Synthesis of Phospholipid by Foam Cells Isolated from Rabbit Atherosclerotic Lesions


ABSTRACT

In a preliminary study, foam cells from atherosclerotic lesions have been isolated by incubation of the intima with collagenase and elastase. The composition and metabolism of homogeneous preparations of foam cells obtained in this way have been compared with other parts of the atherosclerotic intima. About 1% of the cholesterol and phospholipid of the intima was present in the cells obtained. The phospholipids of the foam cells contained a lower percentage of lecithin and of sphingomyelin and a higher percentage of "phosphatidyl ethanolamine" than the other parts of the intima. The isolated foam cells incubated in vitro incorporated P32-phosphate into phospholipid at a rate of about 0.5 mmoles/10^6 cells/hr. The P32 was incorporated mainly into lecithin and phosphatidyl inositol with smaller amounts incorporated into phosphatidyl ethanolamine, sphingomyelin and lysolcithin.

The amount of P32 incorporated into phospholipid by the foam cells was compared with that incorporated into phospholipid by other portions of the intima when the aorta was incubated in vitro prior to disruption. Up to 6.8% of the phospholipid synthesis was brought about by isolated cells. The significance of the findings in relation to phospholipid synthesis in the atherosclerotic lesion was discussed.

ADDITIONAL KEY WORDS phospholipid inositol aortic atheroma cholesterol lecithin sphingomyelin phosphatidyl ethanolamine aortic intima

The presence of large numbers of lipid-containing foam cells in the early atherosclerotic lesion has led to the suggestion that these cells contribute in part to the metabolic changes in the early lesion. These cells have been investigated in situ by histochemical and ultramicroscopic methods and such studies have indicated that metabolism and synthesis of lipid by foam cells may be associated with the development of the atherosclerotic lesion. These studies have obvious limitations, however, in investigations of biochemical changes taking place in the cells. The metabolism of macrophages obtained from the peritoneal cavity or lung of rabbits has been studied previously, but there are difficulties in extrapolating the findings to foam cells in the arterial wall. The separation of foam cells from the aorta for metabolic studies in vitro, however, would provide a means of investigating in detail their biochemical properties and of determining what part these cells play in the lipid changes occurring in the early atherosclerotic lesion. Recently Rodbell has developed a technique in which fat cells were isolated from adipose tissue with the aid of collagenase.

In the present paper a similar method has been described for isolating aortic intimal foam cells from rabbit atherosclerotic lesions and some data are presented comparing the lipid composition and phospholipid synthesis...
of such foam cells with that of other fractions of the arterial wall.

Methods

\textbf{ISOLATION OF FOAM CELLS BY ENZYMIC DISRUPTION OF THE AORTIC INTIMA}

New Zealand albino rabbits, fed daily 100 g Purina chow, containing 1 g of cholesterol and 2.8 g of cottonseed oil for periods ranging from 80 to 123 days, were used in these studies. The rabbits were exsanguinated from the abdominal aorta under ether anesthesia and the thoracic aorta was removed, washed with saline, and bathed in normal rabbit serum. The superficial aorta was then removed and the intima stripped for subsequent incubation as set out in the accompanying flow diagram (fig. 1). The intima was incubated with Krebs Ringer phosphate solution (pH 7.4) containing 4% bovine albumin (fatty acid poor, Pentex, Kankakee, Illinois) 10 mg collagenase (Worthington Biochemical Corp., Freehold, New Jersey), 4 mg elastase (Nutritional Biochemicals Corp., Cleveland, Ohio) and 15 μmoles of glucose in a volume of 5 ml. Incubation was done in 20 ml plastic screw top counting vials at 37°C, the digest being shaken gently every 5 min. After 1 hr the partially disrupted intima was teased apart with forceps and incubation allowed to proceed for an additional 1 hr at 37°C. The digest was then filtered through gauze in a 10 ml plastic syringe, the filtrate was washed with 1 to 2 ml of 4% bovine albumin in Krebs Ringer phosphate and the washings expressed from the residue (fraction I, fig. 1) by means of the syringe plunger. The filtrate was centrifuged at 220 g for 3 min and the deposited cells were washed twice with 0.9% sodium chloride solution and recentrifuged. The initial supernatant (fraction II, fig. 1) was reserved for study while the washings were discarded. The washed cells were then suspended in Hanks solution\cite{19} for counting in a hemocytometer chamber and then suspended in Hanks solution containing 0.5% bovine albumin, dispersed into glass containers (Leighton tubes for metabolic studies or stoppered conical flasks for chemical assay) and incubated for 60 min at 37°C. Most of the foam cells adhered to the glass while other cells and particles remained in suspension. The adhering foam cells were washed with warm saline and the cells which remained in suspension or were washed off were counted to determine loss of cells. The combined solution containing the cell washings was then centrifuged (1250 g for 10 min) and the deposit, washed twice with saline, was reserved for subsequent study (fraction IV, fig. 1). All procedures except the final stage in which the cells were stuck to glass vessels were done in plastic containers. Four fractions were obtained by the above procedure, viz. residue (I), supernatant (II), foam cells (III) and washings (IV). These fractions were used for subsequent chemical or isotope assay or for metabolic studies.

The fractions were extracted with 2:1 chloroform methanol as described by Folch et al.\cite{11} and fractionated into phospholipid and neutral lipid on silicic acid columns.\cite{12} Lipid phosphorus was determined by the method of Bartlett\cite{18} and fractionation of individual phospholipids by thin layer chromatography and elution by the method of Skipski et al.\cite{14} Total cholesterol was determined as described by Zlatkis et al.\cite{15} in the neutral lipid fraction following saponification by the method of Abell et al.\cite{19} Free and ester cholesterol were determined by the same method following separation of free from esterified cholesterol on silicic acid columns\cite{17} and subsequent saponification. Purity of column fractions was checked by thin layer chromatography.\cite{18}

\textbf{SYNTHESIS OF PHOSPHOLIPID BY ISOLATED FOAM CELLS}

Two series of metabolic experiments were performed. In the first series the incorporation of \( \text{P}^{32} \)-labeled phosphate into phospholipid by isolated foam cells was investigated. Approximately 1.5 × 10^6 foam cells were dispensed into Leighton tubes, allowed to adhere to the glass, and washed. The cells which did not adhere to the glass were counted. One ml of medium (1:1 v/v Hanks:normal rabbit serum with 0.1 mg/ml of streptomycin and penicillin) containing up to 30 μc of \( \text{P}^{32} \)-labeled phosphate (Abbott Laboratories) was added. Duplicate batches of cells were

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Flow diagram illustrating separation of foam cells from the atherosclerotic aortic intima.}
\end{figure}
incubated for 1/2, 1, 2, 4 or 18 hours after addition of the labeled medium. After the appropriate incubation time the medium was removed and the adhering cells washed twice with 0.9% sodium chloride solution. The medium was centrifuged and the small amount of deposit washed twice with 0.9% sodium chloride solution and extracted together with the adhering cells. The centrifuged medium was also extracted. Aliquots of the Folch-washed extracts were assayed for P32 with the scintillator described by Gordon and Wolfe and for lipid phosphorus.

In the second series of experiments the rabbit atherosclerotic thoracic aorta was incubated in vitro with P32 as described elsewhere. After incubation the aorta was washed with 0.9% sodium chloride solution, the intima removed by stripping and incubated with collagenase and elastase in order to free and isolate the foam cells as set out above. The four fractions were extracted and assayed for P32 and lipid phosphorus. In some of these experiments the supernatant (fraction II) was filtered through a 1.2 μm millipore filter in order to separate the large numbers of particles present. The particles (which varied up to 3 to 4 μ in diameter) were washed twice with 0.9% sodium chloride solution and extracted.

**Identification of Labeled Phospholipid**

Separation of the lipid extract to identify and quantitate the individual phospholipids labeled by the foam cells was done by thin layer chromatography by the method of Skipski et al. In view of the small amount of lipid phosphorus present in the individual extracts in these metabolic experiments, the cell extract was taken up in a lipid extract of aortic intima as carrier. The phospholipid spots were located by spraying with iodine vapor or by the method of Dittmer and Lester and then scraped into counting vials for counting by the method of Snyder. Identification of spots in aortic extracts by staining reactions has been described elsewhere.

P32-labeled phospholipid was identified by comparison of Rf values with standards and by cochromatography using three systems as follows. The labeled spots, located by radioautography, were eluted and taken up with the appropriate standard for rechromatography (i) by the method of Skipski et al., (ii) with silica gel G and the solvent system chloroform:methanol:water (140:50/9 v/v/v) and (iii) in the case of the phosphatidyl inositol and phosphatidyl ethanolamine spots on an alkaline plate prepared as described by Skipski et al., but using the solvent system di-isobutyl ketone:acetic acid:water (40:25:5 v/v/v). Satisfactory separations of phosphatidyl inositol and phosphatidyl serine were attained by this method. Labeled spots were located by radioautography.

In the cochromatography experiments butylated hydroxytoluene was added to all solvents to minimize oxidation of labile compounds. Standards used were phosphatidyl ethanolamine, phosphatidyl serine and sphingomyelin obtained from Applied Science Laboratories (State College, Pennsylvania), lecithin (containing lysophosphatidyl ethanolamine) from Mann Research Laboratories (New York, New York) and phosphatidyl inositol kindly supplied by Dr. Faure (Pasteur Institute, Paris). These standards were checked by staining reactions as described elsewhere.

**Results**

The number of cells obtained from a single intima varied in 18 experiments from 4.0 to 16.8 x 10^6 (mean 7.8 x 10^6 ± 0.68 SEM). Most of these cells stuck to glass, but 10 to 15% of cells did wash off and were counted in the washings. Over the time period studied (80 to 123 days) there was no obvious correlation between the duration of feeding and the yield of foam cells although the lowest figure was in fact from the 80 day rabbit.

The foam cells are shown, in figures 2 and 3, adhered to glass cover slips. All of the cells observed were mononuclear, varying in size from 15 to 50 μ, and morphologically resembling macrophages. They contained a large number of granules which stained positively for lipid with oil red O. In some prep-

**FIGURE 2**

Foam cells isolated from atherosclerotic intima. Viewed unstained adhering to cover slips. Phase contrast.
arations the granules were absent from parts of the cell. In the final cell preparation shown in figures 2 and 3 there were practically no contaminating particles or other cells. Figure 4 is a free suspension of washed cells (Cells (2) in fig. 1) obtained prior to plating in glass vessels. Contaminating cells were present together with relatively large numbers of particles ranging in size up to 3 to 4 μ. These particles resembled morphologically the granules in the foam cells and, in some areas, clusters of them resembling parts of cells can be seen. Cells present in the supernatant (fraction II) were counted in some experiments and accounted for about 10% of the cells present in the foam cell fraction (fraction III). However, the supernatant contained large numbers of particles both singly and in clusters (fig. 5). These are removed by filtration of the supernatant through millipore filters most being removed by a 1.2 μ filter. They are not appreciably sedimented however, at 1800 g for 15 min.

Some cells could be obtained with collagenase or with elastase alone, but better yields were found when both enzymes were used. When serum was used as incubation medium, the yield was lower than with 4% albumin in Krebs Ringer phosphate.

The distribution of lipid P and of cholesterol between the four fractions obtained is shown in table 1. Most of the lipid P and of the cholesterol was present in the supernatant and residue fractions; only about 1% of the total lipid P and cholesterol was found in the isolated foam cells. The cholesterol:lipid P ratios of the various fractions were similar with the possible exception of the residue in which the cholesterol:lipid P ratio was somewhat higher. The percentages of cholesterol ester present in the foam cells and washings were significantly greater (P < 0.01) than the percentages in the supernatant or the residue.
The per cent distribution of individual phospholipids in the four fractions is shown in table 2. In the foam cells and the washings the percentages of the phospholipid, present as "phosphatidyl ethanolamine" and as the fraction which travels at the solvent front, were greater than the percentages in the supernatant and residue. The percentages of sphingomyelin and lecithin present in the cells and washings were lower than those of the supernatant and residue.

The incorporation of phosphate-P into phospholipid by isolated foam cells is shown in table 3. P-phospholipid increased in the cells fairly uniformly over the time period studied so that after 4 hr incubation almost 0.2% and after 18 hr almost 0.7% of the P added to the medium has been incorporated.

**TABLE 1**

<table>
<thead>
<tr>
<th>Distribution of Lipids Between Fractions of Aortic Intima (means of 4 ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foam cells (III)</td>
</tr>
<tr>
<td>Lipid (% of total in whole intima)</td>
</tr>
<tr>
<td>0.92 ± 0.17</td>
</tr>
<tr>
<td>0.86 ± 0.23</td>
</tr>
<tr>
<td>$74.3 ± 0.99</td>
</tr>
<tr>
<td>$61.5 ± 5.06</td>
</tr>
</tbody>
</table>

*Analysis of variance shows significant differences at the 5% level.
†Analysis of variance shows significant differences at the 1% level.
‡mean of 5.
§mean of 6.

**TABLE 2**

<table>
<thead>
<tr>
<th>Per Cent Distribution of Phospholipid Fractions of Aortic Intima (means of 4 for cells and supernatant and of 2 for washings and residue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Origin</td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>Foam cells (III)</td>
</tr>
<tr>
<td>Washings (IV)</td>
</tr>
<tr>
<td>Supernatant (II)</td>
</tr>
<tr>
<td>Residue (I)</td>
</tr>
</tbody>
</table>

*Analysis of variance shows significant differences at the 5% level.
†Analysis of variance shows significant differences at the 1% level.

**TABLE 3**

<table>
<thead>
<tr>
<th>Incorporation of P-labeled Phosphate into Phospholipid by Isolated Foam Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>Exp. 1</td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>Incubation time</td>
</tr>
<tr>
<td>hr</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>18</td>
</tr>
</tbody>
</table>

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into cellular phospholipid. Table 3 shows that the medium contained very little \( ^{32}P \) labeled phospholipid at the earlier time intervals. Amounts less than 0.001% probably represented traces of \( ^{32}P \)-labeled phosphate present in the lipid extracts. However, the amounts above this were phospholipid \( ^{32}P \) which appeared in the medium in significant, although small, amounts relative to those in the cell extract.

The individual phospholipids labeled by the isolated foam cells are shown in figure 6. Five spots were labeled corresponding in RF value to lysolecithin, sphingomyelin, lecithin, phosphatidyl inositol and phosphatidyl ethanolamine. The latter four spots were eluted and cochromatographed with individual standards as shown in figure 7A, B, C. Sphingomyelin, lecithin and phosphatidyl inositol traveled as single spots together with the standard preparations on both the alkaline plate (fig. 7A) and the silica gel G plate (fig. 7B). In order to determine whether phosphatidyl serine contributed to spot 5, this spot was rechromatographed on an alkaline plate (fig. 7C) with the solvent system di-isobutyl ketone: acetic acid: water (40:25:5) mixed with either phosphatidyl serine or phosphatidyl inositol or with both standards. Spot 5 traveled with, and can therefore be tentatively identified as, phosphatidyl inositol. About 4.0% of the label traveled with phosphatidyl serine, however. Spot 6 which ran with phosphatidyl ethanolamine in the Skipski system.

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**FIGURE 6**
Identification of \( ^{32}P \)-labeled phospholipids synthesized by isolated foam cells. Separation of the labeled foam cell extract by thin layer chromatography followed by autoradiography. Alkaline plate with solvent system chloroform: methanol: acetic acid: water (25/15/4/1.9).

**FIGURE 7**
did not rechromatograph as a single spot. In both fig. 7B and 7C this spot contained, in addition to a spot running with the standard phosphatidyl ethanolamine, other unidentified spots.

The percentage distributions of individual P³²-labeled phospholipids for the cell extracts at different times of incubation are given in table 4. Most of the P³² has been incorporated into cellular lecithin, phosphatidyl inositol and the composite spot "phosphatidyl ethanolamine." The distribution was essentially similar throughout the four-hour time period although in the one experiment at 18 hours the sphingomyelin and "phosphatidyl ethanolamine" accounted for a larger amount and the phosphatidyl inositol for a smaller amount of the P³² than at the earlier incubation times.

The P³² incorporated into phospholipid by each of the four fractions of the aortic intima after in vitro incubation of the intact aorta with P³²-phosphate is shown in table 5. In the first three experiments [table 5 (a)] the aorta was incubated with P³² for four hours. In the last three experiments [table 5 (b)] the incubation of the aorta with P³² was limited to two hours. In the four-hour experiments the cells may have deteriorated since many of the separated cells did not adhere to glass and a relatively large amount of lipid-P³² was present in the washings. In both series of experiments the P³²-phospholipid was distributed mainly in the supernatant and residue fractions, the cells accounting for at best 6.8% of the label. The specific activity of the P³²-labeled phospholipid in

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**TABLE 4**

Incorporation of P³² into Individual Phospholipids by Isolated Foam Cells (% distribution at different time intervals)

<table>
<thead>
<tr>
<th>Incubation time</th>
<th>Origin</th>
<th>Lyso-</th>
<th>Sphingo-</th>
<th>Lecithin</th>
<th>Phospha-</th>
<th>&quot;Phosphatidyl ethanolamine&quot;</th>
<th>Solvent front</th>
</tr>
</thead>
<tbody>
<tr>
<td>hr</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>1/2</td>
<td>3.3</td>
<td>1.3</td>
<td>1.5</td>
<td>58.2</td>
<td>24.5</td>
<td>11.4</td>
<td>2.2</td>
</tr>
<tr>
<td>1</td>
<td>0.6</td>
<td>0.4</td>
<td>1.0</td>
<td>60.3</td>
<td>25.7</td>
<td>8.9</td>
<td>3.2</td>
</tr>
<tr>
<td>2</td>
<td>0.4</td>
<td>1.0</td>
<td>0.9</td>
<td>61.0</td>
<td>26.5</td>
<td>7.5</td>
<td>2.9</td>
</tr>
<tr>
<td>4</td>
<td>0.2</td>
<td>0.7</td>
<td>2.3</td>
<td>65.4</td>
<td>20.7</td>
<td>7.5</td>
<td>2.4</td>
</tr>
<tr>
<td>18</td>
<td>0.1</td>
<td>0.5</td>
<td>8.3</td>
<td>54.3</td>
<td>18.4</td>
<td>17.1</td>
<td>3.2</td>
</tr>
</tbody>
</table>

**TABLE 5**

Contribution of Different Fractions of Aortic Intima to Phospholipid Synthesis in Vitro

(a) Incubation of aorta with P³² in vitro for 4 hr followed by separation into fractions. Mean of 3 ± SEM

<table>
<thead>
<tr>
<th>Lipid-P Specific activity</th>
<th>Cells (III)</th>
<th>Washings (IV)</th>
<th>Residue (I)</th>
<th>Supernatant (II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% distribution</td>
<td>2.0 ± 0.72</td>
<td>9.3 ± 0.35</td>
<td>40.4 ± 3.14</td>
<td>48.2 ± 2.18</td>
</tr>
<tr>
<td>Lipid-P</td>
<td>2.19 ± 0.47</td>
<td>10.13 ± 0.78</td>
<td>341 ± 38.2</td>
<td>420 ± 55.3</td>
</tr>
<tr>
<td>Specific activity</td>
<td>2560 ± 147</td>
<td>2760 ± 67.7</td>
<td>360 ± 9.14</td>
<td>350 ± 24.2</td>
</tr>
</tbody>
</table>

(b) Incubation of aorta with P³² in vitro for 2 hr followed by separation. Mean of 3 ± SEM

<table>
<thead>
<tr>
<th>Lipid-P Specific activity</th>
<th>Cells (III)</th>
<th>Washings (IV)</th>
<th>Residue (I)</th>
<th>Total (II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% distribution</td>
<td>6.8 ± 1.45</td>
<td>6.8 ± 2.09</td>
<td>40.1 ± 1.50</td>
<td>46.3 ± 0.81</td>
</tr>
<tr>
<td>Lipid-P</td>
<td>5.70 ± 1.87</td>
<td>4.85 ± 1.18</td>
<td>252 ± 96.0</td>
<td>294 ± 93.5</td>
</tr>
<tr>
<td>Specific activity</td>
<td>2280 ± 62.0</td>
<td>2460 ± 170</td>
<td>330 ± 50.8</td>
<td>315 ± 53.2</td>
</tr>
</tbody>
</table>

*34.8 X 10⁶ cpm P³²-phosphate added.
†59.0 X 10⁶ cpm P³²-phosphate added.
PHOSPHOLIPID SYNTHESIS BY FOAM CELLS

the cells, however, was six to seven times that in the supernatant and residue. In the three experiments shown in table 5 (b) the distribution of P32 in, and the specific activity of, the particles of the supernatant were also determined. This fraction accounted for 37% of the P32 in the supernatant with a specific activity almost twice that of the supernatant and residue but considerably less than that of the cells.

Discussion

The cells obtained were free of contaminating cells and represented a microscopically homogeneous preparation of lipid-containing foam cells. It is known, that in the rabbit atherosclerotic lesion some of the lipid-containing cells can be identified ultramicroscopically as smooth muscle cells.25 It is possible that some of the cells obtained by the method described may be smooth muscle cells, but morphologically the preparation consisted of rounded mononuclear cells, indistinguishable from macrophages. Further, while the ability to adhere to a glass surface is by no means specific, this property is one well developed in macrophages from other situations and used for their separation from other cell types.28, 27

The lipid content of foam cells was high compared with that of macrophages obtained from the peritoneal cavity of normal rabbits. It can be calculated from the data reported in table 1, knowing the cell count in each preparation, that the total cholesterol content amounted to 72 µg/10⁶ cells, and the lipid phosphorus to 1.1 µg/10⁶ cells. This compared with about 1 µg/10⁶ cells for cholesterol (Day and Fidge, unpublished data) and 0.7 µg/10⁶ cells for lipid phosphorus for peritoneal cavity macrophages derived from normal rabbits.7

The cells obtained were metabolically active as indicated by their ready incorporation of P32-phosphate into phospholipid and can therefore be used to investigate the part played by the foam cell in the development of the early lesion. The incorporation of P32-phosphate into phospholipid was high when compared with that of peritoneal cavity macrophages. It can be calculated that foam cells incubated with P32-phosphate incorporated into phospholipid approximately 0.5 µmoles of the medium phosphate per hr/10⁶ cells. Peritoneal macrophages from normal rabbits incorporated approximately 0.05 µmoles/hr/10⁶ cells which is increased 44% in the presence of a 0.06% cholesterol suspension.7

A further comparison with peritoneal macrophages can be made with respect to the individual phospholipids labeled following incubation. Lecithin represented the major phospholipid labeled in both cell types, but in the case of the foam cells, considerably more phosphatidyl inositol and less sphingomyelin were labeled than was the case with the peritoneal macrophages incubated in the presence or absence of cholesterol.7

The amount of phospholipid present in the foam cells obtained was only about 1% of that in the intima as a whole. If some disruption of cells was occurring, it is possible that the amount of phospholipid present in the foam cells in the intima may account for more than this. However, even if disruption of cells had occurred during preparation, it seems unlikely that the foam cells could contain intracellularly more than a small amount of the phospholipid present in the atherosclerotic intima. In the metabolic experiments for example, if one considers that all the P32-phospholipid present in the supernatant arose from disrupted cells, and this cannot of course be asserted on the data, it can be calculated that the actively metabolizing foam cells could account for 10 to 20% of the phospholipid in the intima. This is in contrast to histochemical data presented previously,1 in which it was shown that much of the phospholipid present in the rabbit atherosclerotic aorta was present intracellularly in foam cells. The data, therefore, with regard to phospholipid distribution in the aorta would support the conclusion of Adams et al.28 who have reported histochemical work demonstrating that phospholipid is distributed predominantly extracellularly in the rabbit lesion.

It is possible that foam cells contribute to the phospholipid accumulation in the atherom-
Phospholipid synthesized by such active metabolic cells as have been obtained in the present experiments may be transferred to the extracellular space. However, very little P\textsuperscript{32}P-labeled phospholipid appeared in the medium in the in vitro experiments, but conditions in the intima may differ from those in the medium. The second possibility is that foam cells degenerate and become necrotic following their active metabolic phase thus leading to the accumulation of extracellular phospholipid. On the other hand, degenerated cells may contain much phospholipid, demonstrable histochemically, but fall apart when the aorta is disrupted with collagenase, etc.

The incorporation of P\textsuperscript{32}P-phosphate into individual phospholipids by the isolated foam cells was almost identical with the incorporation of P\textsuperscript{32}P\textsuperscript{32}P into individual phospholipids by the intact aortic intima.\textsuperscript{20} This could be because the foam cells were responsible for most of the incorporation of P\textsuperscript{32}P into phospholipid that occurs in the aorta, but this conclusion cannot be supported on the basis of the data in table 5, unless one considers that the yield of cells obtained in these experiments was relatively small.

In the present paper it can be concluded that phospholipid can be synthesized by the foam cells present in the atheromatous lesion. The relative contribution to the synthesis of phospholipid by the intima as a whole cannot be assessed satisfactorily, however, until the questions of yield of cells, possible disruption during preparation, and the synthesis of phospholipid by other components of the intima are determined.

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References
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