Incorporation of \(^{14}\text{C}\)-Labeled Acetate into Lipid by Isolated Foam Cells and by Atherosclerotic Arterial Intima


ABSTRACT

The synthesis of lipid by rabbit atherosclerotic intima incubated in vitro has been investigated and compared with that of foam cells isolated from intimal lesions. In the atherosclerotic arterial intima, \(^{14}\text{C}\)-labeled acetate is readily incorporated into the lipid fraction, most of the label being found in the phospholipid and cholesterol ester fatty acid fractions. Minimal incorporation into triglyceride, fatty acid, and cholesterol occurred. The major phospholipid synthesized was lecithin but significant incorporation into phosphatidyl inositol, phosphatidyl ethanolamine, and sphingomyelin also took place. The fatty acids synthesized were predominantly saturated in the phospholipid fraction. More monounsaturated fatty acids were present in the cholesterol ester fatty acids, but in both moieties little polyunsaturated fatty acids were labeled by the acetate. Isolated foam cells were also shown to incorporate \(^{14}\text{C}\)-labeled acetate into both phospholipid and cholesterol ester fatty acids. The foam cells, however, incorporated a significantly higher proportion of acetate into cholesterol ester than did the whole intima. The fatty acids synthesized by the foam cells were more polyunsaturated in both the phospholipid and the cholesterol ester fraction than was the case with the whole intima. The relevance of these findings to the origin of lipid in the atherosclerotic lesion is discussed.

ADDITIONAL KEY WORDS phospholipid lecithin cholesterol ester fatty acid polyunsaturated fatty acids sphingomyelin thin-layer chromatography arterial wall metabolism cholesterol-fed rabbit

■ The appearance of relatively large quantities of phospholipids and cholesterol esters is a characteristic feature of the fatty streak lesion in atherosclerosis (1-3). The origin of these two components is, therefore, of some significance in considering the pathogenesis of the early lesion. The work of Zilversmit and co-workers (4-6) has indicated that the phospholipid which accumulates in the atherosclerotic lesion (both in the human and in the cholesterol-fed rabbit) arises by synthesis in the arterial wall rather than by infiltration from the plasma, and this work, along with that of others, has led to a stimulation of interest in the metabolism of the arterial wall as a factor in the pathogenesis of atherosclerosis. Cholesterol ester being the major component of the early atherosclerotic lesion is of particular interest in this regard. Stein and Stein (7) have shown incorporation of \(^{14}\text{C}\)-labeled linoleic acid into cholesterol ester in the normal rabbit aorta, and Lofland et al. (8) have demonstrated that in the pigeon aorta, incubated in vitro, the amount of \(^{14}\text{C}\)-labeled acetate incorporated into the fatty acid of cholesterol ester increases with increasing atherosclerosis. Recently, Newman et al. (9) have shown that, in eviscerated rabbits injected in vivo with \(^{14}\text{C}\)-labeled acetate, the specific activity of the cholesterol ester fatty acid of the atherosclerotic intima is considerably higher than that of the serum, indicating that the cholesterol is esterified in situ possibly from fatty acid synthesized in the atherosclerotic arterial wall.

The role of arterial foam cells in bringing...
about the synthesis of lipid in the atherosclerotic lesion has been the subject of a number of investigations. It has been shown that phospholipid is synthesized by peritoneal macrophages and such synthesis is stimulated by the uptake of cholesterol by these cells (10). Histochemical observations have demonstrated the presence of phospholipid in foam cells in both rabbit (11) and human lesions (12) and these cells were suggested as active participants in the metabolic processes in the wall. Cholesterol esterase activity has been demonstrated in macrophage homogenates (13, 14) and the suggestion that cholesterol esterification in the atherosclerotic wall might be brought about by the macrophages present was made on the basis of such data (15). Geer and Guidry (16) found that in fatty streaks in man the cholesterol ester fatty acids containing the highest proportion of oleic to linoleic acid were those containing the highest proportion of foam cells and postulate that foam cells synthesize cholesterol ester in the arterial wall. Smith (2, 17), in a similar study, has also demonstrated that foam cell lesions in the human intima contain a high ratio of oleic to linoleic acid in their cholesterol esters, whereas the fatty acid content of the extracellular cholesterol ester resembles that of the serum.

The recent development of a method for isolating viable foam cells from atherosclerotic lesions (18) has provided a means for investigating more directly the role of these cells in the atherosclerotic process. Foam cells isolated from atherosclerotic lesions (18) has provided a means for investigating more directly the role of these cells in the atherosclerotic process. Foam cells isolated from atherosclerotic lesions in rabbits incorporate 32P-labeled phosphate into phospholipid and these cells contribute in part to the phospholipid synthesis that occurs in the atherosclerotic arterial wall (18). In the present paper we have extended these observations and studied the synthesis of phospholipid and of cholesterol ester by isolated foam cells using 14C-labeled acetate as precursor. We have demonstrated the incorporation of 14C-labeled acetate into the fatty acids of both cholesterol ester and of phospholipid by the isolated foam cells and have compared it with the incorporation of 14C-labeled acetate into these moieties by the arterial intima as a whole.

Methods

Male New Zealand white rabbits were fed a diet containing 1 g of cholesterol and 3 ml of peanut oil in 100 g of rabbit chow daily. After 3 to 4 months on this diet the rabbits were killed under ether anesthesia and the thoracic aorta removed.

Isolated foam cells were obtained from the intima by incubation with collagenase and elastase as previously described (18). The cells were dispersed into Leighton tubes so that approximately 0.5 X 10^8 cells were contained in each. After washing with saline to remove contaminating cells, 1 ml of medium (50:50 Hank’s solution: normal rabbit serum containing 20 μg of sodium 1-14C-acetate, Radiochemical Centre) was added and the cells were incubated in vitro at 37°C for 4 hr. The medium was then removed; the foam cells were washed twice with saline and extracted with 2:1 chloroform:methanol according to Folch et al. (19).

For in vitro metabolic study of the intact intima, the superficial fat was removed and the aorta divided longitudinally into halves, each half being incubated separately in vitro in 5 ml of medium (50:50 Hank’s solution: normal rabbit serum containing 20 μg of sodium 1-14C-acetate). After incubation for 4 hr at 37°C, the aorta was washed in saline and the intima stripped from the remaining media and adventitia and extracted with 2:1 chloroform:methanol. In some experiments the aorta was divided longitudinally into thirds, each third being incubated for either 1, 2 or 4 hr with the 14C-labeled acetate as already mentioned.

Thin-layer Chromatography

The lipid extracts from foam cells or intima were separated into phospholipid, cholesterol, fatty acid, triglyceride, and cholesterol ester by thin-layer chromatography on Silica Gel G (Merck), using diethyl ether:acetic acid:hexane (25:2:73 v/v/v) as developing solvent. For the foam cell extract a standard mixture of lipids was added as carrier. The distribution of 14C in the lipid fractions was determined after scraping of the spots and counting by the method of Snyder (20).

Separation of the individual 14C-labeled phospholipids in the total lipid extracts was carried out by thin-layer chromatography using the method of Skipski et al. (21). The foam cell extracts were taken up in a standard phospholipid mixture before chromatography to facilitate identification and location of the labeled spots. The phospholipid spots, located with iodine vapor, were...
LIPID SYNTHESIS BY FOAM CELLS

Scraped into counting vials for counting by the method of Snyder (20). Identification was made by comparison of Rf values with standards. Phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl choline, sphingomyelin and lysolecithin were obtained from Applied Science Laboratories and phosphatidyl inositol was kindly supplied by Dr. Faure (Pasteur Institute). Fuller identification by staining reactions and co-chromatography of similar spots in aortic extracts and in foam cells was described previously (18, 22).

The type of 14C-labeled fatty acids incorporated into phospholipid and into cholesterol ester by both the intima and foam cells was determined following separation of the cholesterol ester and phospholipid fractions of the lipid extracts by thin-layer chromatography as already described. The total 14C-labeled fatty acids in these moieties were converted to their respective fatty acid methyl esters by methanolysis at 60°C for 16 hr with 5% sulfuric acid in methanol in sealed ampoules. The methyl esters obtained were then separated into saturated, mono-, and polyunsaturated groups by thin-layer chromatography on silver nitrate impregnated Silica Gel G as described by Morris (23), using diethyl ether:hexane (1:9 v/v) as developing solvent. The 14C-labeled methyl esters were scraped into counting vials and counted directly (20). For these preparations significant quenching occurred and correction was made using an automatic external standard. All counting was done using a Packard TriCarb Spectrometer.

SAPONIFICATION

In some experiments the proportion of 14C-labeled lipid present as 14C-labeled fatty acid in the cholesterol ester and phospholipid was determined following saponification of these fractions. After initial separation by thin-layer chromatography as set out above, the cholesterol ester or phospholipid spots were scraped into ampoules and saponified directly at 60°C for 16 hr with 2% potassium hydroxide in ethanol.

Results

The pattern of incorporation of 14C-labeled acetate into the individual lipid moieties by the intact intima is given in Table 1. Between 0.210 and 1.22% of the 14C-labeled acetate added to the medium was taken up and converted to lipid by the atherosclerotic aortic halves. Most of this was incorporated into phospholipid (35%), but an appreciable proportion (28.7%) was incorporated into cholesterol ester and lesser amounts into triglyceride, free cholesterol, and fatty acid. Saponification of cholesterol and fatty acid.

The percentage distribution of synthesized 14C-labeled fatty acid between various lipid fractions after incubation of either whole intima or foam cells with 14C-labeled acetate is given in Table 1. The significant differences between the intima and foam cells are discussed below. The total 14C-labeled fatty acids in these moieties were converted to their respective fatty acid methyl esters and separated by thin-layer chromatography. The methyl esters were scraped into counting vials and counted directly (20). For these preparations significant quenching occurred and correction was made using an automatic external standard. All counting was done using a Packard TriCarb Spectrometer.

Table 1

<table>
<thead>
<tr>
<th>Lipid Fraction</th>
<th>% of 14C Acetate converted to lipid</th>
<th>Phospholipid</th>
<th>Cholesterol</th>
<th>Fatty acid</th>
<th>Triglyceride</th>
<th>Cholesterol ester</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intima*</td>
<td>4 hr</td>
<td>0.587 ± 0.139</td>
<td>55.6 ± 1.29</td>
<td>3.6 ± 0.23</td>
<td>10.1 ± 0.15</td>
<td>28.7 ± 0.22</td>
</tr>
<tr>
<td>Foam cells†</td>
<td>4 hr</td>
<td>0.27 ± 0.08</td>
<td>41.0 ± 2.48</td>
<td>3.0 ± 0.32</td>
<td>4.3 ± 0.94</td>
<td>7.2 ± 1.38</td>
</tr>
<tr>
<td>P‡</td>
<td></td>
<td>&lt; 0.001</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Intima§</td>
<td>1 hr</td>
<td>0.129</td>
<td>50.0</td>
<td>4.7</td>
<td>5.5</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td>2 hr</td>
<td>0.331</td>
<td>51.9</td>
<td>2.9</td>
<td>2.6</td>
<td>8.4</td>
</tr>
<tr>
<td></td>
<td>4 hr</td>
<td>0.622</td>
<td>56.7</td>
<td>2.3</td>
<td>1.7</td>
<td>8.6</td>
</tr>
</tbody>
</table>

*Mean of 6 experiments with 6 mean. Aorta halves were incubated separately in 4 of the 6 experiments and these have been treated as duplicates.
†Mean of 5 experiments; duplicate batches of cells incubated separately in each.
‡Significance value based on Student's t-test. Mean percentage distribution in different lipid moieties of intima compared with that in foam cells.
§Mean of 2 experiments at each time interval.
the phospholipid and cholesterol ester of the
14C-labeled extracts established that over 90% of
the 14C-label was present in the fatty acid
portion in each of these two fractions so that
the distribution of label given in Table 1 is
especially that of the fatty acid present in
these fractions. The percentage distribution of
14C-labeled lipid between the various lipid
moieties after 1, 2 and 4 hr of incubation of
the intact intima is also shown in Table 1.
While there are some trends, no gross change
in distribution of label over the 4-hr period
studied could be observed.

The incorporation of 14C-labeled acetate into
lipids by the foam cells is also shown in Table
1. Of the acetate added to the medium,
0.027% was converted to lipid by the 0.5 X 10^6
cells in the isolated foam cell preparations.
The 14C-labeled acetate was incorporated
approximately equally into cholesterol ester and
into phospholipid with smaller amounts of
triglyceride, free fatty acid, and cholesterol
being labeled. Saponification of the phospho-
lipid and the cholesterol ester fractions estab-
lished that more than 90% of the label in these
two moieties was present in the fatty acid por-
tion as was the case in the intima incubations.

From the data given in Table 1, it is appar-
ent, however, that the incorporation of 14C-
labeled acetate into the fatty acid of the phos-
pholipid and cholesterol ester fractions by the
foam cells is significantly different from that
of the intact intima. Of the 14C-labeled acet-
ate incorporated into lipid by the foam cells,
a significantly higher proportion is incorporat-
ed into cholesterol ester, and a significantly
lower proportion into phospholipid than is
the case for the intima. This does not neces-
sarily mean, of course, that more total choles-
terol ester is synthesized by the foam cells but
only that diversion of synthesis to cholesterol
ester occurs in the cells.

The incorporation of 14C-labeled acetate
into individual phospholipids by the arterial
intima is given in Table 2. Most of the label
has been incorporated into lecithin with smaller
amounts in the other phospholipids studied.

The relative incorporation of 14C into different
phospholipids did not differ significantly at 1
or 2 hr to that found after incubation for 4 hr.

The incorporation of 14C-labeled acetate in-
to individual phospholipids by the isolated
foam cells is also given in Table 2. Approx-
mately half of the label has been incorporat-
ed into lecithin with significant amounts of
phosphatidyl inositol and phosphatidyl ethan-
olamine being labeled. It can be seen from the
figures given in Table 2 that a significantly
lower proportion of the 14C-labeled acetate
is incorporated into sphingomyelin and leci-
thin, and a significantly higher proportion
into phosphatidyl inositol and phosphatidyl
ethanolamine by the foam cells than is the
case for the intima.

The fatty acids synthesized and incorp-
orated into the phospholipid fraction by the in-
tima are predominantly saturated (Table 3).
On the other hand, fatty acids incorporated
into the cholesterol ester fraction by the intima
are about equally distributed between the
saturated, mono-, and polyunsaturated groups.
The fatty acids synthesized by the foam cells,

<table>
<thead>
<tr>
<th>Incubation time</th>
<th>Lysolecithin</th>
<th>Sphingomyelin</th>
<th>Lecithin</th>
<th>Phosphatidyl inositol</th>
<th>Phosphatidyl ethanolamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intima*</td>
<td>4 hr</td>
<td>0.5 ± 0.05</td>
<td>8.5 ± 0.38</td>
<td>65.5 ± 0.75</td>
<td>14.4 ± 0.36</td>
</tr>
<tr>
<td>Foam cells†</td>
<td>4 hr</td>
<td>2.0 ± 0.72</td>
<td>6.3 ± 0.61</td>
<td>49.3 ± 2.28</td>
<td>21.2 ± 0.46</td>
</tr>
<tr>
<td>P‡</td>
<td></td>
<td>n.s.</td>
<td>&lt; 0.02</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Intima§</td>
<td>1 hr</td>
<td>0.6</td>
<td>6.1</td>
<td>64.0</td>
<td>17.5</td>
</tr>
<tr>
<td></td>
<td>2 hr</td>
<td>0.4</td>
<td>7.3</td>
<td>85.7</td>
<td>14.8</td>
</tr>
<tr>
<td></td>
<td>4 hr</td>
<td>0.6</td>
<td>8.4</td>
<td>66.3</td>
<td>14.0</td>
</tr>
</tbody>
</table>

For explanation of footnotes see Table 1.
however, are predominantly polyunsaturated, both in the phospholipid and in the cholesterol ester fractions (Tables 3, 4). This difference between the distribution of label in the intima and the foam cells is more apparent, however, in the case of the phospholipid fatty acids. With this moiety the diversion of $^{14}$C-labeled acetate to polyunsaturated fatty acids by the foam cells is in marked contrast to the predominant incorporation of $^{14}$C-labeled acetate into saturated fatty acid by the intima. These differences are highly significant statistically (Table 3).

In the case of the cholesterol ester (Table 4), the $^{14}$C-labeled acetate was approximately equally distributed between the saturated, monounsaturated, and polyunsaturated fatty acids by the intima, and although there was a shift towards the polyunsaturated side by the foam cells, there was too much variability to produce statistically significant differences between the respective fatty acids of intima and foam cells in the small series studied.

**Discussion**

The incorporation of appreciable amounts of $^{14}$C-labeled acetate into cholesterol ester fatty acids in the atherosclerotic intima confirms for the rabbit lesion what has already been demonstrated for the pigeon lesion by Lofland et al. (8), i.e. the significant diversion of fatty acid to cholesterol ester in the atherosclerotic lesion. The observations have been extended in the present work, however, to

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**TABLE 3**

Percentage Distribution of Synthesized Phospholipid $^{14}$C-Labeled Fatty Acid Between Saturated, Mono- and Polyunsaturated Groups After Incubation of Either Whole Intima or Foam Cells with $^{14}$C-Labeled Acetate

<table>
<thead>
<tr>
<th>Incubation time</th>
<th>Saturated</th>
<th>Monounsaturated</th>
<th>Polyunsaturated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intima*</td>
<td>4 hr</td>
<td>58.9 ± 4.1</td>
<td>18.7 ± 1.56</td>
</tr>
<tr>
<td>Foam cells†</td>
<td>4 hr</td>
<td>31.4 ± 3.58</td>
<td>23.0 ± 1.00</td>
</tr>
<tr>
<td>$P$</td>
<td>&lt; .001</td>
<td>&lt; 0.05</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>Intima§</td>
<td>1 hr</td>
<td>55</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>2 hr</td>
<td>57</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>4 hr</td>
<td>65</td>
<td>17</td>
</tr>
</tbody>
</table>

*Mean of 6 experiments with se of mean (3 duplicates + 3 singles).
†Mean of 4 experiments, duplicate batches of cells in each.
Other footnotes as in Table 1.

**TABLE 4**

Percentage Distribution of Synthesized Cholesterol Ester $^{14}$C-Labeled Fatty Acid Between Saturated, Mono- and Polyunsaturated Groups After Incubation of Either Whole Intima or Foam Cells with $^{14}$C-Labeled Acetate

<table>
<thead>
<tr>
<th>Incubation time</th>
<th>Saturated</th>
<th>Monounsaturated</th>
<th>Polyunsaturated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intima*</td>
<td>4 hr</td>
<td>33.7 ± 3.59</td>
<td>37.4 ± 2.64</td>
</tr>
<tr>
<td>Foam cells†</td>
<td>4 hr</td>
<td>22.9 ± 5.29</td>
<td>35.3 ± 1.87</td>
</tr>
<tr>
<td>$P$</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Intima§</td>
<td>1 hr</td>
<td>42</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>2 hr</td>
<td>41</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>4 hr</td>
<td>42</td>
<td>31</td>
</tr>
</tbody>
</table>

*Mean of 6 experiments with se of mean (4 duplicates, 2 singles).
†Mean of 4 experiments, duplicate batches of cells in each.
Other footnotes as in Table 1.
demonstrate the type of fatty acid involved. In the intima as a whole saturated \(^{14}\)C-labeled fatty acids predominate in the phospholipid fraction and monounsaturated \(^{14}\)C-labeled fatty acids predominate in the cholesterol ester fraction. This distribution corresponds reasonably closely to that of the fatty acids that accumulate in these two moieties as demonstrated by analytical chemical studies (24, 25) and is consistent with the conclusion that the fatty acid pattern of these two moieties is determined by their active synthesis in the intima. However, information regarding the rate of formation and of removal of the different fatty acids is necessary before the role of synthesis in the wall can be adequately assessed.

The incorporation of \(^{14}\)C-labeled acetate into the individual phospholipid fatty acids by the intima has also been demonstrated in the present work. Most of the phospholipid labeled is present in the lecithin fraction with about 8.5% in the sphingomyelin fraction. Chemically, however, sphingomyelin comprises approximately one-third of the total phospholipid in rabbit lesions and the incorporation into this moiety is considerably below this level. This is true not only for the incorporation of \(^{14}\)C-labeled acetate but in previous studies in which \(^{32}\)P-labeled phosphate was incubated in vitro with atherosclerotic intima it was shown that the \(^{32}\)P-labeled sphingomyelin comprised only 2% of the labeled phospholipid (unpublished data). Most of the sphingomyelin, however, arises by synthesis in the atherosclerotic lesion (26) so that its rate of removal from the intima must occur at a slower rate than is the case for lecithin, phosphatidyl inositol, and phosphatidyl ethanolamine, all of which appear to be synthesized more rapidly in the present experiments over the period studied than is the case for the sphingomyelin.

The present work was designed mainly, however, to investigate the possible role of the foam cell in bringing about lipid synthesis in the atherosclerotic lesion. Isolated foam cells have been previously shown to incorporate \(^{32}\)P-labeled phosphate into phospholipid and to contribute by such synthesis to the phospholipid accumulation in the atherosclerotic lesion (18). In the present paper it has been demonstrated that these cells are also able to synthesize fatty acid and to incorporate this into both phospholipid and cholesterol ester. Under the conditions of the present experiments, the foam cells divert a higher proportion of the synthesized fatty acid to cholesterol ester than does the intima. This may be explained in part by the finding that the foam cells synthesize fatty acids which are more unsaturated than is the case for the intima, and such fatty acids are more likely to go to cholesterol ester than to phospholipid.

The role of the foam cell in the synthesis of the cholesterol ester in the lesion remains an open question however. The present data provides the first direct evidence for the synthesis of cholesterol ester by foam cells. Thus the foam cell, at least in the rabbit lesion, possesses the metabolic machinery to synthesize fatty acid and to esterify cholesterol with such synthesized fatty acid. The role of these cells in forming at least some of the cholesterol ester in the fatty streak lesion as suggested by the morphological correlative studies of Geer and Guidry (16) and Smith et al. (2, 17) becomes, therefore, a distinct possibility, and some of the synthesis in the intima in the present study doubtless occurs in the foam cells present in the lesion. It will be apparent, however, that no estimate of the quantitative contribution of foam cells to the intimal lipid synthesis can be obtained from this work.

The more polyunsaturated pattern of fatty acids synthesized by the foam cells and the incorporation of these fatty acids into phospholipid and cholesterol ester, however, does not support the concept that these cells are contributing by active synthesis to the more saturated pattern shown for the early atherosclerotic lesion. The intima as a whole synthesizes fatty acids which are predominantly saturated and monounsaturated. Foam cells by contrast incorporate acetate into fatty acids which are predominantly polyunsaturated. In view of this, it seems unlikely that these cells are responsible for cholesterol ester deposition.
in the atherosclerotic lesion, and may in fact indicate a more active role of the cells in removal of cholesterol from the lesion. Polyunsaturated esters of cholesterol are removed from the liver more readily than are saturated cholesterol esters (27), and it has been shown that a shift of cholesterol ester to the more polyunsaturated type in the atherosclerotic lesion is associated with a decrease in severity (28-30). Whether synthesis of polyunsaturated and monounsaturated fatty acids by the foam cell and the resultant incorporation of these fatty acids into cholesterol esters is associated with resolution of the plaque, however, awaits further clarification.

Acknowledgments

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