*Biochimica et Biophysica Acta*, 556 (1979) 408–422


BBA 78506

# DIFFERENCES IN MEMBRANE UNSATURATED FATTY ACIDS AND ELECTRON SPIN RESONANCE IN DIFFERENT TYPES OF MYELOID LEUKEMIA CELLS·

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(Received January 31st, 1979)

*Key words: Unsaturated fatty acid; Cloning; Differentiation; Fluidity; Lipid composition; (Myeloid leukemia cell)*

## Summary

The fatty acid composition and some physical properties of intact cells and isolated plasma membranes of two types of mouse myeloid leukemia cell clone grown in culture have been examined. One clone type, $MGI^+D^+$, can be induced by the macrophage and granulocyte-inducing protein (MGI) to differentiate into mature macrophages and granulocytes. The other clone type, $MGI^+D^-$, could not be induced to differentiate into mature cells. A two-fold increase in the ratio of saturated fatty acid to unsaturated fatty acid was found in the $MGI^+D^-$ compared to the $MGI^+D^+$ clones. The $MGI^+D^-$ clones produced an unusual polyunsaturated $C_{20:5}$ fatty acid at 28°C, whereas the $MGI^+D^+$ clones did not grow at this temperature. The cells and their isolated plasma membranes were studied by electron spin resonance. The motion of the 5-nitroxide stearate spin label was found to be higher in the intact cells and in the membranes of $MGI^+D^-$ clones than of the $MGI^+D^+$ clones. The cells of $MGI^+D^+$ clones showed a similar freedom of motion to normal myeloblasts from the bone marrow. The results indicate that myeloid leukemia cells which differ in their competence to be induced to differentiate into mature cells have different physical properties of their plasma membranes and that this is correlated with their fatty acid acyl chain composition.

## Introduction

It had been shown that there are certain surface membrane differences

Abbreviations: MGI, macrophage and granulocyte-inducing protein; $S$, order parameter; $\tau_0$, motion parameter; $T'_{\parallel}$, outer hyperfine splitting; $T'_{\perp}$, inner hyperfine splitting.

between two types of myeloid leukemia clones [1—4] which differ in their ability to differentiate into mature cells. One type of clone, $MGI^+D^+$, was induced to differentiate into mature cells by the protein inducer, MGI (macrophage and granulocyte-inducing protein), whereas the other type of clone, $MGI^+D^-$, could not be induced to differentiate by MGI into mature cells [5].

The membrane physical properties resulting from the fatty acid acyl chains of the phospholipids in the plasma membranes are important factors in the regulation of cellular activities in living cells [6—8]. Studies have shown that changes in lipid composition can modify the rate of enzyme activities [9,10], concanavalin A agglutination [11], cell adhesion [12,13] and phase transition temperature [14]. The degree of unsaturation of the fatty acyl groups in phospholipids can be modified either by medium supplementation [15,16] of by growing the cells at different temperatures [17,18]. It is, therefore, of interest to determine if the fatty acid differences exist in the plasma membrane of these myeloid leukemia cell types, and what effect they have on these cells' physical and biological properties.

The ESR spectra of nitroxide-free radicals have been used in the physical studies of biological membranes [19—21], and fatty acid-nitroxide spectra could provide conformational and dynamic data about the physical state of the membrane lipids [22]. In this study, ESR spectra were used to determine the relative molecular motion parameters, and the order parameters of the environments in the region of the nitroxide-free radical anisotropic motion. Although the actual values of the motion parameters and the order parameters are merely a close estimation, the relative values can be used for comparative study of the molecular motion in the biomembrane [24,38]. The rapid incorporation of the spin probe does not inhibit cell viability [20] and, with a low label concentration [25], spin probe technique is reproducible and valid to study the physical properties of plasma membranes.

## Materials and Methods

*Cells and cell cultures.* The clones of myeloid leukemia cells employed in this study were established in vitro [5] from a myeloid leukemia in a SL mouse [52]. Cells were grown in suspension in Dulbecco's modified Eagle' medium (H-21, Grand Island Biological Co.) with a four-fold concentration of amino acid and vitamins, with 10% heat-inactivated fetal calf serum, (Grand Island Biological Co.) at different temperatures. Cells were grown in petri dishes (Nunc, Denmark) in suspension as myeloblasts and subcultures made every 3—4 days. Normal bone marrow myeloblasts were isolated from the femurs of 2—3-month-old SL mice. The mice were injected intraperitoneally 3 days earlier with 10% sodium caseinate (Difco) and the myeloblasts were obtained by isolating the non-rosette femur cells after $C_3$ rosette formation and Ficoll-Hypaque density centrifugation [53]. To obtain a higher yield, only one of $C_3$ rosette formation was used. The yield was about $20 \cdot 10^6$ cells from ten SL mice.

*Preparation of plasma membranes.* The myeloid leukemia clones were grown in large petri dishes and harvested after 5—6 days, yielding about $6 \cdot 10^9$ cells for the initial plasma membrane isolation. The cells were washed twice with

phosphate-buffered saline, then resuspended in Hanks' balanced salt solution, and were disrupted by nitrogen cavitation (Pan Inst. Co.). The cell suspension was incubated in a chilled pressure vessel for 15 min of equilibration with the 500 lb/inch$^2$ of $N_2$ under constant gentle stirring [26]. The cells were disrupted by the slow release of pressure. The resulting homogenate was centrifuged at 6000 × *g* for 15 min (4°C) to remove intact nuclei, mitochondria and non-disrupted cells [27]. The supernatant was further centrifuged at 105 000 × *g* for 75 min (Beckman ultracentrifuge rotor type 30). The supernatant was discarded and the microsomal pellet resuspended in 10 mM Tris-HCl buffer, pH 8.6, using a small, hand-driven homogenizer (20 ml) and washed twice. 2 ml of the microsomal fraction was layered on top of 8 ml of Ficoll solution (Pharmacia) ($\rho$ = 1.01 at 25°C) [27], in cellulose nitrate tubes and centrifuged at 82 500 × *g* for 15 h (Beckman ultracentrifuge with rotor sw-27 at 17 000 rev./min). The fraction at the Ficoll-buffer interface was collected by using a Pasture pipette with a bent tip, diluted five-fold with 10 mM Tris-HCl, 10 mM $MgSO_4$, pH 8.6, centrifuged at 141 000 × *g* for 30 min (Beckman sw-36 rotor at 35 000 rev./min). The precipitated material was suspended in 1 mM Tris-HCl buffer, pH 8.6, and was immediately used for analysis or stored at −20°C.

*Assays for marker enzymes.* Ouabain-sensitive ($Na^+ + K^+$)-ATPase (EC 3.6.1.3) was assayed as described by Schimmel et al. [50] with some modification. The assay mixture contained 30 mM imidazole/HCl buffer (pH 7.5), 120 mM NaCl, 20 mM KCl, 5 mM $NaN_3$, 0.5 mM ethylene glycol bis($\beta$-aminoethyl ether)-*N*,*N*′-tetraacetic acid (EGTA), 4 mM $MgCl_2$ and 3 mM Tris/ATP (Sigma). The ($Na^+ + K^+$)-ATPase activity was measured after 30 min at 37°C in the absence and the presence of 1 mM ouabain. The reaction was stopped with 10% cold trichloroacetic acid, the protein sedimented by centrifugation and the inorganic phosphate in the supernatant was determined by the method of Ames [51]. ($Na^+ + K^+$)-ATPase was measured within two days of preparation of the plasma membrane. 5′-Nucleotidase (EC 3.1.3.5) was assayed essentially as described by Beldfield [47], with 10 mM $MgCl_2$, 10 mM Tris-HCl, pH 7.9, 50 mM sodium $\beta$-glycerophosphate and 1.2 IU of calf intestinal adenosine deaminase (Sigma). The sample solution was incubated 15 min at 37°C, and 0.1 mM 5′-AMP was added and the production of adenosine was monitored spectrophotometrically (Zeiss) at 265 nm with this system. The change in absorbance is linear with time and with adenosine production.

Glucose-6-phosphatase (EC 3.1.3.9) was assayed with 50 mM maleate buffer (pH 6.0), 4 mM EDTA, 1 mM KF and 50 mM glucose 6-phosphate [28]. After 15 min of incubation at 37°C, the reaction was stopped with 10% trichloroacetic acid the mixture centrifuged and $P_i$ was determined in the supernatant; blanks contained the buffer, EDTA and KF. The amounts of $P_i$ liberated in the absence of the substrate and the enzyme, respectively, were subtracted from that of the complete system.

Acid phosphatase (*p*-nitrophenyl phosphatase) (EC 3.1.3.2) was assayed with 50 mM sodium acetate buffer (pH 5.0), 0.1% (w/v) Triton X-100, 5 mM *p*-nitrophenyl phosphate (Sigma) and the enzyme solution [29]. The assay mixture was incubated for 30 min at 37°C and was stopped with 2 ml of 0.1 M NaOH. The absorbance was determined at 400 nm and the acid phosphatase activity

was calculated from a standard curve of commercial *p*-nitrophenol (Sigma).

Succinate : cytochrome *c* reductase (EC 1.3.99.1) was assayed with 0.3 mM of fresh KCN and 50 nM phosphate buffer (pH 7.5). The reaction was started with the addition of the substrate and was incubated at room temperature for 5 min, the $\Delta A$ was read at 550 nm [30].

NADH : lipoamide oxidoreductase (EC 1.6.4.3) was assayed by the method of Wallach and Kamat [27].

Protein was estimated by modified method of Lowry et al. [23], using Folin reagent, the test tubes were capped and placed in boiling water for 45 s, and immediately placed in an ice bath. The samples were read at 750 nm in a Zeiss spectrophotometer. Bovine serum albumin (Sigma fraction V) was used for the standard curve.

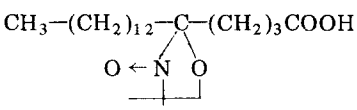
*Fatty acid analysis.* Harvested cells and membrane fractions were washed twice with 0.85% NaCl. Lipids were extracted from the cells with chloroform/methanol (2/1, v/v) [31], in Virtis '23' homogenizer for 5 min at room temperature, followed by 2 h of slow stirring. The lipid extract filtered through filter paper (Whatman No. 1) and dried under reduced pressure in a rotary evaporator. The extracted lipids were then washed according to Folch et al. [31] to remove the water-soluble non-lipid contaminants, and anhydrous sodium sulfate was added to the chloroform layer to remove all traces of water. The lipids were subjected to further separation on column chromatography on 1 g of silicic acid (Sigma SIL-R) in a disposable pasteur pipette. The neutral lipids were washed out with 30 ml of chloroform, followed by 30 ml of methanol to extract the polar lipids. The lipid fractions were stored at −20°C in 1 ml of chloroform under $N_2$ or were used immediately for analysis. The fatty acids were converted to their methyl ester derivatives by using boron fluoride-methanol (Supelco, Inc.), according to the method of Morrison and Smith [49]. The samples of the polar lipid fraction were evaporated to dryness under nitrogen, and 2 ml of $BF_3$ reagent was added and the test tubes were tightly closed and heated to 80°C for 30 min, cooled, and 1 ml of glass-distilled water was added. The fatty acid methyl esters were extracted three times with 4 ml of hexane, dried under $N_2$ and stored in 1 ml of hexane at −20°C.

The fatty acid methyl ester were analyzed with a Beckman gas-liquid chromatograph (GLC) equipped with a hydrogen flame ionization detector, on a polar column (15% DEGS coated on 80/100 mesh of Supelcoport, Supelco, Inc.,) and on a non-polar column (3% OV-1 coated on 80/100 mesh Supelcoport, Supelco, Inc.,). Both columns consisted of coiled glass tubing (2.5 m long, 4 mm inner diameter). Injector temperature was 250°C and the column at the isothermic temperature of 180°C, detector at 250°C with a nitrogen flow rate of 36 ml/min. Fatty acids were identified by their retention time relative to that of standard methyl ester mixtures and by a Finnian model 1011 masspectrometer equipped with a 1% OV-1 GLC column [32]. The percent of the total extracted fatty acids was calculated from the area under the peaks of the GLC spectra [33].

*Electron spin resonance (ESR) spectroscopy.* ESR measurements were carried out on a Varian E-12 or E-3 spectrometers that were equipped with a variable temperature-controlled unit. The spectra were recorded as the first derivative of the absorption curves, with field modulation of 100 KHz, and the

microwave power was kept at 10 mW to avoid signal saturation or sample heating. The scanning time was 4—8 min, and the time constants were 0.1—0.1; the temperatures throughout the ESR recording was monitored by a copper-constant thermocouple that was placed above the sample in the ESR cavity. The recorder scan width was calibrated with Fremy's salt [34] with center field of 3320, and was operating in the X band that was linear and accurate to ±0.1 G.

The spin label, 5-nitroxy stearate I [3,12] (2,3-carboxypropyl-4,4-dimethyl-2-tridelyl-3-oxazolidinyloxyl):



was purchased from Syva and was kept at a stock solution of 0.1 M in 100% ethanol. The cells and the membrane fractions were dispersed in 10 ml of phosphate-buffered saline containing 5—10 μM of the spin label [20], and were incubated for 15 min in 37°C with a gentle shaking. The samples were washed three times with phosphate-buffered saline to remove the free spin label, and used immediately for the ESR measurements. In the glutaraldehyde study, the cells and the membrane fractions were fixed with 2.5% glutaraldehyde at 0°C for 30 min and the spin probe was introduced as in the unfixed cells. In some experiments 0.5 mM $K_3Fe(CN)_6$ was used to reactivate the spin probe signal [20], after 60 min of incubation at 37°C the oxazolidine ring decayed with oxydation by the biological material. The ferricyanide does not penetrate into the cytoplasm so only the spin label present in the plasma membrane was recorded [20]. The results were expressed in terms of the motion parameter and the order parameters calculated from the ESR spectra.

*Electron microscopy.* Pellets of freshly prepared cell membrane fractions were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, for 2 h at room temperature. After washing the pellets with cacodylate buffer, 2% $OsO_4$ was added for 1 h postfixation. After the dehydration process (with 50%, 75%, 96% and 100% ethanol) the pellets were inbedded in a mixture of Epon 812 and Araldite. Ultrathin sections were obtained with Sorval Potter Blub MT-2-B Ultra-Microtome and were placed on 300—400 mesh uncoated copper grids, further stained with 1% uranyl acetate and 1% lead citrate, and were examined with Phillips 300 electron microscopy at 80 kV.

## Results

*Plasma membrane isolation and marker enzyme activity*

About $6.5 \cdot 10^9$ cells were used for the plasma membrane isolation and the estimation of contamination of the membrane fraction by marker enzymes are given Table I. The procedure for preparation of plasma membrane involved with the disruption of the plasma cell membranes by nitrogen cavitation which gave a 30% cell shearings. This method was reproducible and under the optimal conditions described in Materials and Methods only the plasma membrane was disrupted leaving the nuclei intact, as seen by phase-contrast microscopy observation.

The enrichment of the specific activity of $(Na^+ + K^+)$-ATPase of the mem-

TABLE I

ESTIMATION OF CONTAMINATION OF $MGI^+D^+$ AND $MGI^+D^-$-ISOLATED PLASMA MEMBRANES BY SPECIFIC ACTIVITY OF MARKER ENZYMES

$(Na^+ + K^+)$-ATPase, glucose 6-phosphate is expressed in mg protein. 5′-Nucleotidase is expressed as $\Delta A$/60 min per mg protein/8.1. Acid phosphatase is expressed in μmol *p*-nitrophenol/min per mg protein. Succinate:cytochrome *c* is expressed as μmol cytochrome *c*/min per mg protein. NADH diaphorase is expressed as μmol NADH/min per mg protein. n.d., not determined.

| | Cell type | Clone No. | $(Na^+ + K^+)$-ATPase | 5′-Nucleotidase | Glucose 6-phosphate | Acid phosphatase | Succinate cytochrome *c* reductase | NADH diaphorase |
|---|---|---|---|---|---|---|---|---|
| Cell homogenate | $MGI^+D^+$ | 12 | 0.229 | 0.360 | 2.455 | 4.7 | 2.717 | 6.89 |
| | | 9 | 0.148 | n.d. | 2.083 | 4.3 | 3.896 | 5.53 |
| | $MGI^+D^-$ | 19 | 0.746 | 0.463 | 1.068 | 2.5 | 2.300 | 7.45 |
| | | 13 | 1.216 | n.d. | 0.849 | 2.7 | 3.635 | 4.54 |
| Isolated membranes | $MDI^+D^+$ | 12 | 4.608 | 0.556 | 0.250 | 0.00 | 0.648 | 1.67 |
| | | 9 | 2.236 | 0.632 | 0.158 | 0.00 | 0.720 | 0.99 |
| | $MGI^+D^-$ | 19 | 7.255 | 0.240 | 0.000 | 0.18 | 0.486 | 4.67 |
| | | 13 | 7.859 | 0.072 | 0.096 | 0.25 | 0.474 | 2.31 |

brane fraction of $MGI^+D^+$ and $MGI^+D^-$ were 18.15 and 7.7-fold, respectively, relative to the whole cell homogenate. There was about 5.2-fold increase in this enzyme activity in the $MGI^+D^-$ cells and membrane. The $(Na^+ + K^+)$-ATPase specific activity showed some variation with the different clone types as shown

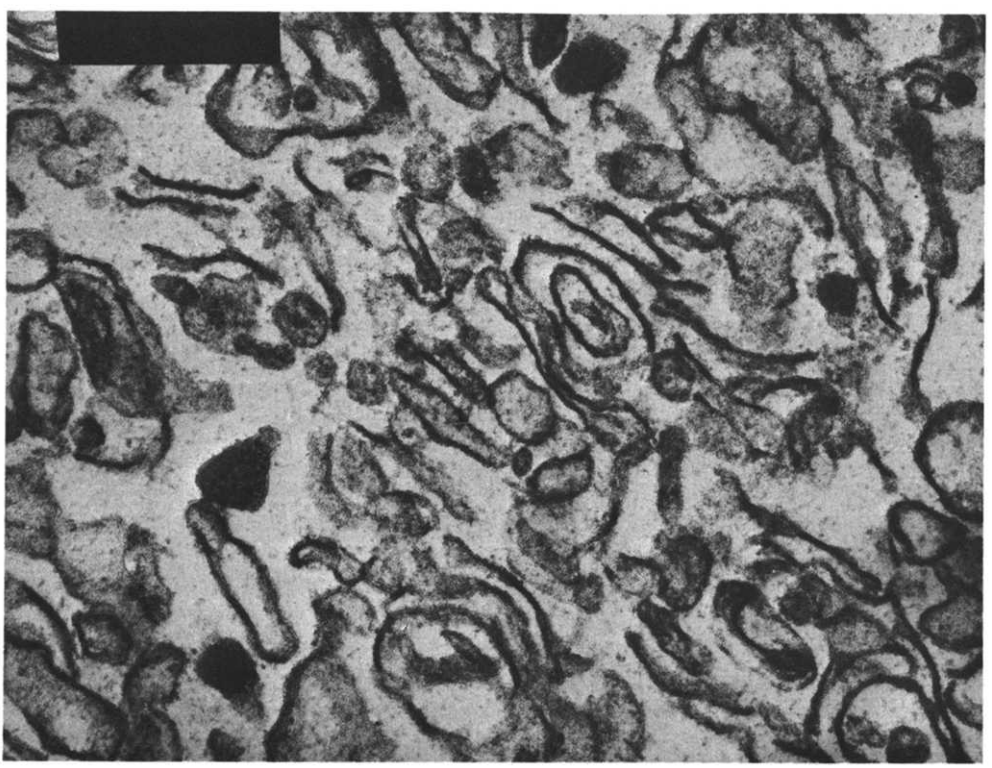


Fig. 1. Fraction of plasma membranes isolated from a $MGI^+D^+$ clone of myeloid leukemia cells. X105 280.

TABLE II

FATTY ACID COMPOSITION OF POLAR LIPIDS FROM CELLS AND ISOLATED PLASMA MEMBRANES OF $MGI^+D^+$ AND $MGI^+D^-$ CLONES GROWN AT 37°C

Fatty acid nomenclature: chain length:number of double bonds in the fatty acid.

| Cell type | Clone No. | Fatty acid (%) | | | | | | | | | Total saturated fatty acid | Total unsaturated fatty acid | % saturated / % unsaturated |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $C_{14}$ | $C_{16}$ * | $C_{16}$ | $C_{16:1}$ | $C_{18}$ | $C_{18:1}$ | $C_{18:2}$ | $C_{20:4}$ | $C_?$ | | | |
| $MGI^+D^+$ | 12 | 3.00 | 2.85 | 28.71 | 14.21 | 14.73 | 29.96 | 2.74 | 3.80 | <0.5 | 49.29 | 50.71 | 0.97 ** |
| | 21 Cells | 4.48 | 1.77 | 21.35 | 11.44 | 14.28 | 40.27 | 1.83 | 2.75 | 1.83 | 41.88 | 56.29 | 0.74 |
| | 11 | 2.45 | <0.5 | 25.11 | 10.25 | 20.82 | 28.66 | 2.06 | 7.00 | 3.64 | 48.38 | 47.97 | 1.01 ± 0.193 *** |
| | 11 (membranes) | 2.14 | 4.27 | 21.90 | 10.28 | 18.04 | 30.72 | 2.39 | 7.85 | 2.40 | 46.35 | 51.24 | 0.90 ± 0.042 |
| $MGI^+D^-$ | 19 Cells | 1.62 | 0.28 | 10.85 | 8.54 | 21.35 | 50.21 | 2.76 | 4.38 | <0.5 | 34.10 | 65.89 | 0.52 |
| | 5 | 2.38 | <0.5 | 13.89 | 19.56 | 8.06 | 45.57 | <0.5 | 4.75 | 5.78 | 24.33 | 69.88 | 0.35 |
| | 13 | 1.30 | 1.01 | 15.95 | 14.27 | 12.43 | 47.69 | 1.08 | 2.91 | 3.32 | 30.69 | 65.95 | 0.47 ± 0.073 |
| | 13 (membranes) | 1.90 | 1.32 | 15.71 | 12.83 | 14.82 | 48.40 | 0.69 | 4.33 | <0.5 | 33.75 | 66.25 | 0.51 ± 0.037 |

* Iso branched fatty acid chain.

** Values are mean of three experiments.

*** Values are S.D. of five experiments.

on for two clones of $MGI^+D^+$ and $MGI^+D^-$ (see Table I). Although over investigators used the 5′-nucleotidase as a marker for plasma membrane [34], it was found that its activity is very low in these myeloid leukemia cells as in some other cultured cells [35]. In order to estimate the degree of contamination of subcellular organelles in the plasma membrane fraction, the following marker enzyme were assayed. The glucose-6-phosphatase, acid phosphatase, and succinate : cytochrome *c* reductase specific activity in the membrane fraction were low, indicating that only trace amounts of smooth endoplasmic reticulum, lysosome or mitochondria were contaminating the $MGI^+D^+$ and $MGI^+D^-$-isolated plasma membrane. It is interesting to note that the activity of acid phosphatase in $MGI^+D^+$ cell homogenates is two-fold higher than that of the $MGI^+D^+$ cells. The high NADH diaphorase enzyme activity in the membrane fraction indicates about 50% contamination by rough endoplasmic reticulum in $MGI^+D^-$, and about 20% in $MGI^+D^+$.

The isolation of the plasma membrane was followed by electron micrographs of thin sections of the plasma membrane pellets (Fig. 1). The membrane fraction consisted of smooth membranes arranged in vesicles and vacuoles and the bilayer structure of the membranes are visible. Few vesicles contained amorphous unidentified material and contaminants, but no mitochondria, lysosomes, nuclei or ribosomes were detected.

*Fatty acid composition*

The fatty acid composition of the polar lipids of the different clones of $MGI^+D^+$ and $MGI^+D^-$ cells and respective membrane fraction are shown in Table II. All the clones were grown at 37°C and the data presented were obtained by GLC with the polar column and confirmed with the non-polar column (±1%). Mass spectra of the individual fatty acid methyl esters were identical to those described in the literature [36]. There was no significant difference in the fatty acid composition of the saturated to unsaturated fatty acid ratio of the membranes compare to the whole cells in clone 13 ($MGI^+D^-$) and clone 11 ($MGI^+D^+$). There is some variation of the fatty acid composition among the different clones but the average ratio of unsaturated to unsaturated fatty acid is 0.9 in $MGI^+D^+$ clones and 0.46 in $MGI^+D^-$ clones, a two-fold increase of unsaturated fatty acid in $MGI^+D^-$. The increased amount of the unsaturated fatty acid in $MGI^+D^-$ cells are predominantly in the oleate ($C_{18:1}$) which is 47% in $MGI^+D^-$ cells and 30% in $MGI^+D^+$ (Table II. Palmitate ($C_{16}$) is the predominant saturated fatty acid in $MGI^+D^+$ cells where it is 25% of the fatty acid composition, versus 13% in the $MGI^+D^-$ cells.

The correlation between the increased amount of unsaturated fatty acid composition in the $MGI^+D^+$ and $MGI^+D^-$ cells with the decrease in the temperature of growth is shown in Table III. When the growth tempeature was lowered to 32°C both clones had a lower ratio of saturated to unsaturated fatty acid in $MGI^+D^+$ (0.76) and in $MGI^+D^-$ (0.28). At this temperature oleate made up 61% of the fatty acid composition of $MGI^+D^-$ cells. When the growth temperature was lowered to 28°C, only $MGI^+D^-$ clones could grow, with a peculiar polyunsaturated 5,8,11,14,17-eicosapentenoate ($C_{20:5}$) as 18% of the total fatty acid composition. The ratio of saturated to unsaturated fatty acid of $MGI^+D^-$ was 0.23.

TABLE III

FATTY ACID COMPOSITION OF POLAR LIPIDS ISOLATED FROM CELLS OF $MGI^+D^+$ AND $MGI^+D^-$ CLONES GROWN AT 37, 32 AND +**EC

Fatty acid nomenclature: chain length:number of double bonds in the fatty acid.

| Cel typel (clone number) | Tempera-ature (°C) | Fatty acid (%) | | | | | | | | | | Total saturated fatty acid | Total unsaturated fatty acid | % satur-ated/% unsatur-ated |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $C_{14}$ | $C_{16}$ * | $C_{16}$ | $C_{16:1}$ | $C_{18}$ | $C_{18:1}$ | $C_{18:2}$ | $C_{20:4}$ | $C_?$ | $C_{20:5}$ | | | |
| $MGI^+D^+$ (11) | 37 | 2.45 | <0.5 | 25.11 | 10.25 | 20.82 | 28.66 | 2.06 | 7.00 | 3.64 | <0.5 | 48.38 | 47.97 | 1.01 ** |
| | 32 | 2.33 | 1.61 | 25.10 | 14.63 | 14.2 | 33.51 | 2.33 | 6.28 | <0.5 | <0.5 | 43.24 | 56.75 | 0.76 |
| | 28 | No growth | | | | | | | | | | | | |
| $MGI^+D^-$ (19) | 37 | 1.62 | 0.28 | 10.85 | 8.54 | 21.35 | 50.21 | 2.76 | 4.38 | <0.5 | <0.5 | 34.10 | 65.89 | 0.52 |
| | 32 | 0.57 | 0.34 | 10.11 | 7.81 | 10.08 | 61.89 | 0.16 | 5.70 | 3.33 | <0.5 | 21.10 | 75.56 | 0.28 |
| | 28 | 1.45 | <0.5 | 9.42 | 12.20 | 7.73 | 50.70 | <0.5 | <0.5 | <0.5 | 18.44 | 18.60 | 81.34 | 0.23 |

* Iso branched fatty acid chain.

** Values are mean of three experiments.

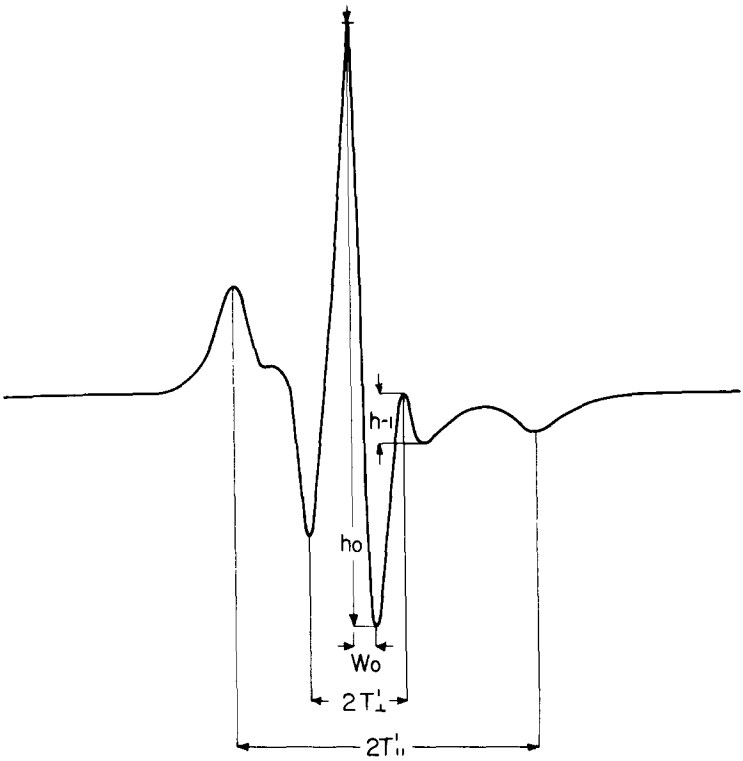



Fig. 2. The parameters used in the determination of the molecular motion of 5-nitroxy stearate spin label in the cells and isolated plasma membrane fraction.

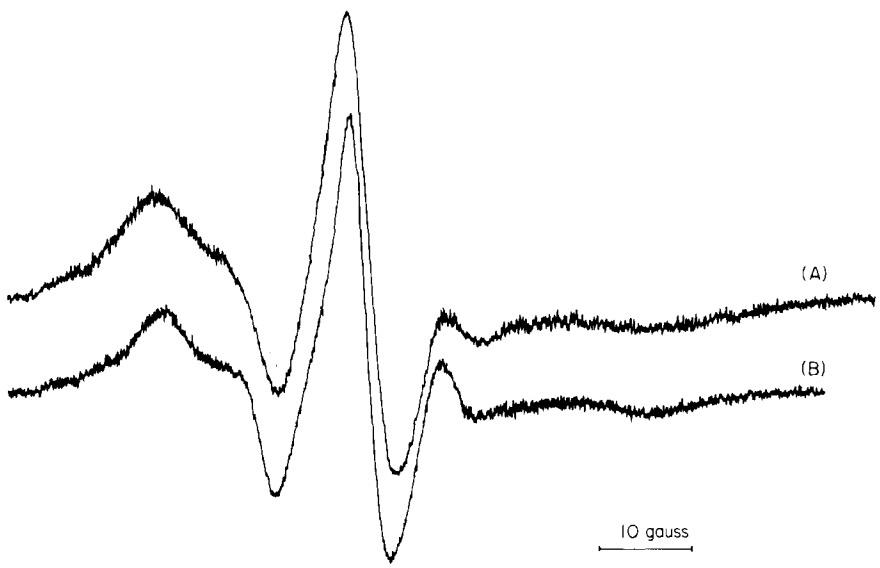



Fig. 3. ESR spectra of the 5-nitroxy stearate spin label in (A) $MGI^{+}D^{+}$ and (B) $MGI^{+}D^{-}$ cells at 30°C.

*ESR spectroscopy analysis*

The ESR spectra of the fatty acid-nitroxide spin label embedded in the membrane bilayer can be used to detect changes of the freedom of motion in lipid membranes [37]. The motion parameters of the spin label which designate the average tumbling time, depend on the temperature, membrane fluidity and position of the oxazolidine ring on the hydrocarbon chain [19]. Three stearic acid I-[2,14], -[5,10] and -[12,3] spin labels were used in the initial study and all were readily incorporated into cells and the membrane fractions. These three spin probes exhibited the flexibility gradient in the motion parameters with the position of the oxazolidine ring on the alkyl fatty acid chain. The 5-nitroxy stearate I-[12,3] was selected for its best ESR spectra measurements in the 0—40°C range; it allowed a good resolution of the outer and the inner hyperfine splittings. Fig. 3 shows the ESR spectra of $MGI^+D^+$ and $MGI^+D^-$ intact cells taken at 30°C with the 5-nitroxy stearate. The spin label signal decayed in the intact cells and in the membrane fraction at room temperature or at 37°C incubation after 30 min, and could be restored by adding 0.5 mM $K_3Fe(CN)_6$. This would suggest that in the cells the spin label was predominantly present in the outer side plasma membrane [20].

The observed values of the outer ($2T_{\parallel}$) and the inner ($2T_{\perp}$) hyperfine splitting (in gauss) were used to calculate the order parameter ($S$) [24].

$$S = \frac{T'_{\parallel} - T'_{\perp} + -c}{T'_{\parallel} + 2T'_{\perp} + +2c} \times 1.723 \qquad (1)$$

were $c = 1.4G - 0.053(T'_{\parallel} - T'_{\perp})$.

The motion parameter ($\tau_0$) was calculated from the formula of Henry and Keith [38], where $W_0$ is the linewidth, $h_0$ is the mid-field height and $h_{-1}$ is the high-field height (Fig. 2).

$$\tau_0 = K \cdot W_0 \left[ \left( \frac{h_0}{h_{-1}} \right)^{1/2} - 1 \right] \qquad (2)$$

The constant $K = 6.5 \cdot 10^{-10}$ s is dependent on the anisotropic hyperfine coupling values and the $g$-tensor terms when the correlation time $\tau_c > 10^{-9}$ [38,39]. Although Eqn. 2 was derived for isotropic motion, the empirical term of $\tau_0$ could be used as parameters for the 5-nitroxy stearate anisotropic mobility for comparison purpose and not as the correlation time ($\tau_c$).

Greater freedom of motion of the spin label in the biomembrane bilayers are associated with the smaller values of the order parameters and the motion parameters. When the ferricyanide was added to the cells after 60 min of incubation at 37°C the reactivation of the spin label signal had about 80% recovery.

Arrhenius plots of the motion parameter $\tau_0$ (Fig. 4) of the 5-nitroxy stearate in the intact cells indicate a lower $\tau_0$ value for $MGI^+D^-$ compared to $MGI^+D^+$ in all the temperatures studied. These differences are smaller in the higher temperature (40°C) and up to two-fold in the lower temperature (20°C). In Table IV the data of the order parameters ($S$) indicate a similar spin-label motion in the $MGI^+D^+$ and the normal myeloblast cells (0.66) and a higher mo-

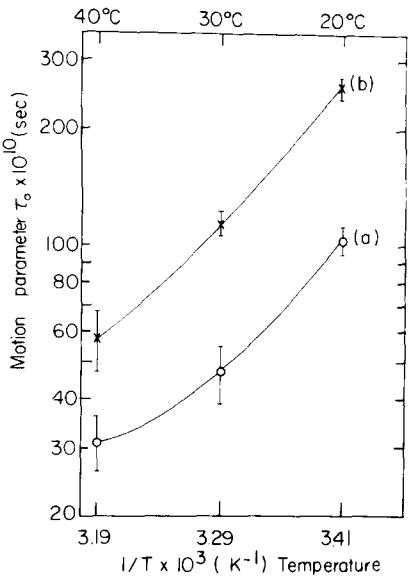



Fig. 4. Arrhenius plot of the motion parameter ($\tau_0$) with different temperatures of the 5-nitroxy stearate spin label in (a) $MGI^+D^+$ and (b) $MGI^+D^-$ cells.

tion amplitude in the $MGI^+D^-$ cells (0.63). In the isolated membrane fraction there are differences in the order parameter of $MGI^+D^+$ and $MGI^+D^-$ membranes but both had higher values in the order parameter. The slower motion of the spin label in the membrane fractions could be due to the difference in the inner and outer half of bilayer lipids that are exposed in the isolated membrane fraction.

There was no effect of 0.1 μg/ml of vinblastine and 0.1 M $NaN_3$ on the spin label motion. 2.5% glutaraldehyde slowed down the amplitude of the 5-nitroxy

TABLE IV

ORDER PARAMETER ($S$) OF 5-NITROXY STEARATE SPIN LABEL IN CELLS AND ISOLATED PLASMA MEMBRANES OF $MGI^+D^+$ CLONES, $MGI^+D^-$ CLONES AND NORMAL BONE MARROW MYELOBLASTS

The ESR spectra were taken at 30° C.

| Cell type | Clone No. | Observed hyperfine splitting | | Order parameter ($S$) |
|---|---|---|---|---|
| | | $2T_\parallel$ | $2T_\perp$ | |
| $MGI^+D^+$ | 12 (cells) | 53.25 ± 0.228 * | 17.75 ** | 0.6578 |
| $MGI^+D^-$ | 13 (cells) | 52.00 ± 0.132 | 18.00 | 0.638 |
| Normal Cells | | 53.00 | 17.75 | 0.6553 |
| $MGI^+D^+$ | 12 (membranes) | 55.50 | 17.50 | 0.6964 |
| $MGI^+D^-$ | 13 (membranes) | 53.63 | 17.375 | 0.6762 |
| $MGI^+D^+$ | 12 (cells + 2.5% glutaraldehyde) | 55.375 | 17.625 | 0.6903 |
| $MGI^+D^-$ | 13 (cells + 2.5% glutaraldehyde) | 52.88 | 17.75 | 0.6527 |
| $MGI^+D^-$ | 13 (membranes + 2.5% glutaraldehyde) | 56.125 | 17.00 | 0.7224 |

* Values are S.D. of six samples.

** Values are the mean of three separate determinations.

stearate motion in the intact cells and in the membrane fraction. This observation could be the result of the cross-linking of phospholipids containing amino groups (phosphatidylethanolamine and phosphatidylserine) [40,41].

## Discussion

The fatty acid and the phospholipid composition of the plasma membrane can regulate the structure and the function of the lipids in the biomembrane [6,7,42,43]. In the $MGI^+D^+$ and $MGI^+D^-$ myeloid leukemia cells, the study of the plasma membrane lipid composition indicate a two-fold increase in the ratio of saturated to unsaturated fatty acid in the $MGI^+D^-$ compared to the $MGI^+D^+$ cells. The $MGI^+D^+$ cells can be induced to differentiate to mature macrophages and granulocytes, whereas the $MGI^+D^-$ cells could not be used to differentiate to mature cells [4,5,44]. A small variation in the ratio of saturated to unsaturated fatty acid in the different clones of $MGI^+D^+$ cells and the $MGI^+D^-$ cells could be the result of small individual differences of the clones. The increased activity of the $(Na^+ + K^+)$-ATPase in the $MGI^+D^-$ clones [2] could be correlated with the plasma membrane fatty acid composition [10]. The temperature dependence of the mobility of the spin-labeled fatty acids in the cells and in the plasma membrane fraction and the increase in mobility with the position of the nitroxide ring from the polar head group of the probe, suggested that the spin label is associated with the lipid environment of the bilayers [45]. The exact location of the 5-nitroxy stearate in these experiments is not clear and the influence of the membrane protein is not defined, but the fast cellular destruction of the spin label signal indicate that most of the spin label was in the plasma membrane and gave information about the amplitude of motion of lipid acyl chains [24]. The treatment of the cells with ferricyanide indicated that most of the spin label was in the outer cell membrane [20,46], as well as the results of the isolated plasma membrane. The differences of the motion parameter shown by the Arrhenius plots and the order parameter values are correlated with the higher freedom of motion in the $MGI^+D^-$ clones and with the higher degree of unsaturated fatty acid in the membrane fatty acyl chains. From other studies [14,46] it was shown that the introduction of polyunsaturated fatty acid to the lipid bilayers produced a greater perturbation on the ordered packing of the fatty acyl chains, and with it more fluid membrane. The similar results of the freedom of motion of the spin probe in the normal bone marrow myeloblasts and $MGI^+D^+$ cells show a further similarity between these two types of cells [4]. The effect of low temperature on the lipid modification and on the proliferation of the $MGI^+D^+$ and $MGI^+D^-$ cells as shown in this study, could indicate that lowering the temperature produced an increase in the unsaturated fatty acid contents of the phospholipid [17,18]. The cells and the membrane fractions that were fixed with glutaraldehyde had a lower freedom of motion due to the cross-linking of the phospholipids contamining amino groups [41] but still showed differences between the $MGI^+D^+$ and $MGI^+D^-$ cells.

Studies on the lipid composition of mammalian plasma membrane have shown that modification of specific fatty acids are associated with changes of membrane enzymes activities [9,10,47], structural changes that were detected

by ESR probes [16,22,48]. In this study the differences of the lipid composition were not associated with a modification of the culture medium but with a difference in certain biological properties of the cells. The results indicate that the differences in the competence of $MGI^+D^+$ and $MGI^+D^-$ cells to undergo differentiation to mature cells may result from different physical properties of their plasma membranes associated with a different fatty acid composition of the plasma membranes.

## Acknowledgments

I am very grateful to Prof. L. Sachs for his continued encouragement, to Prof. Z. Luz and Dr. R. Poupko for their help with the ESR analysis, and to Dr. E. Yavin and J. Lipton for reading the manuscript.

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