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THE RELATIONSHIP BETWEEN PLASMA MEMBRANE LIPID COMPOSITION AND PHYSICAL-CHEMICAL PROPERTIES

II. EFFECT OF PHOSPHOLIPID FATTY ACID MODULATION ON PLASMA MEMBRANE PHYSICAL PROPERTIES AND ENZYMATIC ACTIVITIES

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The fatty acid composition of plasma membrane phospholipids of the murine T lymphocyte tumor EL4 were systematically modified in an attempt to understand the relationship between lipid bilayer composition and plasma membrane physical and biological properties. Two plasma membrane enzyme activities, adenylate cyclase and ouabain-sensitive ($Na^+ + K^+$)-ATPase, were measured in normal and fatty acid-substituted EL4 plasma membrane fractions. The fatty acid effect on enzyme activities was similar to previously reported effects of fatty acids on cytotoxic T cell function. The activity of both enzymes was inhibited by saturated fatty acids, while unsaturated fatty acids had a moderate enhancing effect on both enzyme activities. Using two different nitroxide derivatives of stearic acid, the order parameter and approximate rotational correlation times were calculated from ESR spectra of normal and fatty acid-modified plasma membranes. No significant difference was found in either parameter in these membranes. These results, in conjunction with earlier data from our laboratory and others, suggest that caution should be exercised in inferring changes in membrane 'fluidity' based on lipid modulation of biological membranes.

Introduction

In previous publications, we described a method for altering the fatty acid composition of the plasma membrane phospholipids of EL4 tumor cells [1]. Such alterations were found to have a marked effect on the rate of patching of EL4 surface H-2 proteins [2]. We subsequently presented evidence that modification of the fatty acyl residues of membrane phospholipids also has a profound effect on the expression of cytotoxic function in cytotoxic effector T lymphocytes [3,4]. On the other hand, we have also shown that such fatty acid alterations, even when quite substantial, do not cause physical changes in T cell plasma membranes that can be detected with the fluorescent probes diphenylhexatriene or perylene [5].

Another way to measure the effects of lipid alteration on biological membranes is to analyze the impact of such treatments on the activity of intrinsic membrane proteins. The effect of membrane lipids on the activities of a number of such proteins has been studied in reconstitution studies in vitro [6,7], and by lipid alteration studies with bacteria [8] and mammalian cells in tissue culture [9–12]. In the studies reported here, we show that alterations in the fatty acid composition of plasma membrane phospholipids

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of the murine T cell tumor EL4 do indeed cause substantial alterations in the activities of two intrinsic membrane enzyme systems, adenylate cyclase and ouabain-sensitive ($Na^+ + K^+$)-ATPase. The pattern of sensitivities of both of these enzymes to the incorporation of fatty acids is (probably coincidentally) identical to the pattern of effects seen with cytotoxic T cells: unsaturated fatty acids enhance activity, while saturated fatty acids inhibit. Nevertheless, using a second physical technique, electron spin resonance (ESR) spectroscopy, we again failed to detect changes in two different spectral parameters (order and approximate rotational correlation time). These findings reinforce our previous conclusion that physical techniques that provide useful information about simple model membranes may be of limited value in the analysis of real cell membranes.

Materials and methods

Materials. [α-^{32}P]ATP, cyclic [2,8-^{3}H]AMP, [^{3}H]-ouabain were obtained from ICN Radiochemicals. Fatty acids were obtained from Nu-Chek Corp. All other chemicals were of the highest purity available from Sigma Chemical Co.

Cell culture. Fatty acid substitution of EL4 cells was done in spinner cultures as previously described [5]. Fatty acid concentrations during the 24-h culturing period were chosen to give maximum incorporation of the fatty acid into plasma membrane phospholipids while maintaining a minimum viability of 95% and approximately one doubling of cell number (see Refs. 1–5 and Table I).

Preparation of plasma membranes. Following culture, the washed cells were lysed by nitrogen cavitation as described before [5]. Alternatively, cells were lysed by two passages through a Stansted Cell Disruptor using a forward pressure of 40 lb/inch^2 and a back pressure of 32 lb/inch^2. The resultant cell lysate contained less than 0.1% intact cells, was not viscous (indicating that nuclei had not been extensively disrupted), and displayed numerous nuclei and mitochondria. The supernatant fraction from a 10-min, 4000 $\times g$ centrifugation of this cell lysate was centrifuged at 50 000 $\times g$ for 30 min, and the pellet (microsomal fraction) was resuspended in 15 ml 10 mM Tris-HCl, pH 7.4 with a Dounce homogenizer. Plasma membrane was prepared from this homogenate according to the fractionation scheme of Crumpton and Snary [13] and was stored in 10 mM Tris-HCl, pH 7.4 under nitrogen at –20°C until use.

Adenylate cyclase assay. The adenylate cyclase assay was performed as described previously [14]. Assays contained 0.32 mM disodium ATP, 1 mM disodium EDTA, 5 mM $MgCl_2$, 25 mM Tris-HCl (pH 7.4), 250 units/ml creatine kinase, 10 mg/ml phosphocreatine and sufficient [α-^{32}P]ATP to yield a specific activity of 50–80 cpm/pmol ATP. Incubations were for 10 min at 30°C. All assays were terminated by the addition of 0.9 ml of stopping solution which contained 1 mM cyclic AMP, 0.1% SDS and 0.01 μCi cyclic [2,8-^{3}H]AMP/ml. The quantitative detection of cyclic AMP formed in this assay is as described in a previous paper [14].

Ouabain-sensitive ($Na^+ + K^+$)-ATPase assay. Total and ouabain-resistant ($Na^+ + K^+$)-ATPase were measured in an enzyme-coupled, spectrophotometric assay as described by Jorgensen [15]. 50–100 μg of plasma membrane protein was added to 1 ml solution containing: 0.25 mg NADH, 1.82 mg Tris-ATP, 0.5 mg phospho*enol*pyruvate, 8 units pyruvate kinase, 5 units lactate dehydrogenase, 3 mM $MgCl_2$, 130 mM NaCl, 20 mM KCl and 30 mM histidine-HCl, pH 7.5, with or without 1 mM ouabain. Samples were mixed and transferred to quartz cuvettes and the absorbance at 340 nm was continuously monitored at 37°C in a temperature-controlled Beckman spectrophotometer. Total and ouabain-resistant ($Na^+ + K^+$)-ATPase activities were calculated using an A_M of $6.2 \cdot 10^{-3}$ for NADH. The ouabain-sensitive ($Na^+ + K^+$)-ATPase was calculated as the difference between these determined activities.

Ouabain binding. The number of ATPase molecules per mg membrane protein was determined as described by Jorgensen [15]. Membrane samples containing 100 μg protein were incubated at 37°C for 30 min in 1 ml solution containing 3 mM ATP (Tris salt), 3 mM $MgCl_2$, 120 mM NaCl, 25 mM Tris-HCl (pH 7.5), 1 μCi [^{3}H]ouabain, and 1 mM, 1.25 μM, 0.3 μM, 0.15 μM, 39 nM, 10 nM, 2.5 nM, or 1 nM unlabeled ouabain. Following incubation, tubes were cooled on ice and centrifuged at 50 000 $\times g$ for 30 min. The pellet and supernate were analyzed for radioactivity by liquid-scintillation spectrophotometry and free and bound ouabain was calculated. Ouabain-binding capacity was determined by the method of Scatchard [16].

Electron spin resonance measurement. The two probe molecules used were spin-labeled stearic acid derivatives; 2-(3-carboxypropyl)-4,4-dimethyl-2-tridecyl-3-oxazolidinyloxyl (5 NS) and 2-(10-carboxydecyl)-2-hexyl-4,4-dimethyl-3-oxazolidinyloxyl (12 NS) which have a nitroxide reporting group 5 and 12 carbons removed from the carboxyl terminus, respectively. These probe molecules were dissolved in ethanol at 10^{-2} M and added directly to membrane samples in 10 mM Tris-HCl, pH 7.4. The probe concentration in all samples was not more than 1 mol% of membrane phospholipids. The labeled samples were pelleted in sealed capillary tubes of Wiretrol 50-μl micropipettes. The aqueous supernate was removed and ESR spectra were obtained on the pellet in a Varian E104 spectrometer operating at x-band and equipped with a variable temperature accessory and an X-Y recorder. The temperature inside the sample cavity was monitored directly with a copper-constantan thermocouple sensor. Each spectrum consisted of a 4-min scan at 20 mW power. Two motional parameters were calculated according to the following equations.

$$\text{order parameter } (S) = \frac{T'_{\parallel} - T'_{\perp}}{T_{zz} - T_{xx}}$$

where $2\,T'_{\parallel}$ and $2\,T'_{\perp}$ are the separations between the outer and inner hyperfine extrema on the 5 NS spectrum [17], and T_{zz} = 32 G and T_{xx} = 6 G.

$$\text{rotational correlation time } (\tau_c) = (h_0/h_{-1})^{1/2} - 1\ W_0 \kappa$$

where h_0 and h_{-1} are the heights of the mid- and highfield lines, respectively, and W_0 is the width of the midfield line in gauss of the 12 NS spectrum [18]. κ is a constant ($6.5 \cdot 10^{-10}$) calculated from crystal parameters.

Fatty acid analysis. The fatty acid composition of plasma membrane phospholipids was analyzed as described previously [1]. Briefly, plasma membranes from approx. 10^{10} EL4 cells was extracted with chloroform/methanol (2 : 1, v/v). The phospholipids were resolved by thin-layer chromatography and were transmethylated with boron trifluoride (14% in methanol). The methylated fatty acids were analyzed by gas-liquid chromatography.

Results

($Na^+ + K^+$)-ATPase activity in normal and fatty acid-altered EL4 plasma membrane

The extent of incorporation of exogenous fatty acids under the conditions used for fatty acid substitution in these experiments is shown in Table I. As in our previous studies [5], the exogenously supplied fatty acids were incorporated by the cells to the extent of 20–50% of total plasma membrane phospholipid fatty acid.

Plasma membrane ATPase activity in control and fatty acid-modified EL4 cells was examined in the presence and absence of ouabain, which specifically binds to and inactivates the ($Na^+ + K^+$)-ATPase membrane enzyme complex. The ouabain-resistant ATPase activity is usually attributed to nonspecific phosphate-cleaving activities such as phosphatases, kinases, etc. The results of these experiments are shown in Table II. The effect of fatty acid substitution is evident when the ouabain-sensitive activity is normalized against the amount of ouabain bound, which can be taken as a direct measure of the amount of ATPase in the membrane sample. When this is done, it is clear that the effects of the fatty acids fall into two groups: the saturated fatty acids inhibit ($Na^+ + K^+$)-ATPase activity, while unsaturated fatty acids enhance it.

Adenylate cyclase activity in normal and fatty acid-altered EL4 plasma membrane

We measured plasma membrane-bound adenylate cyclase activity under three different conditions: basal activity, prostagladin E_2-stimulated activity, and fluoride-stimulated activity. Prostaglandin E_2 presumably stimulates adenyl cyclase by interaction with a hormone-specific site on the enzyme complex [19,20]. The mode of stimulation by F^- is less clear, but both prostaglandin E_2 and F^- appear to alter the V of the enzyme reaction rather than the K_m for substrate [21,22].

The results of two representative experiments are shown in Table III. Again, the effects of fatty acid alteration fell into two clearly distinct groups. The saturated fatty acids 17 : 0 and 19 : 0 both had a marked suppressive effect on all three adenyl cyclase activities. The effect of unsaturated fatty acids, although distinct, is less clear. In most cases, there

TABLE I

CONCENTRATIONS OF FATTY ACIDS IN EL4 PLASMA MEMBRANE

Lipids from normal and fatty acid-substituted plasma membranes were extracted as described in Ref. 1. The degree of incorporation of the fatty acid into plasma membrane phospholipids was determined by gas-liquid chromatography as described in Materials and Methods.

Fatty acid	Concn. in culture (μg/ml)	Plasma membrane fatty acids in unsubstituted EL4 (%)	Plasma membrane fatty acids after 24 h culture (%)
$18:1_t$	20	0	20
$18:2_c$	35	12	40.7
$18:2_t$	50	0	53.9
17 : 0	20	0	23.5
19 : 0	20	0	33.9

was a statistically significant increase in the basal and prostaglandin E_2-stimulated activities of unsaturated fatty acid-substituted plasma membrane compared to the normal control, although in other cases no significant effect was observed. However, there was, if anything, a slight decrease in the F^--stimulated enzyme activity.

ESR motional parameters in normal and fatty acid-altered EL4 plasma membrane

The order parameter (S) is by definition a ratio of the observed hyperfine anisotropy to the maximum theoretically obtainable, which corresponds to a completely rigid orientation. It therefore has values ranging from unity, for completely rigid order, to

TABLE II

$(Na^+ + K^+)$-ATPase ACTIVITY IN NORMAL AND FATTY ACID-ALTERED EL4 PLASMA MEMBRANES

$(Na^+ + K^+)$-ATPase activity was measured in the absence and presence of ouabain using an enzyme-coupled, spectrophotometric assay as described in Materials and Methods. 'Molecular activity' was determined as ouabain-sensitive ATPase activity in nmol/min per mg protein divided by pmol ouabain bound per mg protein.

Addition	ATPase activity (nmol/min per mg)			Ouabain-bound (pmol/mg)	Molecular activity
	Total	Ouabain-resistant	Ouabain-sensitive		
None	295	202	93	1.4	65
None	185	78	107	1.4	76
None	195	118	77	0.82	94
					Av. = 78 ± 15
17 : 0	129	80	49	1.2	42
17 : 0	316	151	165	5.6	29
19 : 0	187	123	64	1.1	56
19 : 0	123	65	58	2.6	23
19 : 0	150	56	95	5.9	16
					Av. = 36 ± 17
$18:2_c$	259	137	122	0.61	200
$18:2_t$	230	118	112	0.59	189
$18:2_t$	233	197	36	0.35	104
$18:1_t$	191	103	88	0.64	138
$18:1_t$	215	102	113	0.31	364
					Av. = 199 ± 100

TABLE III

ADENYLATE CYCLASE ACTIVITY IN NORMAL AND FATTY ACID-ALTERED EL4 PLASMA MEMBRANE

Adenylate cyclase activity was assayed by direct quantitation of cyclic-[^{32}P]AMP formed from [α-^{32}P]ATP as described in Materials and Methods. The concentrations of effectors used were 10 mM fluoride and 10 μg/ml prostaglandin E_2. Two representative experiments are shown in the table. The data is the mean and ±S.D. of triplicate determinations.

Addition	Adenylate cyclase activity					
	Basal		Prostaglandin E_2		Fluoride	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2
None	4.2 ± 1.4	5.4 ± 1.1	12.3 ± 1.0	11.0 ± 1.3	92.2 ± 17.3	72.1 ± 2.4
17 : 0	0.8 ± 0.3	0 ± 1.0	6.9 ± 0.2	2.1 ± 1.1	55.7 ± 5.0	10.4 ± 1.5
19 : 0	1.7 ± 0.7	0.8 ± 1.0	6.7 ± 0.5	0.8 ± 1.1	50.1 ± 1.4	3.3 ± 2.3
18 : 1_t	6.4 ± 2.0	7.6 ± 1.8	13.4 ± 3.1	19.8 ± 1.6	88.1 ± 8.2	70.4 ± 1.9
18 : 2_t	7.6 ± 1.2	9.3 ± 1.6	15.0 ± 1.1	15.1 ± 2.3	74.9 ± 3.8	65.2 ± 3.6
18 : 2_c	6.6 ± 1.1	9.8 ± 2.6	16.5 ± 1.4	12.9 ± 1.2	76.5 ± 2.5	67.1 ± 5.6

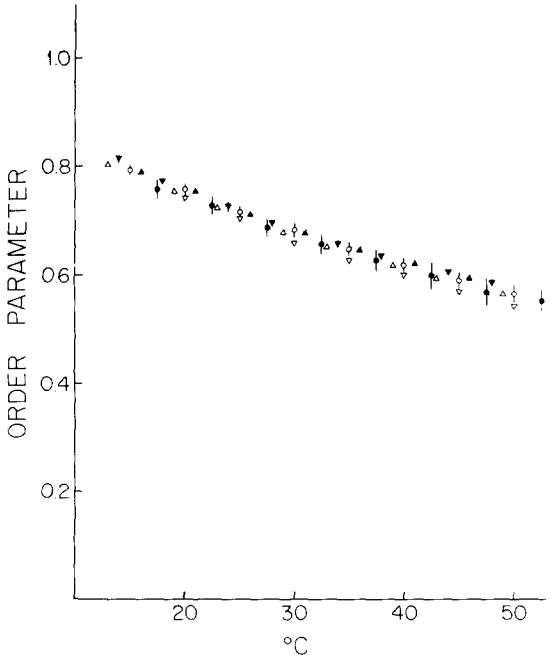

Fig. 1. Temperature dependence of the order parameter, S, in normal and fatty acid-substituted EL4 plasma membranes. Plasma membrane was isolated and labeled with the stearic acid spin probe, 5 NS, as described in Materials and Methods. Each spectrum was recorded as a 4-min 100 G scan at 20 mW power. S was calculated using the equation given in Materials and Methods. (○) normal EL4 plasma membrane; (●) 18 : 2_c-; (▲) 18 : 2_t-; (▲) 18 : 1_t-; (△) 17 : 0-; and (△) 19 : 0- substituted plasma membranes.

zero, for completely isotropic motion. We have found no significant difference in this parameter, which reflects the packing order of fatty acyl chains of membrane lipids, over a broad temperature range in plasma membranes enriched individually with all six fatty acids in our studies (Fig. 1).

The equation for calculating rotational correlation time (τ_c) is derived for the theoretical isotropic motion of a spherical molecule and its value should be in the order of 10^{-9} s. It should be noted that experimental values of this parameter are greater than 10^{-9} s and the application of this equation to the structural probes we used gives only an approximate motional parameter (τ_0) (see Discussion). Our results are shown in Fig. 2. Differences among the values of τ_0 of control and the various fatty acid-modified membranes at the lower end of the temperature range may be more apparent than real. τ_0 is a function of the ratio, h_0/h_{-1}, and the peak height of the highfield line (h_{-1}) becomes very small and difficult to measure accurately at lower temperatures. Instead, we wish to draw attention to the close agreement of τ_0 values around the growth temperature of these cells (37°C). This observation has been reported for other ESR motional parameters in the membranes of other organisms [23–27] subjected to temperature shifts with or without experimental alteration of membrane fatty acid composition.

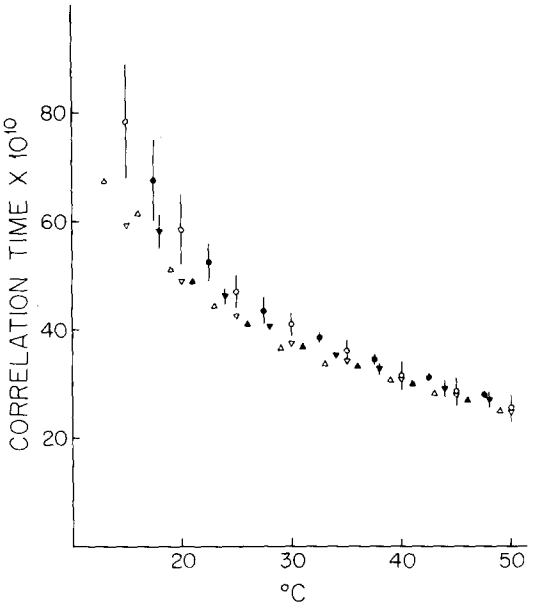

Fig. 2. Temperature dependence of the approximate rotational correlation time, τ_0, in normal and substituted EL4 plasma membranes. Samples were prepared as in Fig. 1, except that the stearic acid spin probe used was 12 NS. Each spectrum was recorded as a 4-min 40 G scan at 20 mW power. τ_0 was calculated using Kivelson's equation (see Materials and Methods). Symbols are the same as in Fig. 1.

Discussion

In previous studies, we found that alteration of the fatty acid composition of the membrane phospholipids of cytotoxic T lymphocytes could dramatically alter the ability of these cells to carry out their cytotoxic function [3,4]. On the other hand, we found that such alterations, even when quite substantial, did not significantly affect the polarization or fluorescence lifetimes of two lipid probes, diphenylhexatriene and perylene [5]. The present study was undertaken to gain further insight into the relationship between structure and function in the plasma membranes of lymphoid cells. Because cytotoxic effector cell populations are very heterogeneous with respect to cell population (less than 10% being actual cytotoxic T lymphocytes in most cases), physical and chemical studies carried out on such populations, or membranes derived from them, would be ambiguous. The studies reported here were therefore carried out on EL4, a murine T cell tumor line.

It is interesting to note that the effects of fatty acid substitution on the two plasma membrane enzymes examined in this study were almost identical to the effects of fatty acid substitution on cytotoxic T lymphocytes: unsaturated fatty acids enhance, while saturated fatty acids inhibit. The significance of this correlation is unclear at present. The effect of fatty acid modification on ATPase activity deserves additional comment. It is clear from Table II that the main effect of either saturated or unsaturated fatty acids is to alter the amount of ouabain bound to the enzyme in the cell membrane. This could represent either a change in the actual number of molecules of $(Na^+ + K^+)$-ATPase present in the plasma membrane, or some change in the ability of the enzyme to bind ouabain. The latter alteration could perhaps result from a slight conformational distortion in the ouabain-binding site of the enzyme. The present experiments provide no insight into these various possibilities. While glucose transport and utilization are required for cytolysis to be expressed [28], it is not clear from the data presented here that the sugar transport facilitation function of the sodium pump is actually affected by fatty acid substitution.

Our results on the effects of unsaturated fatty acid on plasma membrane-associated adenyl cyclase are in general agreement with those of Engelhard et al. [11]. They found that basal, NaF-stimulated and prostaglandin E_1-stimulated activities of adenyl cyclase in LM cell plasma membranes were all enhanced by unsaturated fatty acid. They did not test the effects of saturated fatty acids. They also reported that the degree of enhancement correlated with the total amount of fatty acid unsaturation introduced, a correlation not immediately apparent from our own data. Although some changes in behavior of fluorescent probes was noted, such changes were irregular and could not be correlated with the physical properties of the fatty acid used. They concluded that structural features of membrane lipids are more important in affecting enzyme activity, probably by direct molecular interactions, than are properties of fatty acids that could affect bulk properties of the membrane lipid milieu such as 'fluidity'.

As with $(Na^+ + K^+)$-ATPase, the significance of the apparent correlation between the effects of fatty acid on adenyl cyclase and on cytolysis is also unclear.

Agents that increase intracellular levels of cyclic AMP in lymphocytes tend to depress their ability to carry out cytolysis [29]. While we have not actually measured the levels of intracellular cyclic AMP resulting from membrane lipid alterations, it is curious that an increase in the activity of adenyl cyclase should correlate with an increase in lytic activity.

It must be noted that neither of the enzymes studied here have been directly implicated in any of the steps involved in cell-mediated cytolysis. Thus, for the time being we consider the fact that unsaturated fatty acids enhance cytolysis and the activity of both enzymes, while saturated fatty acids inhibit all of these activities, to be most likely coincidental. The real significance of these studies is the demonstration that lipid alterations that affect cytolysis, which is presumably a membrane-mediated process, also have a measurable effect on other known plasma membrane activities. This strengthens our conclusion [4] that the incorporation of fatty acids into plasma membrane phospholipids, which affects T cell cytolytic function, is indeed affecting plasma membrane function.

We have gathered data on two ESR parameters (S and τ_0) and find that there are no significant differences in these parameters between normal and fatty acid-substituted plasma membranes. These two motional parameters were chosen for study because they are based on well-defined motions exhibited by the probe molecules, and because we feel that the term membrane 'fluidity' can be meaningfully discussed only if it is related to a particular type of molecular motion. It is unlikely that all possible forms of molecular motion will be affected to the same degree by the same factors. Due to orientational constraints imposed by the molecular organization of the membrane bilayer, the structural probe molecules we used can be visualized as undergoing anisotropic motion by the flexing of the carbon backbone within the confines of a conical space, anchored by the carboxyl terminus of the probe near the head group region of the lipid bilayer. The flexing of the carbon backbone is made possible by the rapid isomerization between the *gauche* and *trans* conformations of carbon-carbon single bonds. The order parameters for these probe molecules is related to the magnitude of the cone angle, and is therefore a measure of the degree of freedom of this type of motion. The approximate rotational correlation time τ_0 is a measurment of the rate of rotational motion about the long axis of the molecule. Although the approximate rotational correlation time is widely used for this kind of measurement, we feel that it may be a poor choice. The approximate parameter is calculated using Kivelson's equation which is derived for an isotropically tumbling molecule [18]. The application of this equation to the anisotropic motion of a hydrocarbon probe represents a significant departure from one of the most important assumptions in the original theoretical treatment.

Our basic finding that fatty acid enrichment did not alter the average thermotropic properties of the plasma membrane bulk lipid matrix is in agreement with data presented in recent reports where membrane physical properties were measured using fluorescence polarization techniques [5,30] or fluorescence photobleaching-recovery [31]. On the other hand, it is somewhat at odds with other findings reported in the literature. Using ESR measurements similar to those used in the present study, and with the same hydrocarbon probes, King and Spector [32] analyzed the properties of fatty acid-substituted Ehrlich ascites cell plasma membranes. They reported small changes in S and τ_0 values as a result of fatty acid substitution. We also find marginal differences in S and τ_0 values in fatty acid-substituted EL4 plasma membrane, but in view of the standard deviations in our data, we are of the opinion that the magnitude of these differences is not sufficient to be considered significant. Standard deviations were not reported by King and Spector [32], who reached an opposite conclusion. However, it is difficult to make a direct comparison between our work and theirs since their point of reference was based on 18 : 1_c-substituted plasma membranes rather than on normal unsubstituted controls. Moreover, they exposed their cells to exogenous fatty acid for only 3–4 h; the resultant degree of incorporation of various fatty acids into phospholipids was quite modest compared to those reported here. In our experience, the degree of fatty acid incorporation into phospholipids at 3 h is also very low, and no effect of fatty acid on the cytotoxic function of T cells is apparent before about 6–8 h of fatty acid incorporation [4]. Since their method of feeding free fatty acid into the membrane was dif-

ferent from ours, it may be that the effects they report in whole plasma membrane are due to free (rather than phospholipid-incorporated) fatty acid.

Fatty acyl composition has been altered with concomitant changes in membrane physical properties as judged by the partitioning of ESR probes and subsequent derivation of the so-called 'characteristic temperatures,' determined as discontinuities in Arrhenius plots. Such findings have been reported for *Escherichia coli* fatty acid autotrophs [33–35]. We have not studied partitioning in part because it is not a measurement of any defined type of motion. More importantly, however, biological membranes at physiological temperatures are probably at or above the inferred phase transitions, and motions due to lateral phase separation induced by temperature changes are probably irrelevant to whatever motional freedom may be required by membrane protein functions. Lastly, the interpretation of discontinuities in Arrhenius plots may be open to question since there are cases of poor correlation with the endothermic peak detected in differential scanning calorimetry [7,33] which measures gel to liquid-crystalline transitions.

Our inability to detect any changes in the degree of flexing or the rate of rotational motion of our probes in the fatty acyl-altered membrane environment could be related to a number of factors. As fatty acid analogues, these probes may selectively partition into specific domains of the bilayer not affected by the substituted fatty acid, as was shown for free fatty acids by Klausner et al. [36]. Another possibility is that the cells are compensating for induced physical changes by altering other membrane lipid compartments, as has been suggested in studies involving shifts in growth temperatures [37,38]. We are currently analyzing the impact of fatty acid alteration on overall membrane lipid composition, and will report on this in a separate publication. It is entirely possible, however, that changes in the physical state of the plasma membrane are in fact being induced by fatty acid modification, but cannot be detected by the probes or spectral parameters we have employed. We feel that data gathered by ourselves and others, using both fluoresence polarization and ESR, suggests great caution in inferring changes in membrane 'fluidity' based on lipid alterations of real cell membranes.

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