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Diet-Induced Changes in Plasma Membrane Fatty Acid Composition Affect Physical Properties Detected with a Spin-Label Probe†

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ABSTRACT: The fatty acid composition of plasma membranes derived from Ehrlich ascites tumor cells was altered in vivo by changing the diet fed to the tumor bearing mice. After isolation, membranes prepared from cells grown in mice fed either a regular rodent chow (PM_{RC}) or a fat-deficient chow (PM_{FD}) were spin labeled with 12-nitroxide stearic acid (12NS). Discontinuities in Arrhenius plots indicated that the transition temperatures with the 12NS label were 31 and 19 °C for the PM_{RC} and 31.5 and 24.5 °C for the PM_{FD}. The order parameter, S, at 37 °C was higher for PM_{FD} than for PM_{RC}. The phospholipid composition, ratio of phospholipid to cholesterol, and distribution of fatty acyl chain lengths were similar in PM_{RC} and PM_{FD}. Marked differences were observed, however, in the degree of unsaturation of the two membrane preparations. PM_{FD} contained 34% saturated and 49% monoenoic fatty acids. Because of their high monoenoic acid content, the PM_{FD} contained a large band of unsaturation in the middle of the bilayer leaflet. By contrast, the fatty acid composition of the PM_{RC} was much more heterogeneous, with 36% polyenoic and only 23% monoenoic fatty acids. The overall unsaturation of the PM_{RC} was 1.4 times greater than that of the PM_{FD}, and the PM_{RC} contained large amounts of unsaturation in both the 9,10 and 12,13 positions of the fatty acyl chains. We conclude that the lower transition temperature and the smaller S values in PM_{RC} result from the increased amount and broader distribution of fatty acid unsaturation. These data indicate that diet-induced lipid modifications in a mammalian tumor cell are of sufficient magnitude to alter the physical properties of the plasma membrane.

The physical properties of membrane lipids are important in determining the biological activity of membrane related functions. For example, Eletr et al. (1974) have shown that Arrhenius plots of O_2 uptake and of spin-label motion in yeast exhibit discontinuities at the same temperature. Ca^{2+}-ATPase activity in sarcoplasmic vesicles also depends on the fluidity of the membrane lipids (Seelig and Hasselbach, 1971). In addition, poikilotherms have been shown to adjust their lipid composition as the temperature is lowered in order to prevent lipid fluidity from decreasing below a point detrimental to enzyme function (Marr and Ingraham, 1962; Lewis, 1962; Pearson and Raper, 1972). One important parameter that affects the physical properties of membrane lipids is fatty acyl chain unsaturation. Alterations in phase transition temperatures in phospholipid vesicles (Chapman, 1968) and yeast (Eletr and Keith, 1972) have been correlated with the degree of unsaturation. It has been suggested, however, that these types of changes might have less of an effect in the much more complex and heterogeneous mammalian membrane systems (Eletr and Keith, 1972).

We have observed that the fatty acid composition of Ehrlich ascites tumor cells can be altered appreciably by feeding the tumor-bearing mice diets containing different kinds of fats (Liepkalns and Spector, 1975; Brenneman et al., 1975). This is associated with large changes in the fatty acid composition of the Ehrlich cell plasma membrane (Awad and Spector, 1976). Therefore, it was of interest to determine whether these types of lipid changes in a heterogeneous mammalian system like the Ehrlich cell membrane would produce the same kinds of physical effects as in phospholipid vesicles and bacteria. Very large quantities of Ehrlich cells can be grown routinely, making the preparation of enough plasma membrane for electron spin resonance (ESR)[1] measurements feasible.

ESR of nitroxide free radical probes has been used extensively in physical studies of membranes (Raison et al., 1971; Seelig, 1970; Hubbell and McConnell, 1971; Eletr and Keith, 1972). Fatty acids labeled with a nitroxide group at various positions along the hydrocarbon chain can be added in vitro to membrane preparations (Hubbell and McConnell, 1971), and the ESR spectra of these probes can provide conformational and dynamic information about the physical state of the membrane lipids. These spectra may be interpreted in terms of relative molecular motion and the order of the environment in the region of the label, and they can be used to infer the existence of temperature-dependent phase separations. In the present work, we have modified the degree of lipid unsaturation in Ehrlich cell plasma membranes and utilized ESR spectra

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[1] Abbreviations used are: ESR, electron spin resonance; PM_{RC}, plasma membrane fraction from cells grown in mice fed regular mouse chow; PM_{FD}, plasma membrane fraction prepared from cells grown in mice fed a fat-deficient mouse chow; 12NS, 12-nitroxide stearic acid; S, order parameter; A^n, electron spin resonance nitrogen nuclear hyperfine splitting constant; Tris, tris(hydroxymethyl)aminomethane; Tricine, *N*-tris(hydroxymethyl)methylglycine; P_i, inorganic phosphate; TLC, thin-layer chromatography.

of a nitroxide-labeled stearic acid probe to assess the effect of these modifications on the physical properties of the membrane.

Experimental Procedure

Growth and Isolation of Cells. Ehrlich ascites tumors were grown in male CBA mice (McGee and Spector, 1974). Weanling animals were placed on either a control diet containing 4.5% fat, made up of 35% saturated, 31% monoenoic *n*-9 and 30% polyenoic *n*-6 fatty acids or an experimental diet that was fat deficient (Liepkalns and Spector, 1975). Mice were maintained on these diets for 6–8 weeks prior to intraperitoneal implantation of the tumor and during tumor growth. After 14 days of growth, tumor cells were harvested, separated from the ascites plasma, and washed (McGee and Spector, 1974).

Preparation of Plasma Membranes. Washed Ehrlich cells were homogenized three times by nitrogen cavitation in a 0.25 M sucrose solution containing 0.04 M NaCl, 0.1 M KCl, 0.005 M $MgSO_4$, and 0.02 M Tris-HCl (pH 7.6) (Molnar et al., 1969). The cell suspension was incubated in a Parr pressure bomb for 20 min at a N_2 pressure of 900 psi and then slowly expelled, causing disruption due to rapid decompression of intracellular N_2 (Wallach and Kamat, 1966). The homogenate was centrifuged at 27 000*g* for 10 min at 4 °C. The pellet was suspended in 0.25 M sucrose and sedimented again as described above. This was repeated twice. The three supernatant solutions were combined and centrifuged for 1 h at 75 000*g*. After the supernatant solution was removed, the pellet was suspended in 0.25 M sucrose with a glass homogenizer. This microsomal fraction was layered on top of a 1.1 M sucrose solution (ionic composition as above) and centrifuged for 16 h at 83 000*g* in a Beckman SW-27 swinging bucket rotor. The plasma membrane fraction was isolated from the rough and smooth endoplasmic reticulum as a band between the two sucrose layers (Molnar et al., 1969) and pelleted at 114 000*g* for 2 h. Two types of plasma membranes were studied: one obtained from cells grown in mice fed the regular control diet containing a mixture of fatty acids (PM_{RC}) and the other obtained from cells grown in mice fed the fat deficient diet (PM_{FD}).

Characterization of Plasma Membranes. The purity of the isolated plasma membranes was checked by marker enzymes. ATPase activity was assayed in the presence of 1 mM ouabain (Mg-ATPase) and without ouabain (total ATPase). The difference between the two activities was taken as the (Na^+-K^+)ATPase activity. The ATPase assay mixtures contained 3 mM ATP (Na salt), 3 mM $MgCl_2$, 120 mM NaCl, 20 mM KCl, and 30 mM Tricine-NaOH buffer (pH 8.1) (Solomonson et al., 1976). The amount of ATP hydrolyzed (P_i liberated) was determined by the method of Fiske and Subbarow (1925). Succinic dehydrogenase activity was assayed by the method of Green et al. (1955). NADPH-cytochrome *c* reductase activity was determined by the method of Sottacasa et al. (1967).

The protein content of the membrane fractions was analyzed by the method of Lees and Paxman (1972), using bovine serum albumin as the standard. Lipids were extracted from the plasma membranes with a 2:1 (v/v) chloroform–methanol mixture (Folch et al., 1957). Aliquots of the chloroform phase were taken for analysis of cholesterol (Driscoll et al., 1971) and phospholipid (Raheja et al., 1973). The procedure of Skipski et al. (1964) was used to separate classes of phospholipids from the chloroform extracts by thin-layer chromatography. Fractions isolated, in order of migration, were lysophosphoglycerides (origin), sphingomyelin, choline phosphoglycerides, serine plus inositol phosphoglycerides and ethanolamine phosphoglycerides. The lipids were extracted from the silica with chloroform:methanol:distilled water (10:10:9) (Raheja et al., 1973). Additional aliquots of the membrane lipid extracts were saponified and methylated (Morrison and Smith, 1964). The fatty acyl composition was determined using a Hewlett-Packard gas chromograph with a 6 ft × 0.25 in. glass column containing 10% Silar 10C on Gas Chrom Q (100–200 mesh). Fatty acids were identified by using standards obtained from Applied Science Laboratories.

Synthesis of Spin Labels. The method of Jones (1947) was employed to oxidize 12-hydroxystearic acid to the ketone. Methylation was carried out by dissolving the keto acid in methanol–HCl and refluxing for 30 min (Waggoner et al., 1969). The 12-nitroxide methylstearate was prepared from the keto compound by the method of Keana et al. (1967). After purification by preparative TLC on silica gel G using hexane–ether (7/3) (Waggoner et al., 1969), the 12-nitroxide derivative gave an elemental composition of: C, 69.40; H, 11.09; N, 3.50 (calculated composition of $C_{23}H_{44}NO_4$: C, 69.36; H, 11.04; N, 3.51). Infrared spectra showed a carbonyl ester bond at 5.78 μm. The 12-nitroxide stearic acid (12NS) was then prepared (Waggoner et al., 1969). After purification by preparative TLC (Waggoner et al., 1969), infrared absorptions characteristic of carboxylic acids were observed: a broad peak at 2.7–4.4 μm and a strong peak at 5.82 μm.

Spin Labeling of Plasma Membranes. The spin-labeled fatty acid (12NS) was added to bovine serum albumin by first dissolving the labeled compound in ethanol and then evaporating the solvent to produce a thin film on the glass surface (Hubbell and McConnell, 1969). A solution of fatty acid poor albumin in Krebs–Ringer bicarbonate buffer was then added and the solution mixed at 22 °C for 1 h. The molar ratio of 12NS to albumin was 0.2. The freshly isolated membrane preparations were labeled at 22 °C for 20 min by exchange from the previously labeled albumin, and they then were washed three times with fresh buffer. The final concentration of spin label in the membranes was approximately 1 per 100 phospholipid molecules, and this concentration was the same in every preparation labeled. ESR spectra were recorded with a Varian V-4502 spectrometer at X-band equipped with a variable temperature accessory. The temperature was maintained within ±0.5 °C, measured with a copper-constantin thermocouple. Spin-label concentrations in the bovine serum albumin solution and plasma membranes were measured by calculation of the integrated signal intensity, using diphenylpicrylhydrazine as a standard.

Results

Composition of the Membrane Preparations. The purity of the membrane preparations was assessed by comparison of marker enzyme activities in the plasma membrane and crude microsomal fractions (Table I). Both (Na^+-K^+)- and Mg^{2+}-ATPase activities were enriched 4.6- and 5.5-fold, respectively, in the isolated plasma membranes. The enrichment of the Mg^{2+}-ATPase in plasma membranes from Ehrlich cells has previously been observed (Awad and Spector, 1976) and is due to the presence of this enzyme on the Ehrlich cell surface (Ronquist and Agren, 1975). Both the NADPH-cytochrome *c* reductase and succinic dehydrogenase were greatly reduced in the plasma membrane fraction.

The phospholipid to cholesterol ratio and phospholipid head-group composition of the plasma membrane preparations obtained from cells grown on the regular and fat deficient diets,

TABLE I: Specific Activities of Marker Enzymes in Membrane Fractions.[a]

Subcellular fraction	Activity (μM (mg of protein)$^{-1}$ min^{-1})			
	Mg^{2+}-ATPase	(Na^+-K^+)-ATPase	NADPH cytochrome *c* reductase	Succinic dehydrogenase
Plasma membranes	190×10^{-3}	42.5×10^{-3}	6.2	0.23×10^{-3}
Microsomes	34×10^{-3}	9.6×10^{-3}	189	70×10^{-3}

[a] Values are the mean of three determinations.

TABLE II: Lipid Composition of Plasma Membranes of Ehrlich Ascites Tumor Cells Grown in Mice Fed Either the Control or Fat Deficient Diet.[a]

Lipid fraction	Units	PM_{RC}	PM_{FD}
Phospholipids/cholesterol	mole/mole	2.3 ± 0.1	2.4 ± 0.1
Cholinephosphoglycerides	%[b]	44.5 ± 1.6	43.9 ± 1.4
Ethanolaminephosphoglycerides	%	24.1 ± 1.0	23.9 ± 1.1
Serine plus inositolphosphoglycerides	%	9.0 ± 0.6	8.3 ± 0.6
Lysophosphoglycerides	%	3.5 ± 0.5	4.1 ± 0.5
Sphingomyelin	%	18.9 ± 0.7	19.8 ± 1.3

[a] Values are the mean ± SE from three membrane preparations. [b] Percent of total membrane phospholipids.

TABLE III: Fatty Acyl Group Composition of Plasma Membrane Lipids of Ehrlich Ascites Tumor Cells.

Fatty acid (% of total)	PM_{RC}	PM_{FD}
Composition		
14:0[a]	0.64	0.44
16:0	17.82	15.46
16:1	1.74	3.19
18:0	19.76	14.09
18:1	20.20	43.01
18:2	19.87	4.94
20:3	0.79	4.00
20:4	9.92	5.57
20:5	ND[b]	2.55
22:5	2.05	0.40
22:6	3.77	2.20
Others	3.44	4.15
Saturation class		
Saturated	39.01	33.99
Monounsaturated	23.08	49.05
Polyunsaturated	35.60	15.66
Chain length		
14 C[c]	0.64	0.44
16 C	19.56	18.65
18 C	59.83	62.04
20 C	11.00	9.82
22 C	7.39	7.75

[a] Chain length: degree of unsaturation. [b] Not detected. [c] Total amount of fatty acid containing this number of carbon atoms, including both saturated and unsaturated species.

PM_{RC} and PM_{FD}, are shown in Table II. In agreement with previous studies (Awad and Spector, 1976; Solomonson et al., 1976), the type of dietary fat fed to the host had no significant effect on the membrane cholesterol content, phospholipid content, or cholesterol to phospholipid ratio. Likewise, the phospholipid head-group composition was not altered.

The fatty acid composition of the total lipid extracts from PM_{RC} and PM_{FD} is shown in Table III. Approximately similar amounts of saturated fatty acids were present in both membrane preparations. The major difference was in the monoenoic and polyenoic fatty acid composition. The PM_{RC} contained 23% monoenoic acid, most of this being 18:1.[2] They also contained 36% polyenoic acids, more than half of which is 18:2. By contrast, PM_{FD} contained more than twice as much monoenoic fatty acids (49%) but less than half as much polyenoic fatty acids (16%). The PM_{RC} contained almost 40% more unsaturated bonds than the PM_{FD}. Moreover, the differences in the location and distribution of the unsaturation in the two membrane preparations are even more striking than the differences in total amount of unsaturated bonds. The PM_{RC} have large amounts of unsaturation in both the 9,10 and 12,13 positions of the fatty acyl chains, while the PM_{FD} have unsaturation predominantly in the 9,10 position. Although the diet appreciably altered the degree of unsaturation, the chain length of the membrane fatty acids remained relatively constant (Table III).

Spin-Label Data. ESR spectra recorded from the PM_{FC} and PM_{FD} spin labeled with 12NS are shown in Figure 1. The calculated values for the nitrogen hyperfine coupling constant (A^n), which correlate with the dielectric constant of the solvent system in which the nitroxide is dissolved (Tourtellotte et al., 1970), are shown in Table IV. These values have been determined by averaging the distance between the central and outer lines of the ESR spectra. These results indicate that the 12NS spin-label resides in a strongly hydrophobic environment in the isolated plasma membrane (Tourtellotte et al., 1970).

TABLE IV: Values for the Nitrogen Hyperfine Coupling Constant and the Order Parameter for Membranes Labeled with 12NS.

Parameter[a]	PM_{RC}	PM_{FD}
A^n (gauss)	14.2	14.3
$2T_\perp'$ (gauss)	21.0	21.9
S	0.40	0.46

[a] These values were measured at 37 °C. Each value represents the mean of two closely agreeing determinations on separate membrane preparations. A^n and $2T_\perp'$ values agreed within 0.1 G and S within 0.01.

The location and environment of the label were further tested by the substitution of 20 mM sodium ascorbate for 20 mM NaCl in the solutions bathing the membranes. Since ascorbate is insoluble in organic phases, it should have no effect on the ESR spectra of a spin label if it is oriented perpendicular to the membrane surface and rotating within the lipid phase about its long molecular axis (Hubbell and McConnell, 1969). None of the ESR spectra were significantly affected by the presence of ascorbate, indicating that appreciable quantities of the spin label were not exposed to the aqueous environment.

The ESR spectra of both PM_{RC} and PM_{FD} were predominantly isotropic and qualitatively similar in the high temperature range. Between 40 and 32 °C, there was no evidence of outer hyperfine extrema indicative of axial symmetry and anisotropic motion. Similar results have been reported for lobster nerve and canine erythrocytes (Hubbell and McCon-

[2] Fatty acids are abbreviated as chain length:number of unsaturated bonds. Thus, 18:1 is an 18-carbon-atom fatty acid having one double bond.

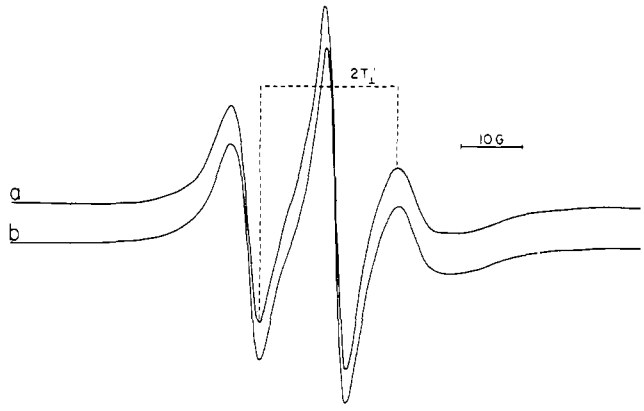

FIGURE 1: Paramagnetic resonance spectra of 12NS spin label in plasma membranes isolated from Ehrlich ascites tumor cells grown in mice fed the regular mouse chow (a) or the fat-deficient chow (b).

nell, 1969). As the temperature was lowered, the appearance of the hyperfine extrema occurred at different temperatures for the PM_{RC} and PM_{FD}. The first detectable signal for the extrema occurred at 24 °C for the PM_{RC} and 31 °C for the PM_{FD}. Since the 12NS motion remained more isotropic over a larger temperature range in PM_{RC}, its lipid environment posed smaller restraints on the molecular motion of the spin probe than did that of PM_{FD}.

The molecular freedom of motion is related to the rotational correlation time (τ_c) of the nitroxide spin-labeled molecule (McConnell, 1956; Kivelson, 1960). Since the ESR spectra in the Ehrlich cell membranes are partially immobilized and somewhat anisotropic (Figure 1), inaccuracies result in the τ_c calculation. Consequently, an extremely useful empirical motion parameter, τ_0, termed the "approximate" rotational correlation time, has been used to characterize changes in spin label motion:

$$\tau_0 = 6.5 \times 10^{-10} W_0[(h_0/h_{-1})^{1/2} - 1]$$

where W_0 is the width of the mid-field line and h_0 and h_{-1} are the heights of the mid- and high-field lines, respectively (Keith et al., 1970). Although the actual numerical values of τ_0 may be in error, the relative values for purposes of comparison of the molecular motion within PM_{RC} and PM_{FD} are accurate (Keith et al., 1970). An increase in label mobility produces a decrease in τ_0. Additionally, discontinuities in Arrhenius plots of the motion parameter have been used to identify the transition temperatures at which conformational changes are believed to occur in the lipid bilayer of biological membranes (Raison et al., 1971; Eletr and Inesi, 1972; Eletr and Keith, 1972).

Arrhenius plots of τ_0 for 12NS in PM_{RC} and PM_{FD} were widely divergent above 15 °C (Figure 2). τ_0 for PM_{RC} was lower at every temperature tested indicating a marked difference in the physical characteristics of the two membranes. This was especially evident in the physiologic temperature range. Two discontinuities were noted in the Arrhenius plots in each membrane preparation: 31 and 19 °C in PM_{RC} and 31.5 and 24.5 °C in PM_{FD}.

The above observations are qualitative, based on changes in an empirical motion parameter and the first detectable appearance of anisotropic motion. A quantitative expression based on an axial model for spin-label motion in membrane systems nas been developed, the order parameter S (Hubbell and McConnell, 1971). $S = 1$ in the most ordered state possible, i.e., if all of the C-C bonds preceding the spin-labeled 12-position carbon are trans (perpendicular to the plane of the

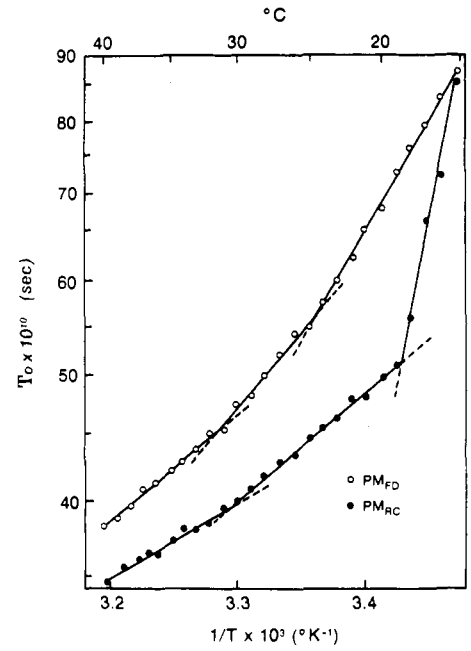

FIGURE 2: Arrhenius plots of the motion parameter τ_0, of the 12NS spin label in plasma membranes of Ehrlich ascites tumor cells obtained from host mice fed diets of either regular mouse chow (PM_{RC}) or fat-deficient mouse chow (PM_{FD}). The values of τ_0 were determined over the temperature range 40-15 °C. All of the points for both PM_{RC} and PM_{FD} are the average of two closely agreeing (within 1%) determinations on separate membrane preparations. The data were analyzed by the method of least squares and are fitted best by three straight lines intersecting at two transition temperatures: 31.5 °C and 24.5 °C for PM_{FD}; 31 °C and 19 °C for PM_{RC}. The regression coefficients for the slopes of the three lines (>31.5 °C, 31.5-24.5 °C, <24.5 °C) were 0.993, 0.991, and 0.990 for PM_{FD}. The regression coefficients for PM_{RC} (>31 °C, 31-19 °C, <19 °C) were 0.987, 0.994, and 0.982.

bilayer). If the motion of the electron is isotropic, indicating the most fluid state possible, $S = 0$. S can be determined directly from ESR spectra of less fluid systems as follows (Gaffney, 1975, 1976):

$$S = \frac{T_\parallel' - T_\perp' - c}{T_\parallel' + 2T_\perp' + 2c} \times 1.723$$

where $T_\parallel'$ and $T_\perp'$ are the separations between outer and inner hyperfine extrema, and $c = 1.4\ \text{G} - 0.053(T_\parallel' - T_\perp')$. Such a direct measurement cannot be made from the spectra obtained here because the spin-label motion is too rapid for accurate measurement of the outermost hyperfine extrema ($T_\parallel'$) in the temperature range of interest. However, Gaffney (1976) has shown that the measurement $2T_\perp'$ is inversely proportional to S for nitroxide labels in biological membranes over the entire range of order ($S = 0$ to $S = 1.0$). Furthermore, the quantity ($T_\parallel' + 2T_\perp'$) depends on molecular fixed parameters of the spin probe and has a value of 44.5 G, and $c = 0.8$ G, for the 12-position label in biological membranes (Gaffney, 1975, 1976). An extremely good estimate of S over its entire range of values can then be determined using the resulting equation (Bales et al., 1977):

$$S = \frac{43.7\ \text{G} - 3T_\perp'}{46.1\ \text{G}} \times 1.723$$

The absolute values of S obtained from this equation will depend on the choices of the molecular fixed parameters and c, but the relative values of S are valid indications of changes in fluidity independent of these choices (Gaffney, 1975; Bales et al., 1977).

The quantity $2T_\perp'$ was measured at 37 °C in the spectra of

each membrane preparation, and S was calculated as a second means (in addition to changes in τ_0) of estimating the effects of fatty acyl enrichment on membrane fluidity. As the data in Table IV indicate, the lipid bilayer in PM_{FD} appears to be more ordered than in PM_{RC} at physiologic temperatures, in agreement with the τ_0 values.

Discussion

Double bonds provide steric hindrance to the tight packing and ordered arrangement of fatty acyl chains in lipid bilayers (Eletr and Keith, 1972). The introduction of a cis double bond into a saturated lipid bilayer forms a kink which perturbs the axial symmetry of fatty acyl chains, thus lowering the phase transition temperature (Chapman, 1968). Vandenhuval (1968) has shown from x-ray data that the effective volume of a fatty acid increases greatly with increasing unsaturation. Therefore, polyunsaturated fatty acids would be expected to produce larger decreases in the transition temperature as a result of the greater perturbation on ordered packing produced by the disruptive effect of many kinks.

For these reasons we attribute the differences in physical properties of PM_{RC} and PM_{FD}, as demonstrated by the Arrhenius plots and S values, to alterations in the degree and location of unsaturation within the membrane fatty acyl chains. The extent of unsaturation appears to be the main factor, for the fatty acyl chain length and phospholipid–cholesterol ratio are not significantly different in PM_{RC} and PM_{FD}. Likewise the phospholipid head-group composition is not different in the two membrane preparations. The PM_{FD} fatty acyl profile is 85% binary, consisting primarily of saturated and monoenoic fatty acids. Since the predominant monoenoic acid is oleate ($18:1n-9$), the PM_{FD} have for the most part a single band of unsaturation across the middle of the bilayer leaflet, with the regions on either side being largely saturated. By contrast, the PM_{RC} have 40% more unsaturated bonds than PM_{FD}, and much of the additional unsaturation is between carbon atoms 12 and 13. This increase in unsaturation in the $n6$ position, resulting in the formation of larger kinks, would be expected to disturb the packing order of the acyl chains to a greater extent toward the center of the bilayer. The relative values of τ_0 and the temperatures at which discontinuities in Arrhenius plots occurred in PM_{RC} and PM_{FD} are consistent with this interpretation. Moreover, the differences in S and τ_0 persisted even when the temperature was raised to 37 °C. This indicates that the changes in membrane physical properties that were produced are likely to be manifest in the physiologic state.

Recent studies have established that the lipid composition of a mammalian cell plasma membrane can be modified extensively by exposure of the intact cell to different kinds of fatty acids (Wisnieski et al., 1973; Ferguson et al., 1975). Some of these modifications have been associated with changes in the activity of membrane bound enzymes (Coleman, 1973; Engelhard et al., 1976). Others have produced structural changes detected by physical or chemical probes (Rittenhouse et al., 1974; Wisnieski et al., 1974; Horwitz et al., 1974; Schroeder et al., 1976). These lipid modifications have been produced in cell culture where the composition of the media can be carefully controlled. Often, the lipid content of the media employed is quite unphysiologic. The novelty of the present work is that the lipid modifications were produced by dietary manipulation while the cells were growing in an intact animal. Our results indicate that the degree of membrane lipid modification achieved under these physiologic conditions is sufficient to alter certain of the physical properties of the membrane.

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Glycosphingolipids of Purified Human Lymphocytes†

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ABSTRACT: Biochemical analysis of the glycosphingolipids (GSLs) of human lymphocytes revealed qualitative and quantitative variations among purified lymphocytes from different tissues. The major neutral GSLs of tonsil lymphocytes are glucosyl ceramide (CMH), lactosyl ceramide (CDH), trihexosyl ceramide (CTH), and globoside. Thymocytes and peripheral blood lymphocytes (PBL) contain only traces of CTH and globoside, and PBL contain more CMH and CDH per cell than tonsil lymphocytes. Thymocytes and PBL contain relatively large amounts of more complex neutral GSLs that are present in only trace amounts in tonsil lymphocytes. Peripheral blood lymphocytes contained three and five times more lipid-bound sialic acid than thymocytes and tonsil lymphocytes, respectively. Thymocytes and PBL contained mostly hematoside, whereas tonsil lymphocytes contained more complex gangliosides in addition to hematoside. The observed differences in GSL content among these cells may be related to their content of B cells, which comprise approximately 50% of tonsil lymphocytes, 10% of PBL and 0–2% of thymus cells, and/or the known differences in functional capacities of cells in different lymphoid organs. These findings suggest that cell surface GSLs may serve as markers for identification of functional subpopulations of human lymphocytes.

One of the most important advances in immunology in recent years was the recognition of the functional specialization of lymphocyte subpopulations. The use of alloantisera against theta antigen (Reif and Allen, 1966) and the Ly antigens (Cantor and Boyse, 1975) led to the identification of T cells and T cells subsets in the mouse. More recently Huber et al. (1977) and Ahmed et al. (1977) described cell surface antigens present on murine B cell subpopulations. Comparable markers for human lymphocytes are not as well defined but there is evidence for subsets of human T cells (Moretta et al., 1975; Evans et al., 1977).

Our studies of murine lymphocytes (Stein-Douglas et al., 1976; Stein et al., 1977) demonstrated that purified antibodies to glycosphingolipids (GSLs)[1] can be used to identify subpopulations of T cells and immunoglobulin-bearing lymphocytes (B cells). We have undertaken a chemical and immunological study of human lymphocyte GSLs, and we report here differences in the GSL content of normal lymphocytes from different tissues.

Materials and Methods

A. Cell Preparation and Characterization

Peripheral Blood Lymphocytes (PBL). Leukocyte-rich plasma (acid-citrate-dextrose anticoagulant) was obtained by passage of multiple units of human blood from a single donor through a Haemonetics Model 30 cell separator (Haemonetics Corp., Natick, Mass.; Segel et al., 1977). The leukocyte-rich plasma was centrifuged at 150*g* for 8 min at 4 °C to remove platelets and the lymphocyte-rich pellet was mixed with an equal volume of freshly drawn plasma (of the donor blood type) to enhance coagulation. The cell suspension was recalcified by the addition of 0.1 volume of 10% $CaCl_2$ and defibrinated by manual rotation for 40 min at room temperature in an Erlenmeyer flask containing 5 glass beads (2-mm diameter) per 10 mL of cell suspension. The fibrin clot was removed and the cells were diluted with Eagle's minimum essential medium (MEM) to a concentration of 2×10^8 leukocytes per 25 mL. The diluted cell suspension was centrifuged in aliquots of 25 mL of cells on 10 mL of Ficoll-Hypaque (F-H)

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[1] Abbreviations used: F-H, Ficoll-Hypaque; PBS, phosphate-buffered saline, 0.02 M PO_4, pH 7.3, 0.15 M NaCl; FCS, fetal calf serum; MEM, minimal Eagle's medium; TLC, thin-layer chromatography; GSLs, glycosphingolipids; PBL, peripheral blood lymphocytes.