The incorporation of saturated fatty acids into the phospholipids of cultured mouse peritoneal macrophages was shown to lead to reduced endocytic activity (Mahoney, E. M., Hamill, A. L., Scott, W. A., and Cohn, Z. A. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 4893-4898). More detailed analyses concerning the kinetics of fatty acid substitution, lipid composition, membrane bilayer structure, and the fatty acid enrichment of a plasma membrane-derived organelle have now been performed. In these studies, macrophages were cultivated in serumless medium containing 10 μg/ml of fatty acid complexed with bovine serum albumin. Under these conditions, the rate of incorporation of 14C-fatty acid into cell phospholipids was determined to be 8.8 nmol/h/mg of cell protein during the first 8 to 10 h, after which incorporation into phospholipids ceased. Although the fatty acyl composition of phospholipids was substantially altered, the relative amounts of the phospholipids and the molar cholesterol/phospholipid ratio remained unchanged during this period. Phagolysosomes, derived largely from plasma membrane, were isolated from macrophages labeled with 14C-fatty acid and similar enrichment of this fraction in the activity of the plasma membrane marker 5'-nucleotidase (4.1-fold) and in 14C radioactivity (3.7-fold) suggested that the fatty acid supplement was incorporated into plasma membrane. Spin label electron spin resonance studies indicated that the membrane lipid bilayer of macrophage membrane remained unchanged during this period. Phagocytosis of radiolabeled fatty acid from cell phospholipids was shown to lead to reduced endocytic activity of these cells to internalize plasma membrane. Both fluid phase pinocytosis and Fc receptor-mediated phagocytosis were markedly depressed when the degree of saturation of cellular lipids was increased (Table I). The extent to which plasma membrane activity had been modified was further expressed in the elevation of both the activation energy for phagocytosis and the threshold temperature at which phagocytosis was initiated.

Although these striking alterations in function were associated with the lipid substitutions described, it was nevertheless difficult to be certain of either the mechanisms or the cell compartments involved. For this reason, we have performed more detailed analyses concerning the kinetics of fatty acid substitution, lipid composition, membrane bilayer structure, and the fatty acid enrichment of a plasma membrane-derived organelle. We have further demonstrated that the fatty acyl modifications induced in culture are reversible as are the effects on pinocytic activity.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—Primary cultures of peritoneal macrophages were established from resident cells of female NIH Swiss mice as previously described (1). Briefly, cultures were separated into three groups following 16-h cultivation in minimal essential medium containing 20% fetal calf serum (GIBCO). Cells to be supplemented with fatty acids were reincubated for 8 h in Neuman and Tytell's serumless medium prepared without bovine or methyl oleate (hereafter referred to as serumless medium), rinsed, and cultivated another 8 h in serumless medium containing fatty acid complexed with bovine serum albumin (BSA) in concentrations of 10 μg/ml of fatty acid and 2.5 mg/ml of BSA. The other two groups of cultures were reincubated in either serumless medium or 20% fetal calf serum for a total of 16 h, with one change of medium after 8 h.

**Fatty Acid-BSA Complexes**—Complexes of 19:0 or trans-18:1 with fatty acid-poor BSA (Miles Laboratories) were prepared by incubating BSA with the potassium salt of the fatty acid (1).

**Radiolabeling**—To assess endogenous fatty acid biosynthesis, macrophages were incubated with [1-14C]acetate (specific activity, 60.1 mCi/mmol; New England Nuclear, Boston, Mass.) at 3 h intervals immediately prior to harvesting (6 μCi/ml) or continuously for the entire 8-h period prior to harvesting (7 μCi/ml). To measure the rate of incorporation of fatty acid supplement, cells were incubated with 10 μg/ml of trans-18:1, including [1-14C]trans-18:1 (specific activity, 27.8 mCi/mmol; New England Nuclear). Separate cultures were harvested at intervals between 1 and 24 h following the addition of fatty acid. To measure the rate of removal of fatty acid following enrichment of macrophage lipids, cultures were incubated with [1-14C]trans-

Macrophage phospholipids may be rapidly enriched in culture with select fatty acids. We previously demonstrated (1) that the enrichment of macrophage phospholipids with saturated fatty acids led to significant alterations in the ability of these cells to internalize plasma membrane. Both fluid phase pinocytosis and Fc receptor-mediated phagocytosis were markedly depressed when the degree of saturation in cellular lipids was increased (Table I). The extent to which plasma membrane activity had been modified was further expressed in the elevation of both the activation energy for phagocytosis and the threshold temperature at which phagocytosis was initiated.

Although these striking alterations in function were associated with the lipid substitutions described, it was nevertheless difficult to be certain of either the mechanisms or the cell compartments involved. For this reason, we have performed more detailed analyses concerning the kinetics of fatty acid substitution, lipid composition, membrane bilayer structure, and the fatty acid enrichment of a plasma membrane-derived organelle. We have further demonstrated that the fatty acyl modifications induced in culture are reversible as are the effects on pinocytic activity.

EXPERIMENTAL PROCEDURES

**Cell Culture**—Primary cultures of peritoneal macrophages were established from resident cells of female NIH Swiss mice as previously described (1). Briefly, cultures were separated into three groups following 16-h cultivation in minimal essential medium containing 20% fetal calf serum (GIBCO). Cells to be supplemented with fatty acids were reincubated for 8 h in Neuman and Tytell's serumless medium prepared without bovine or methyl oleate (hereafter referred to as serumless medium), rinsed, and cultivated another 8 h in serumless medium containing fatty acid complexed with bovine serum albumin (BSA) in concentrations of 10 μg/ml of fatty acid and 2.5 mg/ml of BSA. The other two groups of cultures were reincubated in either serumless medium or 20% fetal calf serum for a total of 16 h.

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The abbreviations used are: BSA, bovine serum albumin; FCS, fetal calf serum; trans-18:1, trans-octadecenoic acid; cis-18:1, cis-octadecenoic acid; 18:0, nonadecanoic acid; 18:1, hexadecanoic acid; C5 spin label, 2-(3-carboxypropyl)-2-tridecyl-4,4-dimethyl-3-oxazolidinylxoyl; C5 spin label, 2-(10-carboxydecyl)-2-hexyl-4,4-dimethyl-3-oxazolidinylxoyl; C6 spin label, 2-(14-carboxytetradecyl)-2-ethyl-4,4-dimethyl-3-oxazolidinylxoyl.

Andrew W. Mellon Foundation Fellow.
Influence of Fatty Acyl Substitution on Macrophage Membranes

Effect of fatty acid enrichment on endocytic activities in macrophage cultures

<table>
<thead>
<tr>
<th>Culture conditions</th>
<th>Index of phospholipid saturation*</th>
<th>Pinocytosis $E_{o}$</th>
<th>Phagocytosis $E_{o}^*$</th>
<th>Temperature range of activity °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>20% fetal calf serum</td>
<td>1.9</td>
<td>1.01</td>
<td>25</td>
<td>142 54 &gt;17-20</td>
</tr>
<tr>
<td>Serumless medium</td>
<td>2.4</td>
<td>0.69</td>
<td>25</td>
<td>128 65 &gt;20-21</td>
</tr>
<tr>
<td>trans-18:1-BSA</td>
<td>3.6</td>
<td>0.51</td>
<td>23</td>
<td>70 75 &gt;24-25</td>
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<tr>
<td>19:0-BSA</td>
<td>3.9</td>
<td>0.39</td>
<td>17</td>
<td>44 90 &gt;24-25</td>
</tr>
</tbody>
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*Calculated as (% saturated fatty acids + % trans-18:1)/(% cis unsaturated fatty acids).

In some cases, fatty acid methyl esters of total cell phospholipids were separated into cis and trans isomers. Neutral lipids and polystyrene by silica gel chromatography. The I4C radioactivity of the phospholipid fraction was then measured.

**RESULTS**

Macrophages cultured in 60-mm dishes in the presence of serum or 19:0-BSA complex as described above were rinsed three times in cold phosphate-buffered saline. Each monolayer was incubated for 5 min at 37°C with 1 ml of phosphate-buffered saline and 0.2 ml of spin label-bovine serum albumin complex prepared as previously described (7). The cells were harvested, washed with phosphate-buffered saline, and centrifuged, the cell pellet was taken up in a 100-μl disposable pipette, and both ends were sealed. The cells were centrifuged again in the capillary and spectra were then recorded using a Varian E-12 spectrometer. Either a Varian or home-built variable temperature accessory was used, and the temperature was measured with a thermocouple.

**Pinocytosis**—Fluid phase pinocytosis was estimated from the uptake of horseradish peroxidase by the method of Steinman et al. (8) as described (1).

**Protein**—Protein was determined by the method of Lowry et al. (9) using bovine serum albumin as standard.

**Incorporation of [14C]Acetate and Long Chain Fatty Acids into Cellular Lipids**—The rates of fatty acid biosynthesis in macrophages cultured in 20% fetal calf serum, in serumless medium, and in medium containing 10 μg/ml of fatty acid

---

**TABLE I**

<table>
<thead>
<tr>
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</tr>
</tbody>
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1. W. Muller, R. M. Steinman, and Z. A. Cohn, manuscript in preparation.
were estimated from the incorporation of \(^{14}\text{C}\)acetate into cellular lipids. Continuous labeling with \(^{14}\text{C}\)acetate during the final 8-h incubation period revealed that only low levels of fatty acid synthesis occurred in any of the cultures (Table II, Lines 1 to 6). One-hour pulses of isotope demonstrated similar results (Table II, Lines 1 to 3). It was further observed that the rate of incorporation of \(^{14}\text{C}\)acetate into the total lipids of cells cultured in medium supplemented with either serum or fatty acid was 2 to 5 times lower than that in serumless medium. The presence of serum or fatty acid in the culture medium had little effect on the incorporation of acetate into neutral lipids but reduced the incorporation into phospholipids by 4- to 10-fold. As a result, only one-third of the total incorporated radioactivity was recovered in the phospholipids of serum- and fatty acid-cultured cells, whereas two-thirds of the radioactivity was incorporated into phospholipids of serum-free cultures. The data therefore suggested that the presence of long chain fatty acids in the medium inhibited the biosynthesis of fatty acids in cultured macrophages and, in particular, limited the incorporation of acetate-derived fatty acid into cellular phospholipids.

**Kinetics of Exogenous Fatty Acid Incorporation into Cellular Lipids**—The time course of incorporation of radioiso-labeled fatty acid into total phospholipids and neutral lipids is presented in Fig. 1. The incorporation of \(^{14}\text{C}\)trans-18:1 into total lipids was linear for 10 h and then proceeded at a reduced rate (top). The rate of incorporation into phospholipid (8.8 nmol/h/mg of cell protein) was constant for 8 to 10 h (middle) and was approximately twice the rate of incorporation into neutral lipids (4.2 nmol/h/mg of cell protein; bottom). Although small amounts of \(^{14}\text{C}\)trans-18:1 continued to be incorporated into neutral lipids after 10 h, incorporation into phospholipids ceased. Since 50 to 75% of the original \(^{14}\text{C}\) radioactivity remained in the culture medium after 8 h, the termination of incorporation into phospholipids could not be ascribed to the depletion of the fatty acid supplement available. This pattern of fatty acid incorporation was similar to that observed for saturated and cis-unsaturated fatty acid.

It should be noted that the rate of incorporation of trans-18:1 into phospholipid during the initial 8 h was 300-fold greater than the corresponding rate observed for acetate incorporation in trans-18:1-supplemented cultures and 20- to 140-fold greater than the rate of acetate incorporation in serum and serum-free cultures (Table II, compare Line 7 with Lines 1 to 6). Similarly, the rate of incorporation of trans-18:
unsaturated fatty acyl components. Furthermore, less than 1% of the incorporated radioactivity was present in nonlipid material. The fatty acid composition of trans-18:1-enriched macrophages determined by gas-liquid chromatography was reported previously (1). Only a small increment was observed in total unsaturated fatty acids containing fewer than 18 carbon atoms. This finding, together with the present data, suggested that metabolites of the trans-18:1 supplement did not constitute a significant contribution to the total fatty acid composition. Instead, the data indicated that trans-unsaturated fatty acids were incorporated intact into cellular lipid.

The distribution of [14C]trans-18:1 among the individual classes of phospholipids is shown in Table III. trans-18:1 was incorporated into all the phospholipids, but not in proportion to the molar amount of each species present. For example, the specific activity in phosphatidylcholine was 2.4-fold greater than that in phosphatidylethanolamine. Whether the nonrandom distribution of the fatty acid label was due to differential incorporation into phospholipid classes or to selective turnover of certain species is not known.

Phospholipid and Sterol Composition—The phospholipid composition of macrophages enriched with 19:0 and trans-18:1 was similar to that of cells cultured in 20% serum or in serumless medium (Table IV). The types and relative amounts of individual phospholipids remained essentially constant regardless of the culture conditions, with phosphatidylcholine and phosphatidylethanolamine being the major phospholipids (>70%) in each case. Similarly, the amount of total cellular phospholipid (~162 nmol/mg of protein) and the amount of cell cholesterol (~34 µg/mg of protein) were not influenced by the composition of the culture medium. As a result, the molar cholesterol/phospholipid ratio in total macrophage lipids was not altered by fatty acid enrichment.

Cell Morphology and Ultrastructure—The phase contrast appearance of macrophages cultured in serumless medium, trans-18:1, or 19:0 was normal when viewed in the living state (10, 11). More than 95% of each of these cell types was viable as measured by dye exclusion methods. After glutaraldehyde fixation, however, the majority of cells in 19:0-enriched cultures exhibited refractile needle-like clefts in the cytoplasm.

Table III: Incorporation of [1-14C]trans-18:1 into macrophage phospholipids

<table>
<thead>
<tr>
<th>Culture conditions</th>
<th>PS</th>
<th>PI</th>
<th>SM</th>
<th>PC</th>
<th>PE</th>
<th>PA</th>
<th>DPG</th>
<th>Na mol lipid phosphorus/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>20% serum</td>
<td>63</td>
<td>58</td>
<td>1.7</td>
<td>45.8</td>
<td>32.6</td>
<td>0.6</td>
<td>6.7</td>
<td>169 ± 11 (7)</td>
</tr>
<tr>
<td>Serumless medium</td>
<td>10.0</td>
<td>7.4</td>
<td>2.6</td>
<td>40.0</td>
<td>29.7</td>
<td>1.3</td>
<td>7.0</td>
<td>164 ± 10 (4)</td>
</tr>
<tr>
<td>trans-18:1-BSA</td>
<td>7.2</td>
<td>8.5</td>
<td>1.0</td>
<td>45.3</td>
<td>27.6</td>
<td>0.5</td>
<td>8.1</td>
<td>161 ± 15 (4)</td>
</tr>
<tr>
<td>19:0-BSA</td>
<td>6.8</td>
<td>7.9</td>
<td>0.9</td>
<td>47.7</td>
<td>28.1</td>
<td>0.8</td>
<td>7.4</td>
<td>160 ± 2 (2)</td>
</tr>
</tbody>
</table>

a Values are the means of two to seven determinations. Abbreviations are those used in Table III.

Values are presented as means ± standard deviations. The number of determinations is given in parentheses.

Fig. 2. Electron micrograph of 19:0-enriched macrophage. Linear arrays of cisternae (large arrow) and clefts devoid of organelles (small arrow) are apparent. Mitochondria (M) and plasma membrane are normal in appearance. × 55,200. Inset, phase contrast micrograph of 19:0-enriched macrophage with large needle-like cleft. × 1,150.
(Fig. 2, inset). Similar clefts were observed in both 19:0- and 16:0-enriched cells at the level of electron microscopy (Fig. 2). These clefts, passing through presumably endoplasmic reticulum, were bounded by unit membranes containing ribosomes on their cytoplasmic surfaces. An additional feature of glutaraldehyde fixation in 19:0-enriched cells was the presence of linear arrays of endoplasmic reticulum not seen in macrophages cultured under other conditions. No other structural alteration was noted in 19:0-enriched cells.

**Phagolysosome Preparation**—Endocytic alterations following fatty acid enrichment of macrophages (1) were thought to result from plasma membrane modifications. Although purified plasma membrane is difficult to obtain from macrophages, phagolysosomes are readily isolated in sucrose gradients and accumulate at the interface between the 10% and 25% layers (10/25 IF)'. Such fractions are readily obtained following interiorization of latex particles by the cells in culture. Since the phagolysosome is largely derived from the plasma membrane (phagocytic vacuole), it is a representative segment of this organelle.

**Table V**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Protein Specific activitya</th>
<th>Lipid radioactivity Specific activityb</th>
<th>Purification</th>
<th>Specific activity Purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate</td>
<td>916</td>
<td>2.43</td>
<td>461</td>
<td>1.0</td>
</tr>
<tr>
<td>10/25 IF, Gradient II (phagolysosomes)</td>
<td>15</td>
<td>10.06</td>
<td>1884</td>
<td>3.7</td>
</tr>
</tbody>
</table>

*Expressed as nanomoles of P, released/40 min/mg of protein.

*Expressed as dpm/mg of protein.

Table V shows the results of an experiment in which phagolysosomes were obtained immediately after latex bead interiorization by [14C]19:0-enriched macrophages (see "Experimental Procedures"). The amounts of protein, radioactivity, and 5'-nucleotidase activity, a plasma membrane marker (12), were determined in the various subcellular fractions. 5'-Nucleotidase activity and 14C radioactivity co-purified with the phagolysosome fraction, indicating that the radiolabel had been incorporated into the plasma membrane during the supplementation period. Increased specific activities of 5'-nucleotidase (4.1-fold) and of the radiolabel (3.7-fold), compared with the cell homogenate, were found in the 10/25 IF, which was the only fraction enriched for these activities. The comparable degree of enrichment of 5'-nucleotidase and of lipid radioactivity suggested that macrophage plasma membrane lipids were enriched with the fatty acid supplement above that of other compartments.

**Cultivation of trans-18:1-enriched Macrophages in Fetal Calf Serum and Its Effect on Cellular Lipids**—Washout experiments with cells enriched with [14C]trans-18:1 were carried out in 20% fetal calf serum, which contains a substantial amount of unsaturated fatty acids. As shown in Fig. 3, serum incubation resulted in the prompt reduction of the specific activity of the radiolabel in total lipids. The amount of trans-18:1 in both phospholipids and neutral lipids was reduced by more than 65% within 24 h. The radiolabel remaining in phospholipids at that time co-chromatographed with trans-unsaturated fatty acids as measured using argentin thin layer chromatography. Comparable results were obtained if the [14C]trans-18:1-enriched cells were reincubated in medium containing [3H]cis-18:1-BSA (data not shown) rather than serum.

In Table VI, the distribution of radioactivity in individual phospholipids immediately after the 8-h exposure to [14C]-trans-18:1 is compared with that observed following 24-h incubation in serum. After the reincubation in 20% serum, a somewhat larger percentage of the total radioactivity was associated with phosphatidylethanolamine than immediately after the enrichment, but, overall, the two patterns resembled one another. This indicated that label was removed from all classes of phospholipids and that the reduction in the 14C

![FIG. 3. Change in specific activity of [1,14C]trans-18:1 incorporated into macrophage lipids. Cells were labeled for 8 h and then transferred (0 h) to medium containing 20% fetal calf serum or to serumless medium. Individual cultures were harvested at various times thereafter, and the lipids were extracted and separated into neutral lipids and phospholipids as described under "Experimental Procedures." The specific radioactivities of the total lipids, phospholipids, and neutral lipids are expressed as the percentage of the specific radioactivity of the corresponding fraction at 0 h.](image-url)
specific activity of total phospholipids was not the result of selective turnover of any one species.

Effect on Fluid Phase Pinocytosis of Cultivating trans-18:1-enriched Macrophages in Fetal Calf Serum—To determine whether the effects of saturated fatty acid enrichment on endocytosis were reversible, the pinocytic activity of macrophages enriched with trans-18:1 was examined after cultivation in 20% fetal calf serum (Fig. 4). Immediately after enrichment, trans-18:1-cultured macrophages pinocytized at a rate which was 50% that of serum control cells. After an additional 24-h cultivation in 20% serum, the level of pinocytic activity in control cultures increased 50%, whereas the pinocytic rate in trans-18:1-enriched cells reincubated in serum increased 123%. The latter activity represented a level of pinocytosis which was 80% that of the serum control. The increase in pinocytosis was not merely the result of additional time in culture, since the pinocytic activity of cells cultivated throughout in serumless medium remained nearly constant (50 to 60% of that of the serum-cultured cells). This suggested that the reversal in pinocytic activity was closely associated with the replacement of trans-unsaturated fatty acid species in cell phospholipids.

Bilayer Structural Changes upon Fatty Acid Incorporation. Spin label ESR experiments were performed to assess the changes in bilayer fluidity induced by saturated fatty acid enrichment. Monolayers of serum-cultured and 19:0-enriched macrophages were labeled with C₅, C₁₂, or C₁₆ nitroxide derivatives of octadecanoic acid. ESR spectra recorded at 4°C from serum-cultured and 19:0-enriched monolayers are shown in Fig. 5. In both cases, the value of \(2\Delta\\alpha\) decreased as the nitroxide moiety was moved from the fifth position on the fatty acyl chain closer to the methyl terminus. This indicated that the spin label probes were situated in an environment where a fluidity gradient existed. Such a fluidity gradient is a property of the lipid bilayer structure (13-15), indicating that the C₅ spin labels in both the serum-cultured and 19:0-enriched macrophages were incorporated into a lipid bilayer, with the C₁₂ label probing the vicinity of the phospholipid-glycerol backbone and the C₁₆ label probing the region near the center of the bilayer (16, 17). The C₁₆ spectra indicated that under the appropriate conditions, binding of the spin label-BSA complex to the macrophages was not observed (18).

In Table VII, the \(2\Delta\\alpha\) values of a representative experiment are given. The \(2\Delta\\alpha\) values for the C₅ and C₁₂ spin labels were increased in the saturated fatty acid-enriched macrophages at each investigated temperature. The greatest difference between the ESR spectra of 19:0-enriched and serum-cultured macrophages was not for the serum-cultured macrophages.

**Fig. 4.** Effect of serum cultivation on pinocytic activity in trans-18:1-enriched macrophages. The end of the fatty acid supplementation period in culture is designated 0 h. The pinocytic activity of each culture is expressed relative to the value determined for the serum control culture at 0 h, which is normalized to the value 1.0. Serum cultures were incubated an additional 24 h in 20% fetal calf serum; serum-free cultures were incubated an additional 24 h in serumless medium; and trans-18:1-enriched cultures were incubated an additional 24 h in 20% fetal calf serum. The values presented are based on the means of duplicate or triplicate determinations.

**Fig. 5.** ESR spectra of spin-labeled macrophages. Monolayers of 19:0-enriched (A) and serum-cultured (B) macrophages were spin-labeled using C₅, C₁₂, or C₁₆ spin label derivatives of 18:0, as described under "Experimental Procedures." Spectra were recorded at 4°C. The parameter \(2\Delta\\alpha\) is defined as the distance (in gauss) between the vertical lines drawn through the extrema of the low and high magnetic field peaks. With each spectral pair is shown the corresponding amplified peaks used for more accurate determination of \(2\Delta\\alpha\). For both C₅ and C₁₂, \(2\Delta\\alpha\), for the 19:0-enriched macrophages is greater than for the serum-cultured macrophages. As the distance between the outermost peaks of the ESR (\(2\Delta\\alpha\)) decreases, the local fluidity of the lipid phase increases (13, 14).
TABLE VII

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>C,S values</th>
<th>FCS 19:0</th>
<th>C,S values</th>
<th>FCS 19:0</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>62.7</td>
<td>63.2</td>
<td>56.9</td>
<td>59.0</td>
</tr>
<tr>
<td>14</td>
<td>59.5</td>
<td>60.0</td>
<td>48</td>
<td>52</td>
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<tr>
<td>23</td>
<td>56.1</td>
<td>56.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE VII lists the C,S values for different spin labels in intact macrophages. Monolayers of serum-cultured and 19:0-enriched macrophages were spin-labeled at 4°C using C,S spin label derivatives of 18:0, as described under “Experimental Procedures.” Spectra were recorded at the temperatures indicated.


cultured macrophages was observed with the C12 spin label, which probes the region of Δ9 and Δ12 double bonds. A smaller, but significant difference was also apparent for the C16 spin label. In contrast, the fluidity of the center of the bilayer was not detectably different, as evidenced by the C16 spectra (Fig. 5).

DISCUSSION

A major role for macrophages in mammalian physiology relates to the uptake and processing of macromolecules (19). In this capacity, macrophages are involved in diverse functions, such as cell-mediated immunity and the clearance of aged erythrocytes. Many of these processes continue to be expressed in primary explants of macrophages and offer the investigator a useful system for the analysis of the structure, composition, and function of plasma membrane and its derived organelles. This article continues our previous reports (4, 20-22) on the role of membrane lipids in macrophage physiology and focuses on the enrichment of the phospholipids of the cell with saturated fatty acids. Employing supplements of fatty acid-albumin complexes, we achieved rapid fatty acid incorporation in these nonreplicating cultures. We found that maximum enrichment of phospholipids was attained within 8 to 10 h. The lack of any appreciable fatty acid biosynthesis in macrophages cultivated in medium containing serum or fatty acids simplified the analysis of phosphatide enrichment and obviated the use of inhibitors of endogenous fatty acid synthesis. Preference for the incorporation of long chain fatty acids available in the medium was displayed, and relative enrichment of a plasma membrane-derived organelle, the phagolysosome, could be demonstrated. Presumably, the lipids of the phagolysosome are derived predominantly from plasma membrane constituents, much as recently completed studies have shown that the phagolysosome polypeptides are similar, if not identical, to those of the plasma membrane.

Other than the modification in the species and relative amounts of fatty acids, there was little or no alteration in the levels of the major lipid classes in the macrophages exposed to lipid supplements. The total amounts and distribution of phosphatides were not changed during the brief enrichment period, nor were sterols affected. We cannot comment on the glycolipids, but we know of no evidence that these would be altered under the conditions employed. Therefore, we have reasonably good and complete evidence that we have selectively modified the fatty acyl composition of the macrophage and that this in turn was responsible for the striking reduction in endocytotic activity.

Our studies do not exclude possible effects of fatty acid enrichment on the function of other subcellular membrane systems such as the mitochondrion, nuclear membrane, endoplasmic reticulum, and Golgi apparatus. Modifications in mitochondrial cristae, for instance, could affect electron transport and energy metabolism, thereby influencing endocytosis and other processes indirectly. The appearance of clefts in association with linear arrays of cisternae in glutaraldehyde-fixed preparations of 19:0-enriched macrophages were similar to those observed in L-cells and human neutrophils incubated with fatty acids in culture (23, 24) and suggested that the endoplasmic reticulum had been altered. However, studies to be reported indicate no influence of saturated fatty acid enrichment on the incorporation of amino acids into protein or on the secretion of two enzymes by macrophages.

The results of ESR experiments supported the idea that reduced fluidity in the lipid bilayer of the plasma membrane of saturated fatty acid-enriched macrophages was responsible for the observed decreases in levels of endocytosis. Spin label ESR measurements at 4°C, 14°C, and 23°C demonstrated that the degree of fatty acid modification achieved affected the mobility of different nitroxide spin labels: an increase in the amount of saturated fatty acid present in phospholipids reduced the fluidity of the lipid bilayer. Other investigators have observed changes in the motion of spin label probes following fatty acid enrichment of 3T3 cells and Ehrlich ascites tumor cells (25, 26). The fatty acyl modifications of these cells as well as LM cells (27) and chick myoblasts (28) have been correlated with alterations in lectin-mediated agglutination (29), lectin binding (30), cell growth (31, 32), fusion (28), and (Na⁺,K⁺)-ATPase activity (33). These changes in cell behavior have consequently been attributed to modified membrane fluidity. In comparison with these studies, it appears that for a given level of saturated fatty acid enrichment, the interiorization of plasma membrane by macrophages appears to be a sensitive correlate to restrictions in membrane fluidity. Since a substantial change in the radius of curvature of the plasma membrane must precede the formation of an endocytic vesicle, it is not surprising that endocytosis is markedly curtailed as membrane fluidity is reduced.

A previous study comparing the ESR spectra of C16 and C18 spin-labeled MDBK and BHK21-F cells, isolated plasma membranes, and various cytoplasmic membrane fractions indicated that the ESR spectra of labeled intact cells were due to spin label in the plasma membrane (34). Since a similar protocol was used, it seems likely that the C1 spin labels were intercalated into the macrophage plasma membrane in the present study. Unfortunately, the preparation of phagolysosomes provides insufficient material for a detailed ESR study. Direct demonstration of the site of spin label localization in intact macrophages awaits the development of a procedure for the preparation of large quantities of purified plasma membrane from cultured macrophages. Furthermore, detailed temperature analyses of spin probe behavior using a single sample of isolated plasma membrane should then be possible. This would circumvent the problem encountered using whole cells where the amplitude of the ESR spectrum rapidly decreases, especially at temperatures above ~20°C, a phenomenon documented in other systems (35, 36) that may be associated with endocytosis and subsequent reduction of the nitroxide radical.

The enrichment of macrophage lipids with select fatty acids was found to be reversible when the supplementation period was followed by incubation in the presence of serum or cisunsaturated fatty acid. Retention of phospholipid fatty acids was demonstrated in the presence of serumless medium devoid of fatty acid. Serum incubation not only reversed the enrichment achieved, but it also reversed the reduction in pinocytic activity associated with the incorporation of saturated fatty acids. These results indicated that macrophages enriched with
fatty acids were capable of regaining a normal lipid composition and pinocytic activity in the presence of the cis-unsaturated species in serum. Neither the reversibility of fatty acid modification nor the reversibility of its functional consequences have been demonstrated for other cell systems. The association of reductions in obviously important endocytic activities in the macrophage with saturated fatty acid enrichment and the relief of inhibition when enrichment is reversed, illustrates the striking influence of fatty acyl composition on physiologically vital membrane processes.

REFERENCES