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MOTION OF SPIN-LABELED FATTY ACIDS IN MURINE MACROPHAGES RELATION TO CELLULAR PHAGOCYTIC ACTIVITY

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SUMMARY

Macrophage membrane fluidity was investigated with respect to cellular phagocytic activity through the use of fatty acid spin labels.

Spin-labeled fatty acid derivatives were incorporated into intact mouse peritoneal macrophages by exchange from bovine serum albumin. The electron spin resonance (ESR) spectra of the spin-labeled fatty acids in the macrophages showed a pronounced temperature dependence and a decrease in the hyperfine splittings ($2T_{||}$) of the spectra as the nitroxide radical was moved away from the polar head group of the fatty acid derivatives.

Spin-labeled macrophages underwent a time- and temperature-dependent decay, which was inhibited by preincubating the cells with mercuric chloride, heating at 56 °C, or by fixing them with 0.25 % glutaraldehyde.

No correlation between the phagocytic activity of macrophages and membrane freedom of motion could be demonstrated. Treatment of macrophages with anti-macrophage serum or extended in vitro cultivation inhibited cellular phagocytic activity but exerted no effect on the motional freedom of the macrophage membrane. Enrichment of the fatty acid composition of the macrophage membrane with *cis*- or *trans*-unsaturated fatty acids had striking effects on cellular phagocytic activity, while no significant changes could be detected in the freedom of motion of incorporated fatty acid spin labels at the degree of specific enrichment achieved here. Thus no correlation between cellular phagocytic activity and lipid motion could be detected.

INTRODUCTION

The use and validity of electron spin resonance (ESR) spectroscopy techniques in the study of the structure and function of biological membranes is well documented [1–3]. ESR spectroscopy of spin-labeled compounds basically involves the introduction of a molecular probe into a physical environment, which in turn reflects data about the molecular matrix in which the spin-labeled molecule is situated. Subsequently, information can be derived about the freedom of molecular motion, anisotropic motion, local environment and the physical state of membrane lipids [4].

The data presently available on spin-labeled biological membranes concern mainly the average lipid fluidity, orientation characteristics of the entire membrane, membrane fine structure and structural alterations induced by foreign molecules. Such information is available on membranes of erythrocytes [5–7], sarcoplasmic reticulum [8, 9], mitochondria [10, 11], plasma membrane fragments [12], intact human lymphocytes [13], mouse L-cells [13] and fibroblasts [14, 15]. However, information is not available regarding the application of spin label techniques to macrophages, cells bearing a highly active plasma membrane [16], which is organized into functionally specific entities and areas [17].

In the present investigation we have used spin label techniques in order to derive information about the freedom of molecular motion and the physical state of macrophage membrane lipids under a variety of environmental and physiological conditions. In particular, the lipid composition of the macrophage membrane has been selectively manipulated by incorporating exogenously supplied fatty acids [18, 19] into cultured macrophages. Such an approach provides a method for altering membrane lipid composition, and thus the role of the membrane lipid phase on cellular phagocytic activity and the effect on overall membrane freedom of motion can be determined. In addition, the preservation of spectral integrity by decreasing nitroxide spin reduction by the cell is reported.

MATERIALS AND METHODS

Animals. 3-month-old C57Bl/6 female mice were used as macrophage donors, except where otherwise noted.

Macrophages. Macrophages were obtained from the peritoneal exudates of mice, 4 days after an intraperitoneal injection of thioglycollate [20]. The cells were washed twice and spin labeled. For in vitro cultivation the cells were suspended in growth medium consisting of 90 % RPMI 1640 (a synthetic culture medium, developed at Roswell Park Memorial Institute) and 10 % newborn calf serum (Microbiological Associates, Bethesda, Md.) or RPMI 1640 alone. $45 \cdot 10^6$ cells were cultured in 14×2 cm glass petri dishes at 37 °C in a 5 % CO_2 humidified incubator.

Macrophage fatty acid replacements. Fatty acid replacement of cultivated macrophages was carried out essentially as described by Horwitz et al. [19] for 3T3 cells (an in vitro cultivated mouse fibroblast line), with some modification. Macrophages obtained as described above were cultured in vitro with Dulbecco's modified Eagles medium [21] (a medium containing no biotin) with either 10 % (v/v) regular newborn calf serum or lipid-depleted newborn calf serum [19]. Fatty acids (25 μg/ml) and avidin (0.04 units/ml) were added to the macrophages cultivated with the lipid depleted serum. $2 \cdot 10^6$ macrophages were cultivated in 3-cm petri dishes (2 ml medium) for the phagocytic assays and $30 \cdot 10^6$ macrophages were cultivated in 9-cm petri dishes (10 ml medium) for the ESR and fatty acid analysis. After 48 h cultivation, the cells were washed and fresh medium added. Following an additional 48 h, the various assays were carried out.

Fatty acid analysis. Cells grown as described for the fatty acid replacements were removed from the petri dishes with a rubber policeman, and washed twice with normal saline. The lipids were extracted from the cells with chloroform/methanol (2 : 1) [22]. Neutral lipids were separated from polar lipids by chromatography

through a column (6×100 mm) of activated silicic acid (100 mesh, Mallinckrodt Chemical Works, St. Louis, Mo.) prewashed with chloroform.

The neutral and polar lipids were eluted from the column with chloroform and chloroform/methanol (1 : 1), respectively. The fatty acids were transmethylated with methanolic boron trifluoride, and were analyzed by gas chromatography in a Packard model 840 instrument equipped with a polar column (200×0.3 cm, 15 % diethyleneglycol adipate on chromosorb W). Fatty acids were identified by their retention time relative to that of standard methyl ester mixtures (Supelco Inc., Bellefonte, Pa.).

Phagocytic assay. The phagocytic activity of peritoneal macrophages was determined as previously described using ^{125}I-labeled *Shigella flexneri* ($4 \cdot 10^7$ bacteria/10^4 cpm) [23].

Antimacrophage serum. Antimacrophage serum was prepared as previously described [23].

Spin labels. Spin-labeled stearic acid analogs (Syva Associates, Palo Alto, Calif.) with the nitroxide ring located at the hydrophilic end (5-nitroxystearate) or the hydrophobic end of the molecule (12-nitroxystearate) were used (see Fig. 1).

Preparation of spin-labeled macrophages. Macrophages obtained as described above or removed from petri dishes with a rubber policeman were washed twice in normal saline and spin-labeled by exchange from bovine serum albumin [5]. $45 \cdot 10^6$ peritoneal cells suspended in 0.5 ml saline were incubated for 15 min at 4 °C with 0.5 ml of 2.5 mM spin label in 5 % bovine serum albumin fraction V (fatty acid poor, Pentax, Kankakee, Ill.). The cells were then sedimented at 400×g for 10 min in the cold, washed once with 5 ml of ice-cold saline and resuspended in 0.3 ml saline. The samples were enclosed in sealed Pasteur pipettes. The ESR spectra were obtained using a Varian E-4 spectrometer.

ESR spectral interpretations. In general, the ESR spectra of nitroxide spin labels consist of three absorption peaks. Alterations in the mobility of the spin-labeled fatty acids are characterized by alterations in the distance or hyperfine splitting ($2T_{||}$) between the low and high field lines [5] or in the mid and high field line heights (h_0 and h_{-1}, respectively) (see Fig. 2). It should be noted, however, that the motion parameter $2T_{||}$ is due to the anisotropic motion of the spin probes and is the result of the time-dependent averaging of the hyperfine coupling tensors, and not

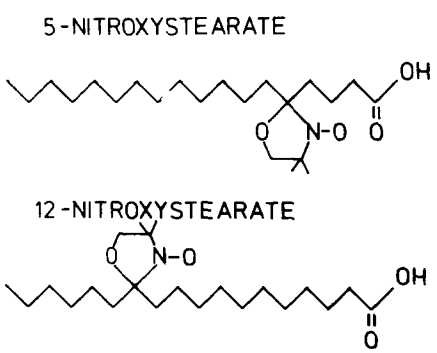

Fig. 1. Spin labels used as cell labels.

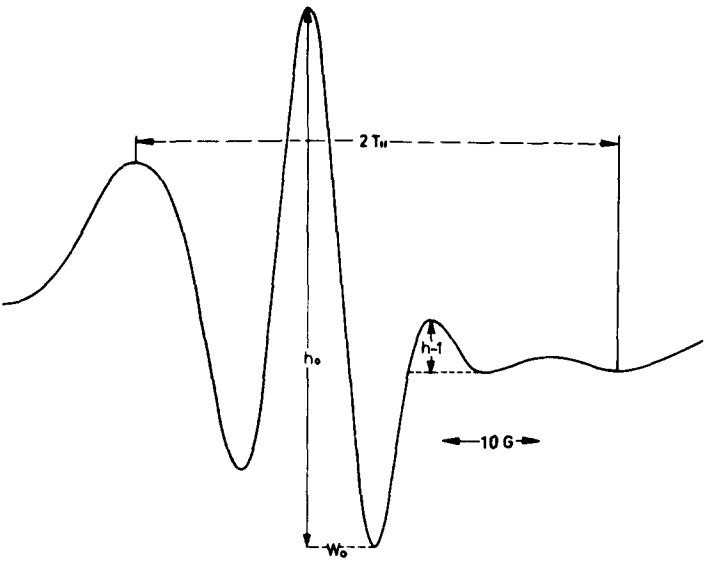

Fig. 2. Spectral parameters used in the determination of molecular motion of spin-labeled fatty acids in macrophages. Cells labeled with 5-nitroxystearate.

of normal hyperfine coupling features.

Moreover, molecular freedom of motion is quantitatively related to the rotational correlation time (τ_c) of the nitroxide spin-labeled molecule [24]. Assuming one lineshape, the correlation time is given [25] by:

$$\tau_c = 6.5 \cdot 10^{-10} W_0 \left[(h_0/h_{-1})^{\frac{1}{2}} - 1\right]$$

The above expression of τ_c is valid for isotropic motion, which would not be expected to originate from nonspherical molecules such as 12-nitroxystearate. However, it has been shown that 12-nitroxystearate moves in a nearly isotropic fashion, as the departure from isotropic motion is related to the proximity of the nitrogen atom to the carboxyl group of the fatty acid chain [27].

Nevertheless, the spectra reported here are too slow for a one lineshape theory to apply ($\tau_c > 10^{-9}$ s) and inaccuracies in τ_c result. Therefore, the empirical term τ_0 is used as a parameter of mobility for comparative purposes and not strictly as a correlation time [3]. Furthermore, the values were calculated only for correlation times which were faster than 10^{-8} s as the high field lines above this value showed nonconsistent variability.

RESULTS

Molecular motion of spin-labeled fatty acids in macrophages

Fig. 3 shows the temperature dependence of the 5-nitroxystearate hyperfine splitting ($2T_{||}$) in murine macrophage membranes. The hyperfine splitting values are related to the freedom of motion of the spin label in the membrane, higher $2T_{||}$ values being associated with a lesser freedom of motion of the probe [5]. Thus, an

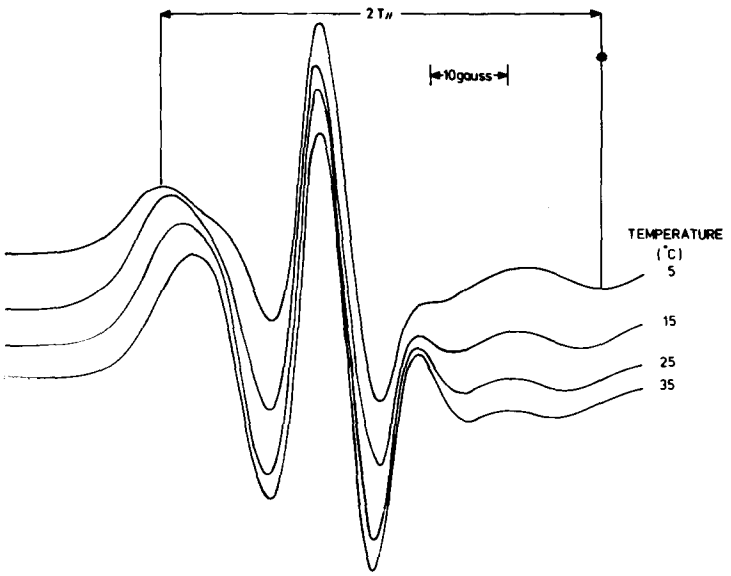

Fig. 3. Effect of temperature on the hyperfine splitting ($2T_{||}$) of spin-labeled fatty acid 5-nitroxystearate in intact mouse macrophages.

increase in the freedom of motion of the spin label in the macrophages with increasing temperature is evident.

Furthermore, the hyperfine splitting ($2T_{||}$) at a given temperature was found to be dependent on the position of the nitroxide group on the fatty acid derivative chain, decreasing as the nitroxide group was moved away from the polar end of the molecule

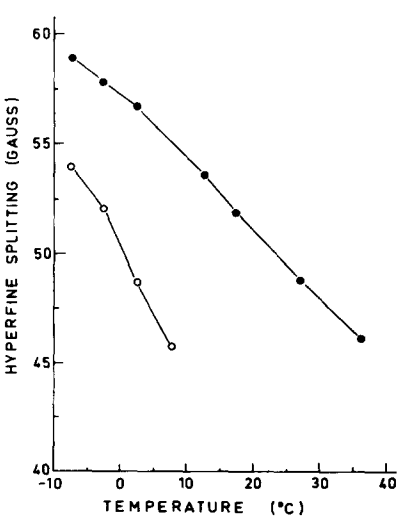

Fig. 4. Temperature dependence of the hyperfine splitting ($2T_{||}$) of spin labels 5-nitroxystearate (●) and 12-nitroxystearate (○) in intact mouse macrophages.

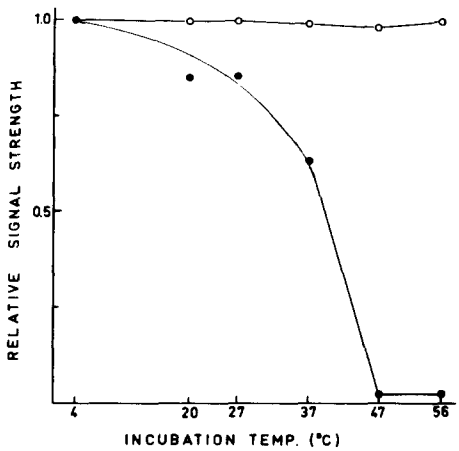

Fig. 5. Effect of fixation (○) and temperature (●) on 12-nitroxystearate signal intensity loss by 12-nitroxystearate-labeled macrophages. Macrophages were labeled with 12-nitroxystearate and fixed with 0.25 % glutaraldehyde for 15 min or heated for 15 min at various temperatures. Thereafter the cells were cooled and ESR signal intensity determined at 10 °C.

(Fig. 4). This positioned dependence demonstrated an increase in the molecular motion of the nitroxide radical with increased distance from the polar head group.

Loss of signal intensity

The ESR signals obtained in our system by incorporating spin-labeled fatty acids into macrophages were extensively destroyed over a period of several minutes to several hours, depending on the temperature of the environment, thus making accurate measurements difficult.

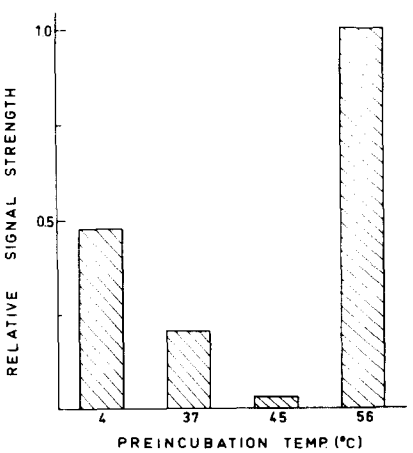

Fig. 6. Effect of macrophages incubated at various temperatures prior to their labeling with 12-nitroxystearate on signal intensity loss. Macrophages were preincubated at various temperatures for 15 min, spin-labeled with 12-nitroxystearate and reincubated at 37 °C for 15 min. ESR signal intensity was then determined at 10 °C.

TABLE I

EFFECT OF METABOLIC INHIBITORS ON SIGNAL DESTRUCTION OF SPIN-LABELED MACROPHAGES

Macrophages were incubated with the inhibitors for 30 min at 4 °C. Thereafter the cells were spin labeled and incubated for 20 min at 37 °C prior to analysis. All spectra were determined at 4 °C.

Inhibitor		Signal intensity (h_0)	% of initial signal*
Control		19.08	100
None		8.59	45
$HgCl_2$	$1 \cdot 10^{-4}$ M	18.75	98
NaN_3	$1 \cdot 10^{-2}$ M	10.1	53
	$5 \cdot 10^{-2}$ M	13.3	70
NaF	$1 \cdot 10^{-2}$ M	8.5	45
	$5 \cdot 10^{-2}$ M	8.9	47
KCN	$1 \cdot 10^{-2}$ M	8.5	45

* Compared to signal intensity of control spin-labeled macrophages kept at 4 °C during the entire experiment.

Fig. 5 shows the signal intensity loss in 12-nitroxystearate-labeled macrophages incubated for 15 min at various temperatures. It can be seen that there was a progressive loss in signal intensity as the incubation temperature was increased, with no detectable signal present at 47 °C. Prior fixation of the cells for 15 min with 0.25 % glutaraldehyde, however, effectively inhibited the loss in signal intensity. Fig. 6 shows that when the cells were incubated at various temperatures prior to their labeling with 12-nitroxystearate, then spin-labeled and transferred to 37 °C for 15 min (conditions which favor rapid signal intensity loss) an increase in signal intensity loss was noted with increasing incubation temperatures up to 45 °C. However, prior incubation of the cells at 56 °C effectively inhibited signal intensity loss.

Signal intensity loss in 12-nitroxystearate-labeled macrophages could also be inhibited by various metabolic inhibitors (Table I). The most active inhibitor was $HgCl_2$; NaN_3 was less effective, while CN^- and F^- had no effect.

TABLE II

MOTION PARAMETERS OF 12-NITROXYSTEARATE SPIN-LABELED MACROPHAGES FOLLOWING VARIOUS TREATMENTS

Pretreatment	Motion parameter ($\tau_0 \cdot 10^{10}$ s) at			
	15 °C	25 °C	35 °C	45 °C
None*	83.9	55.7	41.0	35.4
Heat** (56 °C)	94.3	62.5	46.8	39.7
Glutaraldehyde** (0.25 %)	82.8	54.6	41.8	33.5
$HgCl_2$** (10^{-4} M)	79.6	53.4	39.6	34.4

* Macrophages were kept at 4 °C during all stages of sample preparation.

** Macrophages were heated at 56 °C, fixed with glutaraldehyde or incubated with 10^{-4} M $HgCl_2$ for 15 min, washed and then spin-labeled with 12 nitroxystearate.

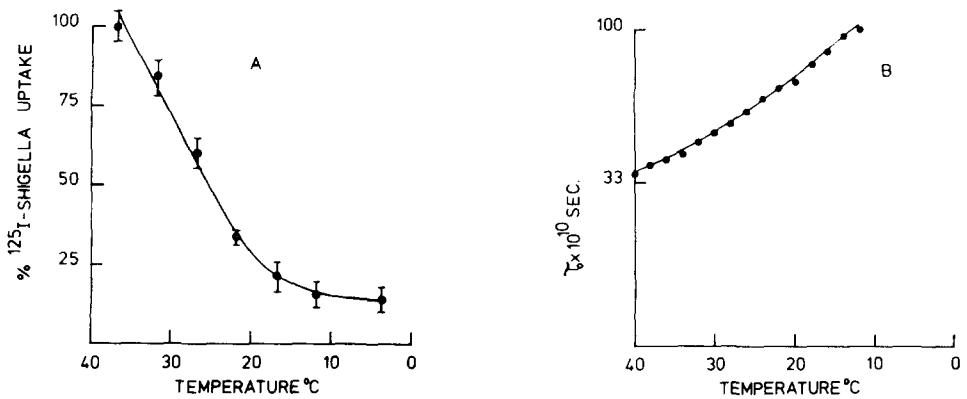

Fig. 7. Phagocytic activity (A) and τ_0 correlation times (B) of 12-nitroxystearate-labeled macrophages at various temperatures.

In order to determine whether the various treatments carried out above had any effect on membrane hydrocarbon properties, τ_0 motion parameters were calculated from the same spectra used to calculate the signal intensities in Table I and Figs. 5 and 6. Table II shows that spin-labeled glutaraldehyde- or mercury-treated macrophages demonstrated τ_0 correlation times similar to control cultures, while heat-treated (56 °C) spin-labeled macrophages demonstrated slightly higher τ_0 changes, reflecting a lower freedom of motion. However, in the above instances the slopes of τ_0 vs. $^\circ K^{-1}$ were the same as in the control cultures (Arrhenius plots based

TABLE III

EFFECT OF ANTI-MACROPHAGE SERUM OR IN VITRO CULTIVATION ON THE PHAGOCYTIC ACTIVITY AND MOLECULAR FREEDOM OF MOTION OF MACROPHAGES

Macrophages ($1 \cdot 10^6$ cells) were incubated with 10^5 cpm ^{125}I-labeled *Shigella* for 1 h at 37 °C for the uptake experiments and at 4 °C for the binding experiments. Thereafter, the cultures were washed and the radioactivity determined. The results presented are the means of 3–5 experiments.

Cultivation time (days)	Growth medium	^{125}I-labeled *Shigella* uptake		^{125}I-labeled *Shigella* bound		12-Nitroxystearate parameter $\tau_0 \cdot 10^{10}$, 37 °C
		cpm	%	cpm	%	
1	RPMI+10% NRS*	9560	100	1323	100	38.7
1	RPMI+10% AMS**	1683	17	1248	94	39.9
1	RPMI+10% NBCS***	12840	100	2060	100	38.6
5	RPMI+10% NBCS	8428	65	1890	92	39.3
9	RPMI+10% NBCS	5830	45	2180	105	39
1	RPMI	6171	100	1328	100	42.9
3	RPMI	6439	104	1267	95	42.4
5	RPMI	6218	100	1023	77	40.9

* NRS: normal rabbit serum.
** AMS: antimacrophage serum.
*** NBCS: new born calf serum.

TABLE IV

FATTY ACID COMPOSITION OF MACROPHAGES GROWN IN REGULAR MEDIUM AND IN LIPID-DEPLETED FATTY ACID-SUPPLEMENTED MEDIA

Macrophages were grown in the various media for 4 days. Fatty acid 18:1 includes the total of oleate (18:1, *cis*) and elaidate (18:1, *trans*).

Medium	Fatty acid (%)										
	10:0	12:0	14:0	15:0	16:0	16:1	18:0	18:1	18:2	Other	% sat./% unsat.
Regular serum	0.5	2.6	1.3	5 .5	25.7	1.9	22.3	17.4	11.1	11.6	65.5/34.4
Lipid-depleted serum											
+palmitate (16:0)	2.1	1.1	4.4	9.0	33.9	1.7	19.4	16.1	5.2	6.6	75.2/24.7
+oleate (18:1)	1.6	9.1	9.0	11.2	20.0	1.2	12.8	25.8	1.7	7.7	68.9/31.0
+elaidate (18:1, *trans*)	5.3	8.0	12.9	10.1	16.3	2.6	7.8	29.4	3.0	4.6	63.3/36.6

on data shown in Table II not presented). Thus, it appears that the various treatments have minimal effects on membrane hydrocarbon properties and may be employed to inhibit spin signal reduction by spin-labeled cells. However, as most of the treatments used have drastic effects on cell viability, they should be used with caution in studies where viable cells are required.

Macrophage phagocytic activity and membrane freedom of motion

As macrophage phagocytosis of particulate matter is an active temperature-energy-dependent process requiring bilayer annealing, it was of interest to determine whether a correlation exists between cellular phagocytic activity and membrane freedom of motion. From Fig. 7 it can be seen that both macrophage phagocytic activity (Fig. 7A) and the freedom of motion of 12-nitroxystearate in the cell membrane (Fig. 7B) decreased at lower incubation temperatures.

As the physical state of the cell membrane will most assuredly affect membrane-oriented physiological processes, it was of interest to determine whether cellular phagocytic activity (a membrane-oriented process) and the freedom of motion of macrophage membranes are interrelated processes.

Table III shows that under conditions known to decrease phagocytic activity, such as treatment with antimacrophage serum or prolonged in vitro cultivation, no change in either the freedom of motion of 12-nitroxystearate in the macrophage membrane or in the binding of ^{125}I-labeled *Shigella* to the cell surface could be demonstrated.

In another set of experiments membrane fatty acid replacements were carried out and their effect on cellular phagocytic activity and membrane freedom of motion was determined.

The data presented in Table IV demonstrate that the exogenously supplied fatty acids present in the growth medium were incorporated into the cells and altered the fatty acyl composition of the membrane lipids. It should be noted, however, that no appreciable changes in the ratios of saturated to unsaturated fatty acids were achieved. The effect of the fatty acyl composition of membrane lipids on macrophage phagocytic activity and membrane freedom of motion is shown in Table V. Macrophages grown in regular serum demonstrated the highest phagocytic activity, whereas

TABLE V

PHAGOCYTIC ACTIVITY AND MOLECULAR FREEDOM OF MOTION OF MACROPHAGES ENRICHED WITH DIFFERENT FATTY ACID SUPPLEMENTS

Experimental procedure as described in Table III.

Growth medium	^{125}I-labeled *Shigella* uptake		^{125}I-labeled *Shigella* bound		12-Nitroxystearate motion parameter $\tau_0 \cdot 10^{10}$, 37 °C
	cpm	%	cpm	%	
Regular serum	10110	100	1987	100	39.0
Lipid-depleted serum					
+ palmitate (16:0)	2487	24.6	1650	83	37.4
+ oleate (18:1)	5114	50.6	1327	66.7	38.9
+ elaidate (18:1, *trans*)	2274	22.5	1130	56.9	38.85

all cells grown in lipid-depleted serum had considerably lower phagocytic levels. However, the oleate-enriched cells demonstrated considerably higher levels of phagocytosis compared to elaidate- or palmitate-enriched cells (Table V). On the other hand, no significant differences in the rotational correlation times (τ_0) of 12-nitroxystearate incorporated into the membrane of these cells could be demonstrated (Table V).

DISCUSSION

The present study was designed to derive information about various lipid-associated physical parameters of the macrophage membrane through the use of fatty acid spin labeling techniques.

It can be seen that spin-labeled fatty acids are readily incorporated into intact viable cells, probably by virtue of their solubility in lipids. However, before interpreting the data from such experiments one must ascertain that spin label incorporation does not interfere with normal cellular physiological function, and that the obtained ESR spectra reflect plasma membrane resonance spectra with only minimal contamination from other intracellular organelles. Both of the above were shown by Kaplan et al. [13], who demonstrated that intact nucleated cells remained viable and were capable of replicating in vitro. Moreover, these authors also showed that 87 % of the probe was found in the nuclear fraction of fractionated cells which contained nuclei and plasma membranes, with no appreciable differences in the spectra of the various fractions detectable. Similar conclusions were reached in our system by finding that spin-labeled macrophages remained viable and were as phagocytically active as unlabeled cells (results not presented).

The present study demonstrated an increase in nitroxide mobility as one goes from 5-nitroxystearate to 12-nitroxystearate in macrophages. A similar effect has been noted in other membrane systems [5, 6, 13, 26, 27] as the nitroxide ring was moved further away from the polar end of the molecule.

These observations lend support to the assumption that the local environment of fatty acid spin labels in macrophages is an associated lipid structure whose properties are those of a bilayer.

Nitroxide spin signal destruction seems to be enzymatic in nature. This conclusion is based on the following findings: (1) reduced spin loss in mercury and azide treated cells, (2) prevention of signal loss by fixation of the cells with glutaraldehyde and (3) preventon of signal loss by preincubating the macrophages at 56 °C prior to their labeling. It should be noted that spin signal destruction appears to be a time- and temperature-dependent process (see Fig. 5) similar to those shown in other membrane systems [13, 15, 28, 29]. Indeed, this problem has been overcome in several of these systems by treating the spin-labeled cells with certain inhibitors [28, 30] or by aerating the cells or treating them with ferricyanide [13].

Although the freedom of motion of fatty acid spin labels is dependent on membrane lipid composition, membrane proteins have a marked influence on the physical state of membrane lipids [26, 31]. In each of the above instances, the presence of protein in the spin-labeled preparations resulted in decreased freedom of motion of the membrane lipids.

However, our data suggest that specific protein binding to the cell surface

has no effect on the fluidity of the lipid domains in the macrophage membranes. This has been suggested by the finding that no changes in the spectra of 5-nitroxystearate- or 12-nitroxystearate-labeled macrophages were observed following the binding of antimacrophage antibodies to the cell surface (see Table III). Such treatment, however, greatly impairs cellular phagocytic function, a membrane-associated process [23]. Furthermore, this finding tends to negate a proposed hypothesis that antibody bound specifically to the cell surface induces protein-lipid interactions which may interfere with normal bilayer fluidity [32].

Although in certain systems it seems that proteins have a marked influence on membrane fatty acid disposition, lipid behavior seems to be only partially dependent on conformational changes of membrane proteins. This was concluded on finding that spin-labeled glutaraldehyde-treated macrophages demonstrated τ_0 correlation times almost identical to those of control cultures, while heat-treated (56 °C) spin-labeled macrophages demonstrated slightly higher τ_0 changes, reflecting a lower freedom of motion. In both the above instances the slopes of τ_0 vs. $^{\circ}K^{-1}$ were the same as in the control cultures (results not presented). Since the slopes were unchanged following the different treatments and τ_0 values were only slightly elevated in the heat-treated samples, it would appear that protein conformational variations have only limited effects on the mobility of lipids. However, it must be emphasized that this conclusion is limited, as the spin-labeled fatty acid probes used may partition into preferentially lipid phases and may not be an indication of the microviscosity in immediate lipid-protein regions.

Although it seems that conditions that result in decreased membrane fluidity (low incubation temperatures) "correlate" with decreased cellular phagocytic activity, conditions which specifically inhibit phagocytosis (antimacrophage serum-treated cells) have no bearing on membrane fluidity. This finding may be explained on the basis that decreased phagocytic activity at low incubation temperatures may be the result of inhibited enzymatic activities essential for the phagocytic process. Indeed, direct correlations between lipid phase transitions and several membrane localized enzymatic activities have been described in other systems [8, 10, 11].

The lack of correlation between phagocytosis and membrane fluidity is evident from the in vitro aging experiments. The in vitro cultivation of macrophages in the presence of serum brought about a decrease in cellular phagocytic activity, while no differences in membrane fluidity or the binding of ^{125}I-labeled *Shigella* to the cell surface were found. However, this finding of decreased phagocytic activity in macrophages cultivated in vitro in the presence of serum may be explained by a decrease in the availability of required intracellular space due to vast vacuolation induced by cellular pinocytosis of serum. Indeed, macrophages cultivated in vitro in the absence of serum demonstrated constant phagocytic activities over a 5-day period.

The lack of correlation between macrophage phagocytic activity and membrane fluidity is most evident from the fatty acid replacement studies. The data presented show that macrophages containing high proportions of cis-unsaturated fatty acids (oleate-enriched) have higher phagocytic potentials than do macrophages containing higher proportions of *trans*-unsaturated fatty acids (elaidate-enriched cells). On the other hand, no significant differences were found in the rotational correlation times (τ_0) of spin-labeled fatty acids incorporated into the membranes of either macrophage population. It should be noted, however, that the degree of

specific fatty acid enrichment achieved here was of the order of 10 %. As macrophages are nonreplicating cells, membrane resynthesis probably occurs to only a limited extent under the in vitro conditions employed here. Moreover, it has been demonstrated that the motion of fatty acid spin labels is sensitive to differences of 10 % in the number of unsaturated fatty acids in fluid lipid bilayers [15]. Indeed, no significant alterations in the ratios of saturated to unsaturated fatty acids were obtained in our system and this may explain why the rotational correlation times (τ_0) of spin-labeled macrophages were similar in the various macrophage populations assayed.

The possible mechanism by which altered membrane lipid composition affects macrophage phagocytic activity is at present unknown. However, if a prerequisite of ingestion by the phagocyte is optimal clustering between the antigen and cell-bound antigen receptors into an aggregate of critical size and shape [33], then the lateral diffusion of specific membrane receptors would be of crucial importance to the rate of the ingestion process [34]. Indeed, in the above context it has been shown that the mobility of membrane glycoprotein receptors as measured by the ability of concanavalin A to agglutinate cells is immensely affected by the lipid composition of the cells being assayed [19].

In conclusion, macrophage phagocytic potential, a parameter characterizing many aspects of membrane activity, cannot be correlated with membrane motional freedom inherent in the lipid bilayer by the ESR fatty acid spin probe technique at the degree of fatty acyl enrichment achieved here. On the other hand, it is possible that other methods, which are sensitive to the microviscosity in mammalian membranes, may reveal differences in lipid viscosity in various cellular populations as they have in normal and transformed systems [35].

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