acids were obtained subsequently using the acidic methanolysis procedure described above.

Individual phospholipids were isolated by preparative two-dimensional thin-layer chromatography (tlc) as described by Glaser *et al.* (1974b). Their fatty acid methyl esters were prepared by methanolysis with 0.5 N NaOH in CH_3OH.

Results

Although a number of authors have shown that fatty acid supplementation can be used to alter the lipid composition of mammalian cells, the method of introduction of the fatty acid into the medium has varied considerably. Stoffel and Scheid (1967) added the ammonium salts of fatty acids to HeLa cells, while others (Williams *et al.*, 1974; Wisnieski *et al.*, 1973) have maintained that fatty acids must be added as Tween esters to BHK_{21} and LM cells in order to prevent fatty acid toxicity. Cowen and Heydrick (1972) utilized fatty acid–bovine serum albumin complexes to supplement L cells.

We have found that LM cells grown in a chemically defined, serum- and lipid-free medium can be supplemented with fatty acid–bovine serum albumin complexes, and that cell survival is not affected under optimal supplementation conditions. The extent of incorporation of linoleate is determined in part by the concentration of linoleate (as the bovine serum albumin complex) present in the medium (Figure 1A). Incorporation of linoleate into phospholipids was increased to about 55% when high levels were present in the medium. As phospholipid linoleate concentrations reached maximal values, more of the exogenously supplied fatty acid was channeled into neutral lipid esters (Figure 1A). This correlated with microscopic observations of lipid droplets within the cells, as reported previously (Moskowitz, 1967). This excess of neutral lipid esters can be minimized by using linoleate concentrations of ≤ 20 μg/ml of medium. The concentration of cells also affected the uptake of linoleate into cellular phospholipids (Figure 1B) in that smaller inocula took up more linoleate from solutions with identical fatty acid–bovine serum albumin concentration. When a flask containing 10 ml of medium was inoculated with 1 mg of cells and after several hours linoleate–bovine serum albumin complex was added at a final concentration of 20 μg/ml of medium, the cells remained attached and grew for at least 3–4 days. Cells which contained above 40% linoleate in their phospholipids were viable for shorter times. Incorporation of linoleate into phospholipids was complete after 12–24 hr of exposure to the supplement (Figure 1C), and therefore in all subsequent experiments cells were harvested 16–24 hr after the addition of fatty acid to the medium. Cells grown under these conditions contained 30–40% linoleate in their phospholipids.

In order to test the general applicability of this supplementation method, fatty acid–bovine serum albumin complexes were prepared using linoleic (18:2 $\Delta^{9,12}$), linolenic (18:3 $\Delta^{9,12,15}$), arachidonic (20:4 $\Delta^{5,8,11,14}$), and nonadecanoic (19:0) acids. All of these fatty acids were incorporated into phospholipids (Table I), and the polyunsaturated fatty acids constituted 25% or more of the phospholipid acyl groups. Nonadecanoic acid was incorporated less well, even though it was supplied at twice the concentration of the other fatty acid–bovine serum albumin complexes (10 μg/ml *vs.* 5 μg/ml). As reported by others (Wisnieski *et al.*, 1973) several metabolic products of 19:0 (17:0, 17:1, 19:1) also accumulated in LM phospholipids.

Arachidonic acid (20:4) was metabolized by LM cells to give significant amounts of its elongation product, 22:4 (Table I). Contrary to a previous report (Cowen and Hey-

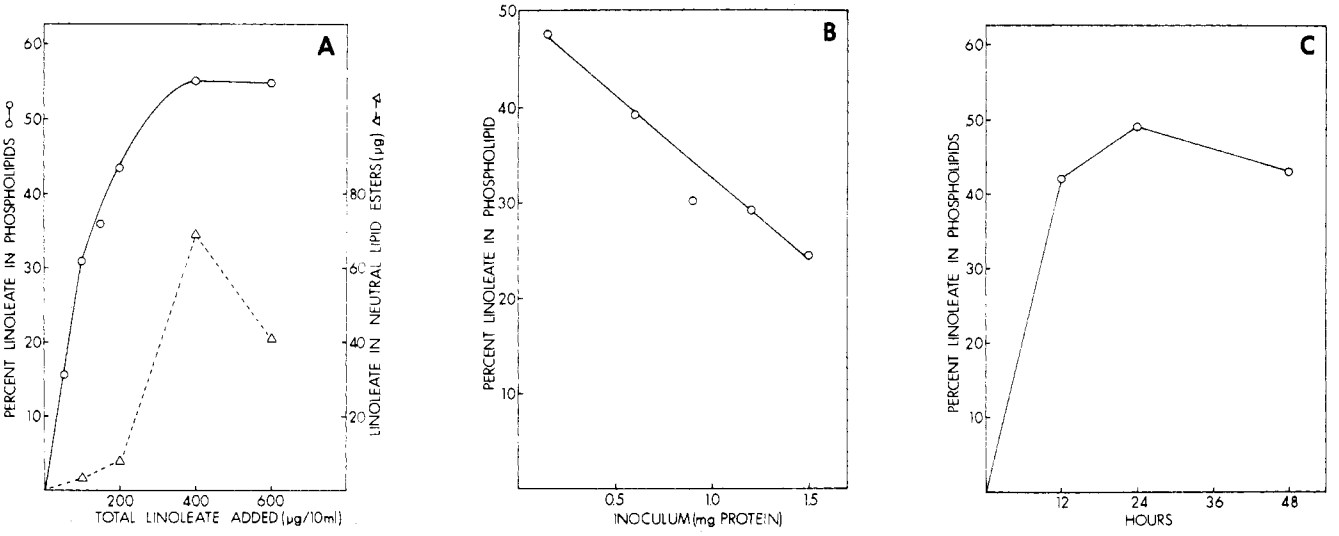

FIGURE 1: (A) Concentration dependence of linoleate incorporation. Identical aliquots of LM cells were grown with increasing concentrations of linoleate in the medium, and per cent linoleate in phospholipid was determined as described under Materials and Methods. Amounts of linoleate in neutral lipids were quantitated relative to a methyl stearate standard. (O—O) per cent linoleate in phospholipid; (Δ- - -Δ) total amount of linoleate in neutral lipid (μg/flask). (B) Cell density dependence of linoleate incorporation. Cultures were set up using increasing numbers of cells in the inoculum. Linoleate was added at a concentration of 20 μg/ml of medium, and the phospholipid composition was determined after 24 hr as described under Materials and Methods. (C) Time dependence of linoleate incorporation. Identical inocula of LM cells were grown for varying periods of time with linoleate present at a concentration of 20 μg/ml. Phospholipid compositions were determined as described.

TABLE I: Fatty Acid Composition of Phospholipids of LM Cells Grown with Different Fatty Acid Supplements.[a]

Fatty Acid Supplement	Fatty Acid Composition (Per Cent by Weight)[b]											
	16:0[c]	16:1	18:0	18:1	18:2	18:3	20:4	22:4	17:0	17:1	19:0	19:1
None	14.9	10.9	8.8	65.4								
18:2 $\Delta^{9,12}$	16.5	6.1	12.3	40.1	24.9							
18:3 $\Delta^{9,12,15}$	13.4	5.9	13.8	42.0		24.8						
20:4 $\Delta^{5,8,11,14}$	17.4	4.7	9.8	33.7			20.7	13.7				
19:0	15.9	7.1	7.7	48.9					2.2	2.7	7.5	7.1

[a] Cells were incubated in medium containing 5 μg/ml of 18:2, 18:3 or 20:4 or 10 μg/ml of 19:0 (all as bovine serum albumin complexes) for 16 hr. [b] Fatty acid compositions of the total phospholipids were determined as described under Materials and Methods. [c] Fatty acid nomenclature : number of carbons:number of double bonds Δ (position of double bonds).

drick, 1972), however, we found only trace amounts (<2%) of metabolic products of linoleic (18:2 $\Delta^{9,12}$) or linolenic (18:3 $\Delta^{9,12,15}$) acids in total LM phospholipids. One study (Williams *et al.*, 1974) reported that LM cells supplemented with linoleate contained only 7% 18:2, but 25% of the total phospholipid fatty acids were longer chain (>C_{20}) unsaturated acids. In the two studies cited above, cells were grown for 3 days in the presence of the supplement, while our cells were harvested after 16–24 hr; this time differential might account for part of the difference in amounts of metabolic products accumulated. In one experiment, in which the cells were exposed to linoleate for 48 hr, linoleic acid and its long chain products (20:2, 20:3) constituted 38.0 and 5.5% of the phospholipid fatty acid composition, respectively.

Individual phospholipids were separated by two-dimensional tlc and eluted from the silica gel. Analysis of phosphatidylethanolamine (PE) and phosphatidylcholine (PC) from control cells (Tables II and III) revealed that these two lipids had different fatty acid compositions. PE contained less palmitate (16:0) and more stearate (18:0) than PC, and also slightly less palmitoleate (16:1) and slightly more oleate (18:1) than PC. This difference is well expressed by the ratio of the per cent fatty acids with 18 or more carbons to the per cent of fatty acids with less than 18 carbons: as shown in Table II, this ratio was much higher in PE than PC. Similarly, the ratio of unsaturated to saturated fatty acids was greater in PE. The values for total glycerophospholipid in control cultures (Table III) were intermediate between those from isolated PC and PE, in keeping with the fact that PC and PE together constituted about 80% of the cellular phospholipids (Glaser, *et al.*, 1974b) of which about 55% was PC and 25% was PE.

LM cells are unable to synthesize PC by methylation of PE (Glaser *et al.*, 1974b), and choline must be supplied in the medium. Interestingly, when choline analogs were incorporated into LM cells, the corresponding analog-containing phospholipids had fatty acid compositions which more often resembled that of PE (Table II) in that the analog lipids in general contained more long chain and unsaturated fatty acids than PC.

The data in Table III show that linoleate was incorporated into both PC and PE to identical levels. It appeared, however, that the minor amounts of metabolic products of 18:2 $\Delta^{9,12}$ formed by these cells were accumulated preferentially in PE.

TABLE II: Fatty Acid Compositions of Phospholipids from LM Cells Grown in Medium Containing Choline Analogs.

	Fatty Acid Composition (Per Cent by Weight)[a]									
	Analog									
	Choline		DMEA		MEA		PA		BA	
Fatty Acid	PC	PE	PDMEA[b]	PE	PMEA	PE	PPA	PE	PBA	PE
14:0	6.0		1.0	2.3	0.5	0.8	0.7	0.9	1.2	0.9
16:0	26.8	8.8	15.0	9.5	8.3	9.2	9.8	6.2	8.0	5.2
16:1	11.6	8.4	13.4	10.8	15.1	12.2	14.5	10.4	12.7	8.2
18:0	2.2	11.8	9.1	16.9	10.1	16.7	15.1	12.6	8.8	24.3
18:1	53.3	69.2	61.5	58.0	66.1	61.0	59.9	70.0	69.3	61.5
20:1		1.7		2.5						
$C_{18,20}/C_{14,16}$	1.3	4.8	2.4	3.4	3.2	3.5	3.0	4.7	3.6	6.0
Unsat/Sat	1.9	3.8	3.0	2.5	4.3	2.7	2.8	4.1	4.6	2.3

[a] Phospholipids were isolated by two-dimensional tlc and their fatty acid compositions were determined as described under Materials and Methods. [b] PDMEA, phosphatidyldimethylethanolamine; PMEA, phosphatidylmethylethanolamine; PPA, phosphatidyl-3-amino-1-propanol; PBA, phosphatidyl-2-amino-1-butanol.

TABLE III: Fatty Acid Composition of Phospholipids from LM Cells Supplemented with Linoleate.[a]

	Fatty Acid Composition (Per Cent by Weight)							
	Control		+18:2		Total Sphingolipid[b]		Total Glycerophospholipid	
Fatty Acid	PC[c]	PE	PC	PE	−18:2	+18:2	−18:2	+18:2
14:0	1.8	0.4	1.8	1.1	2.9	2.8	2.5	2.1
16:0	26.5	8.4	27.3	9.5	77.7	81.9	14.3	20.2
16:1	11.3	8.7	5.6	5.2	9.8	4.1	9.2	6.6
18:0	3.0	14.0	6.6	18.3	5.2	4.4	12.3	12.2
18:1	57.3	66.7	31.6	35.7	4.4	4.0	61.7	30.3
18:2			24.9	24.7		2.8		28.6
20:1		1.8	0.3	1.8				
20:2			1.9	3.2				
20:3				0.6				

[a] Linoleate (as the BSA complex) was added to the medium at a final concentration of 20 μg/ml. [b] Sphingolipid and total glycerophospholipid fatty acid compositions were determined in total phospholipid samples as described under Materials and Methods. [c] PC and PE were isolated by two-dimensional tlc.

Although the total glycerophospholipids contained 29% linoleic acid, negligible amounts of 18:2 $\Delta^{9,12}$ appeared in the total sphingolipids (Table III). In view of the fact that sphingolipid turns over less rapidly than the glycerophospholipids, 24 hr might have been insufficient exposure to the supplement. Identical results were obtained, however, when cells were incubated for 48 hr with linoleate–bovine serum albumin. Weinstein *et al.* (1969) found that in sphingomyelin from L cells grown in medium containing 10% fetal calf serum, linolenic acid (18:3), and lignoceric acid (24:0) represented 60 and 30% of the total fatty acids, respectively. Apparently, long-term exposure to serum results in incorporation of polyunsaturated fatty acids into sphingolipids, but short-term supplementation does not.

It may be noted from the data in Tables I, III, and IV that the percentages of 16:1 and 18:1 decreased from the values found in control cells when linoleate was incorporated into LM phospholipids. This correlates with the results of Geyer (1967) with total cell lipids. Presumably, this adjustment is made in order to keep the lipids within a narrow range of unsaturation (Table IV) so that the membranes retain optimal fluidity or physical properties. It was of interest to determine whether growth at a reduced temperature affected the fatty acid composition of LM phospholipids, and whether supplementation of the medium with a polyunsaturated fatty acid could enhance growth at the lower temperature. As expected from the results of studies with many lower organisms, the lipids contained more unsaturated fatty acids when these mammalian cells were grown at 28° (Table IV). Specifically, there was a significant decrease in 16:0 and increase in 18:1. Identical results were observed when linoleate-supplemented cells grown at 37 and 28° were analyzed. Again, it was noted that cells grown at either temperature contained less 16:1 and 18:1 when linoleate was present. The per cent linoleate incorporated was similar, although 28° cells usually contained slightly higher levels of 18:2. The compensation in LM phospholipids for growth at lower temperatures and the compensation for the

TABLE IV: Fatty Acid Composition of Phospholipids of LM Cells Grown at Different Temperature and with the Addition of Linoleate.[a]

Fatty Acid Supplement	Temp (°C)	Fatty Acid Composition (Per Cent by Weight)[b] 14:0	16:0	16:1	18:0	18:1	18:2	% Unsaturated Fatty Acids
None	37	1.9	20.9	11.2	8.2	57.8		69.0
None	28	1.6	14.2	12.5	9.2	62.3		74.8
	Differences[c]	−0.3 ± 0.3	−6.7 ± 2.6	+1.0 ± 2.4	+1.0 ± 0.9	+4.5 ± 3.5		
18:2 $\Delta^{9,12}$	37	1.8	19.5	4.5	11.6	29.7	32.8	67.0
18:2 $\Delta^{9,12}$	28	2.0	13.3	6.0	11.1	33.4	34.2	73.6
	Differences	−0.2 ± 0.6	−6.8 ± 1.8	+1.5 ± 1.6	−0.5 ± 0.6	+3.7 ± 3.2	+1.4 ± 5.1	

[a] Cells were incubated in medium plus or minus 20 μg/ml of linoleate (complexed to bovine serum albumin) for 16 hr at the indicated temperature. [b] Determination of the fatty acid compositions of phospholipids was carried out as described under Materials and Methods. [c] Differences in each of five experiments were averaged and the standard error was calculated by the formula $\sigma = (\Sigma X^2 - N(\bar{X})^2)/(N-1)$.

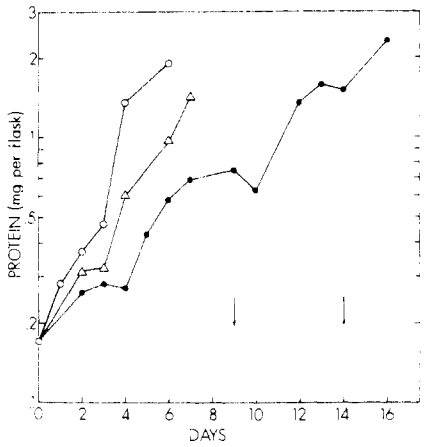

FIGURE 2: Growth of LM cells at different temperatures. Identical aliquots of LM cells were grown for the times indicated at different temperatures, and protein content was determined as described under Materials and Methods. At the times indicated by the arrows, fresh medium was added. (○) 37°; (△) 32°; (●) 28°.

presence of linoleate were both observed at 28° with linoleate supplementation. Therefore, it seems that these compensatory mechanisms are independent of one another. Cells grew more slowly at 28° than at 32 or 37° as expected (Figure 2), but they were viable and could grow to confluency at the lower temperature. No enhancement of growth at 28° was observed when linoleate was added to the medium (data not shown).

Discussion

We have shown that LM cells grown as monolayer cultures in a modified Higuchi medium (see Materials and Methods) can incorporate linoleate and other fatty acids into their phospholipids when the supplements are presented in the medium as the fatty acid–bovine serum albumin complexes. It should be pointed out that cells do not attach to the flasks well in the presence of linoleate. It was, therefore, necessary to allow several hours for the cells to attach before linoleate was added. The optimization of fatty acid incorporation and cell survival requires supplementation under carefully defined conditions, but when these requirements are met, cultures remain viable for at least 3–4 days. No attempt was made to propagate cells in fatty acid-supplemented medium since our objective is to study properties of cell membranes containing specified lipid compositions. For these purposes, incubation of cells with supplement for 24 hr suffices.

A number of different mechanisms for compensation for environmental alterations were observed with LM cells. First, the incorporation of linoleate or other polyunsaturated fatty acids into cells grown at 37° produced a decrease in the endogenously synthesized unsaturated fatty acids, 16:1 and 18:1, leading to little change in per cent unsaturated acids (Table IV). Also, when LM cells were grown at 28°, there was an increase in the overall per cent of unsaturated fatty acids present, similar to the results noted in many other organisms. It is interesting that mammalian cells can compensate for growth at lower temperatures even though they are normally not exposed to such conditions. No increase in unsaturation was observed when cells grown at 28° with linoleate were compared to cells grown at 28° without linoleate. There was no significant stimulation of growth at 28° when linoleate was incorporated, and, therefore, it would appear that the slow growth observed at 28° is not due exclusively to the physical state of the lipids. If this were true, addition of linoleate should have enabled LM cells to increase substantially the unsaturated fatty acid content of their membranes, changing the properties of the lipid bilayer so as to increase membrane fluidity, and to grow more rapidly.

Changing the polar head group of LM cell phospholipids also led to interesting differences in individual phospholipid fatty acid compositions. Lipids containing DMEA, MEA, PA, and BA, which predominantly replace PC in these cells (Glaser *et al.*, 1974a,b), had fatty acid compositions which tended to resemble the PE rather than the PC of normal cells. In view of the fact that some of the analogs (MEA, PA, BA) resemble ethanolamine more than choline, these results suggest that acylation reactions involved in the synthesis of phospholipids within LM cells are responsive to the identity of the polar head group.

Finally, it should be noted that little response of the sphingolipid fatty acid composition to linoleate supplemen-

tation could be detected. This result argues for a somewhat more specific response of LM cells to supplementation than the mere inclusion of linoleate into all fatty acid pools.

Construction of mammalian cells with widely differing lipid compositions is now feasible. Examination of specific membranes from these cells should provide important insight into the precise effects of lipids on mammalian membrane properties.

Acknowledgments

We thank Mrs. Sze-Mei Lau for her excellent technical assistance.

References

Ackman, R. G., and Burgher, R. D. (1965), *J. Amer. Oil Chem. Soc. 42,* 38.
Ames, G. F. (1968), *J. Bacteriol. 95,* 833.
Bligh, E. G., and Dyer, W. J. (1959), *Can. J. Biochem. Physiol. 37,* 911.
Cowen, W. F., and Heydrick, F. P. (1972), *Exp. Cell. Res. 72,* 354.
Dole, V. P. (1956), *J. Clin. Invest. 35,* 150.
Dulbecco, R., and Vogt, M. (1954), *J. Exp. Med. 99,* 167.
Ferguson, K. A., Conner, R. L., Mallory, F. B., and Mallory, C. W. (1972), *Biochim. Biophys. Acta 270,* 111.
Geyer, R. P. (1967), *in* Lipid Metabolism in Tissue Culture Cells, Rothblat, G. H., and Kritchevsky, D., Ed., Philadelphia, Pa., The Wistar Institute Press, p 33.
Glaser, M., Ferguson, K. A., and Bayer, W. H. (1974a), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 33,* 1296.
Glaser, M., Ferguson, K. A., and Vagelos, P. R. (1974b), *Proc. Nat. Acad. Sci. U. S. 71,* 4072.
Higuchi, K. (1970), *J. Cell Physiol. 75,* 65.
Machtiger, N. A., and Fox, C. F. (1973), *Annu. Rev. Biochem. 42,* 575.
Mavis, R. D., and Vagelos, P. R. (1972), *J. Biol. Chem. 247,* 652.
McElhaney, R. N. (1974), *J. Mol. Biol. 84,* 145.
Moskowitz, M. S. (1967), *in* Lipid Metabolism in Tissue Culture Cells, Rothblat, G. H., and Kritchevsky, D., Ed., Philadelphia, Pa., The Wistar Institute Press, p 49.
Munkres, K. D., and Richards, F. M. (1965), *Arch. Biochem. Biophys. 109,* 466.
Oldfield, E., and Chapman, D. (1972), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 23,* 285.
Overath, P., Schairer, H. U., and Stoffel, W. (1970), *Proc. Nat. Acad. Sci. U. S. 67,* 606.
Schairer, H. U., and Overath, P. (1969), *J. Mol. Biol. 44,* 209.
Silbert, D. F., Cronan, J. E., Jr., Beacham, I., and Harder, M. E. (1974), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 33,* 1725.
Spector, A. A., and Hoak, J. C. (1969), *Anal. Biochem. 32,* 297.
Stoffel, W., and Scheid, A. (1967), *Hoppe-Seyler's Z. Physiol. Chem. 348,* 205.
Weinstein, D. B., Marsh, J. B., Glick, M. C., and Warren, L. (1969), *J. Biol. Chem. 244,* 4103.
Williams, R. E., Wisnieski, B. J., Rittenhouse, H. G., and Fox, C. F. (1974), *Biochemistry 13,* 1969.
Wisnieski, B. J., Williams, R. E., and Fox, C. F. (1973), *Proc. Nat. Acad. Sci. U. S. 70,* 3669.