Uptake and Metabolism of ^{14}C-Labeled Oleic Acid by Atherosclerotic Lesions in Rabbit Aorta

A BIOCHEMICAL AND RADIOAUTOGRAPHIC STUDY

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ABSTRACT

The uptake of ^{14}C-labeled oleic acid and its incorporation into combined lipids by aortic intimas from normal and cholesterol-fed rabbits has been investigated in vitro. More than five times as much oleic acid was taken up by the atherosclerotic intima as by the normal intima. About twice as much oleic acid was incorporated into phospholipid, and twenty times as much into cholesterol ester by the atherosclerotic intima as by the normal. Lecithin was the major phospholipid synthesized from oleic acid in both normal and atherosclerotic intimas.

Radioautographs of the atherosclerotic vessels show that the ^{14}C-labeled oleic acid and its metabolic derivatives, principally phospholipid and cholesterol ester, were localized in sudanophilic cells in the intima in both early and advanced lesions. It is concluded that intimal foam cells are primarily responsible for the lipid synthesis that occurs in the atherosclerotic lesion.

ADDITIONAL KEY WORDS fatty acids atheroma foam cells lipid metabolism arterial wall metabolism radioautography phospholipid cholesterol ester

■ In numerous in-vivo and in-vitro studies, lipid precursors have been taken up by the atherosclerotic arterial wall and incorporated into the lipids of the atherosclerotic lesion (1-6). Such studies have indicated that the phospholipid accumulates in the lesion as a result of synthesis in situ (7, 8). Recent evidence indicates that synthesis and hydrolysis of cholesterol ester may also take place in the arterial lesion (9-11). The diversion of fatty acid synthesized from ^{14}C-labeled acetate to cholesterol ester in atherosclerotic lesions in the pigeon (12) and the rabbit (13) has also emphasized the potential role of the arterial wall in the esterification of cholesterol in the atherosclerotic lesion.

In view of the evidence implicating atherosclerotic intimal metabolism in the accumulation of lipid, the question arises as to which portions of the lesion are involved. Geer and Guidry (14) and Smith (15) have demonstrated that the human atherosclerotic lesions which contain large numbers of foam cells have a cholesterol ester fatty acid pattern which differs markedly from that of the serum, being high in oleic acid relative to linoleic acid. On this basis these workers suggest that foam cells synthesize cholesterol ester in the lesion. Day and Wilkinson (13) have shown that isolated foam cells will take up ^{14}C-labeled acetate and incorporate the fatty acid synthesized into both cholesterol ester and phospholipid. Foam cells isolated from rabbit atherosclerotic lesions have also been shown to incorporate ^{32}P-labeled phosphate into phospholipid and to be responsible in part for the phospholipid synthesis in the atherosclerotic intima (16). Parker et al. (17), in a combined biochemical-morphological study, have shown that rabbit atherosclerotic lesions take up and incorporate

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linoleic acid into phospholipid and that the magnitude of such incorporation is related to the increasing number of myointimal cell membranous organelles present in the developing lesion. All of these studies indicate that foam cells may be responsible in part for the lipid synthesis which takes place in the atherosclerotic lesion, but direct evidence implicating these cells is not available.

In the present paper, the in-vitro incubation of atherosclerotic intimas with ^{14}C-labeled oleic acid has been compared with that of normal intimas. The cellular localization of the uptake and incorporation of ^{14}C-labeled oleic acid into phospholipid and cholesterol ester has then been investigated directly by radioautographic techniques to determine which portions of the atherosclerotic arterial lesion are responsible for the lipid synthesis.

Materials and Methods

Oleic acid 1-^{14}C, specific activity 32.5, 43.2, or 57.2 mc/mM (Radiochemical Centre, Amersham, England), was dissolved in 0.05N sodium hydroxide and taken up either in an excess of bovine albumin (Armour) or normal rabbit serum. Radiochemical purity was verified by thin-layer chromatography and by gas liquid radiochromatography as described below.

Atherosclerotic aortas were obtained from male New Zealand white rabbits fed 1 g cholesterol and 3 ml of peanut oil in 100 g rabbit chow daily for 4 months. Normal aortas were obtained from rabbits of the same stock fed a normal rabbit chow diet for the same period.

In the first series of experiments, the rabbits were killed by ether anesthesia. The thoracic aorta was removed and divided longitudinally into halves, and each half was incubated separately in 5 ml of medium (50 : 50 Hank's solution : normal rabbit serum) containing a known amount of sodium 1-^{14}C-oleate : albumin. After incubation for 4 hours at 37°C in an atmosphere of air, the aortic halves were washed in saline and the atherosclerotic or normal intimas stripped from the media and adventitia and extracted with chloroform : methanol (2 : 1 v/v) according to the method of Folch et al. (18). In some experiments the atherosclerotic thoracic aorta was longitudinally divided into thirds, each third being incubated for 1, 2, or 4 hours.

To compare directly the incorporation of ^{14}C-labeled oleic acid into cholesterol ester, phospholipid, and triglyceride, paired experiments were set up. Whole aortas obtained as above from either normal or cholesterol-fed rabbits were incubated together for 4 hours at 37°C in 10 ml of medium containing 50 : 50 Hank's : normal rabbit serum to which had been added a known amount (8.2 μc) of ^{14}C-labeled sodium oleate. No additional albumin was added; the excess serum albumin present was used to bind the tracer amount of ^{14}C-labeled sodium oleate added. The unesterified fatty acid content of the serum used in the medium was determined by the method of Dole (19), and its oleic acid proportion was measured by gas liquid chromatography following separation by thin-layer chromatography as described below. These determinations were used to calculate the amount of oleic acid incorporated into cholesterol ester, phospholipid, and triglyceride by the normal and atherosclerotic intimas in this second series of experiments. Following incubation, the intima was separated from the media and adventitia and extracted as for the first series. The dry defatted weight of the intima was recorded.

In the radioautographic studies, the atherosclerotic aortic halves were incubated for 3 hours in the radioactive medium and then for a further hour in nonradioactive medium. The artery was washed in saline and finally in a solution of unlabeled sodium oleate in 15% albumin (5 mg oleate/ml 15% albumin solution) for 1 hour at 4°C. Both halves were fixed in 1% calcium chloride and 4% formaldehyde in physiological saline for 4 days. The intima from one aortic half was then stripped from the remaining media and adventitia and extracted with chloroform : methanol according to Folch et al. (18), in order to determine the uptake and incorporation of the ^{14}C-labeled oleic acid. Sections (6μ) were cut from the remaining half without prior embedding, using an International Cryostat Model CTI, and radioautographs were prepared with Kodak AR10 Stripping Film. After exposure the radioautographs were developed and stained through the film with Sudan IV and hematoxylin.

THIN-LAYER CHROMATOGRAPHY

After counting the total lipid ^{14}C, the lipid extracts from the intimas, normal or atherosclerotic, were separated into phospholipid, cholesterol/diglyceride, unesterified fatty acid, triglyceride, and cholesterol ester by thin-layer chromatography on Silica Gel G (Merck) using diethyl ether : acetic acid : n-hexane (38 : 3 : 100 v/v/v) as the developing solvent. The distribution of ^{14}C-labeled oleic acid in the individual phospholipids of the intimas was also determined by thin-layer chromatography, using the method of Skipski et al. (20). For this purpose the total phospholipid moiety was first separated by thin-layer chromatography as above. Identification of

phospholipids was made by comparison of Rf values with standards as described previously (21). Following separation of the lipid extracts into the various lipid components or the individual phospholipids, the proportion of the ^{14}C-label in each moiety was determined by scraping the plates and counting directly, using the method of Snyder (22). All counting was done with a Packard Tricarb Spectrometer.

GAS LIQUID RADIOCHROMATOGRAPHY

To determine and compare the specific activity of the oleic acid incorporated into cholesterol ester and phospholipid in the normal and atherosclerotic intimas, the respective fractions were separated by thin-layer chromatography as above, and the fatty acid present in each was converted to methyl esters by heating at 65°C with 5% sulphuric acid in methanol in sealed ampoules. The labeled methyl esters were extracted with petroleum ether and then run on diethylene glycol succinate columns at 185°C in an F & M Model 5750 Gas Chromatograph, using argon as the carrier gas. The gas stream was split, and fractions of the eluant were combusted to $^{14}CO_2$ and its ^{14}C content was monitored with a Pye Radiochromatography unit. All of the ^{14}C-labeled fatty acid in the cholesterol ester and phospholipid moieties was present as ^{14}C-labeled oleate, and its specific activity was determined by comparison with ^{14}C-labeled methyl palmitate of known specific activity. The specific activity of the ^{14}C-labeled oleic acid present in the cholesterol ester and phospholipid moieties of both the normal and atherosclerotic intimas was expressed as counts/min ^{14}C/mμmole oleic acid.

Results

The uptake of oleic acid after incubation for 4 hours in a medium containing ^{14}C-labeled sodium oleate is shown for the first

TABLE 1

Uptake of ^{14}C-Labeled Oleic Acid and Its Incorporation into Combined Lipid by Normal and Atherosclerotic Intimas

	Incubation time	^{14}C Present initially in incubation medium (counts/min × 10^6)	% Uptake	% ^{14}C Initially present incorporated into lipid
Normal*	4 hr	6.5	0.830 ± 0.126	0.702 ± 0.115
Atherosclerotic†	4 hr	5.83 ± 1.00	4.48 ± 0.80	3.51 ± 0.60
Atherosclerotic‡	1 hr	1.19	0.77	0.46
		6.1	1.2	0.71
	2 hr	1.19	1.39	1.09
		6.1	2.3	1.67
	4 hr	1.19	2.05	1.78
		6.1	4.2	3.42

*Mean of four experiments (duplicate aortic halves) with SE of mean; †mean of six experiments (five with duplicate aortic halves) with SE of mean; ‡two experiments as shown.

TABLE 2

Percentage Distribution of ^{14}C-Labeled Oleic Acid Among Lipid Fractions After Incubation with Normal or Atherosclerotic Intimas

	Incubation time	Phospholipid	Diglyceride	Fatty acid	Triglyceride	Cholesterol ester
Normal*	4 hr	48.1 ± 5.0	8.3 ± 1.7	15.8 ± 1.4	25.5 ± 3.4	2.4 ± 0.6
Atherosclerotic†	4 hr	32.2 ± 1.2	2.8 ± 0.3	21.4 ± 2.4	14.2 ± 2.3	29.4 ± 3.3
Atherosclerotic‡	1 hr	28.2	3.7	40.8	5.9	21.5
	2 hr	37.8	3.1	24.6	5.7	29.1
	4 hr	35.3	2.3	16.1	11.9	34.7

*Mean of four experiments (duplicate aortic halves) with SE of mean; †mean of six experiments (five with duplicate aortic halves) with SE of mean; ‡mean of two experiments (both with single aortic thirds).

series of experiments in Table 1. In the normal intima, a mean of 0.830% of the oleic acid was taken up by the intima and most of this (0.702%) was incorporated into combined lipids. In the atherosclerotic intima more than five times as much ^{14}C-labeled oleic acid (4.48%) was taken up, and again most of this (3.51%) was incorporated into other lipids in the intima. The uptake and incorporation of the ^{14}C-labeled oleic acid into lipid was approximately linear over the 4-hour period (Table 1).

The percentage distribution of the ^{14}C-labeled oleic acid among phospholipid, diglyceride, free fatty acid, triglyceride, and cholesterol ester is shown for the first series in Table 2. In the normal artery very little cholesterol ester (2.4%) was labeled; most of the oleic acid taken up was incorporated into phospholipid, and to a lesser extent into triglyceride. In the atherosclerotic artery, however, a mean of 29.4% of the ^{14}C-labeled fatty acid was incorporated into the cholesterol ester fraction. The pattern of incorporation of oleic acid into cholesterol ester and phospholipid is similar at 4 hours to that at the earlier intervals studied. There is, however, relatively more unesterified oleic acid present at the earlier time intervals.

Table 3 shows the distribution of the label between the individual phospholipids for both the normal and atherosclerotic intimas. In both groups the major phospholipid labeled is lecithin. No significant differences are apparent in the distribution of ^{14}C-labeled fatty acid in the phospholipid moieties between the normal and the atherosclerotic intimas. Distribution of the label was similar over the period studied.

In the second series of experiments, normal and atherosclerotic aortas were incubated together in the same medium to compare directly the incorporation of ^{14}C-labeled oleic acid into phospholipid, triglyceride, and cholesterol ester. The percentage uptake by the normal or atherosclerotic intima of ^{14}C-labeled oleic acid present in the medium, together with the distribution of this oleic acid between the various lipid moieties, is given

TABLE 3

Percentage Distribution of ^{14}C-Labeled Fatty Acid in Individual Phospholipids After Incubation with Normal or Atherosclerotic Intimas

	Incubation time	Origin	Lyso-lecithin	Sphingo-myelin	Lecithin	Phosphatidyl inositol	Phosphatidyl ethanolamine	Solvent front
Normal*	4 hr	0.9 ± 0.1	0.7 ± 0.1	1.4 ± 0.1	66.1 ± 1.1	9.5 ± 2.7	15.7 ± 0.8	6.2 ± 1.1
Atherosclerotic†	4 hr	0.2 ± 0.0	0.4 ± 0.1	0.9 ± 0.1	65.5 ± 1.2	11.9 ± 1.0	14.5 ± 0.9	7.1 ± 1.2
Atherosclerotic‡	1 hr	0.1	0.1	1.6	66.1	11.2	15.9	5.2
	2 hr	0.1	0.1	0.7	66.9	11.4	18.6	2.3
	4 hr	0.2	0.1	1.0	67.6	9.6	18.6	3.2

*Mean of four experiments (duplicate aortic halves) with SE of mean; †mean of five experiments (duplicate aortic halves) with SE of mean; ‡mean of two experiments (single aortic thirds).

TABLE 4

Percentage Distribution of ^{14}C-Labeled Oleic Acid Among Lipid Fractions After Incubation with Normal and Atherosclerotic Intimas

	% Uptake of oleic acid	Phospholipid	Diglyceride	Fatty acid	Triglyceride	Cholesterol ester
Normal	0.756	49.3	4.2	24.5	15.2	7.2
Atherosclerotic	4.076	30.1	1.9	21.1	9.6	37.5

Data are means of three paired experiments.

TABLE 5

Amount of Oleic Acid Incorporated into and Specific Activities of Oleic Acid as Combined Lipids for Normal and Atherosclerotic Intimas Incubated in Vitro

Exp. no.		Dry defatted weight (mg)	mμmoles oleic acid incorporated/g dry defatted intima			Specific activity (counts/min/mμmole oleic acid)	
			Phospholipid	Triglyceride	Cholesterol ester	Phospholipid	Cholesterol ester
1*	Normal	79.6	58.6	12.6	12.0	68.9	47.2
	Atherosclerotic	94.5	335.2	102.1	424.5	57.9	11.7
2†	Normal	72.2	116.8	44.2	3.0	92.6	64.6
	Atherosclerotic	102.2	281.4	118.1	339.1	75.2	11.9
3	Normal	54.8	156.8	52.0	30.8	153.0	87.3
	Atherosclerotic	80.8	152.1	34.8	192.5	42.3	11.5

*Four-hour incubations carried out in pairs; normal and atherosclerotic intimas incubated in same medium.

†All incubations had 12.7×10^6 counts/min oleic acid added. There was a total of 1.94 μEq. oleic acid present in 10 ml of medium.

for this series of experiments in Table 4. As for the first series of experiments, a higher proportion of the label was incorporated into cholesterol ester and less into phospholipid and triglyceride in the atherosclerotic intima than in the normal. The distribution of the label between the various moieties has been used to calculate the amount of oleic acid incorporated into phospholipid, triglyceride, and cholesterol ester by the normal and atherosclerotic intima (Table 5). The dry defatted weight of the normal intima was similar to that of the atherosclerotic intima, but the amount of oleic acid incorporated into all of the lipid moieties was considerably greater in the atherosclerotic than in the normal. The amount of oleic acid incorporated into phospholipid and triglyceride was about twice that in the normal. The amount of oleic acid incorporated into cholesterol ester was increased some twenty times. Most of the increased incorporation of oleic acid into lipid in the atherosclerotic intima is therefore accounted for by its increased incorporation into cholesterol ester.

Also in Table 5, the specific activities of oleic acid in phospholipid and cholesterol ester of the normal and atherosclerotic intimas are shown. These are expressed as counts/min/mμmole oleic acid present. Biologically occurring phospholipids have differing fatty acids in the α and β positions so that it can be assumed that only one fatty acid position of the lecithin (the major phospholipid labeled) would be occupied by oleic acid. The specific activities of the cholesterol ester and phospholipid, as expressed in Table 5, are therefore directly comparable on a molar basis. For phospholipid, the specific activity is somewhat less, but of the same order, in the atherosclerotic intima than in the normal. The specific activity of the cholesterol ester fatty acid in the atherosclerotic intima is about one fifth that in the normal intima.

FIGURE 1

Radioautograph of an early atherosclerotic lesion consisting of several closely related foam cells. Granulation representing ^{14}C-labeled oleic acid and its metabolic derivatives is confined to foam cell areas. Aorta from rabbit aged 21 weeks and cholesterol-fed for 15 weeks. Hematoxylin and Sudan IV. Exposure time 7 days.

The percentage distribution of ^{14}C-labeled oleic acid between the individual lipid fractions of the atherosclerotic intimas used for radioautography was determined in the half reserved for analysis. In these experiments only 12.2% of ^{14}C-labeled unesterified fatty acid was still present, most of the label being incorporated into the cholesterol ester and phospholipid as for the above experiments.

RADIOAUTOGRAPHY

The localization of ^{14}C-labeled lipid by the atherosclerotic artery is shown in Figures 1 to 3. No significant film background granulation was present in any of the radioautographs. Early sudanophilic cellular lesions with associated fragmentation of the internal elastic lamina are shown in Figure 1. Granulation, representing essentially ^{14}C-labeled oleic acid and the cholesterol ester and phospholipid formed from it, overlies the foam cell areas. There is little label in intervening relatively normal intima, and little in underlying media. The higher magnification of a similar lesion in Figure 2 confirms localization of ^{14}C to intimal foam cells.

A more advanced lesion with grossly thickened intima is shown in Figure 3. Localization of ^{14}C to the foam cells scattered

FIGURE 2

Early atherosclerotic lesion from the same rabbit as in Figure 1, viewed under oil immersion, confirming the relationship of radioautograph granules to foam cells. Hematoxylin and Sudan IV. Exposure time 8 days.

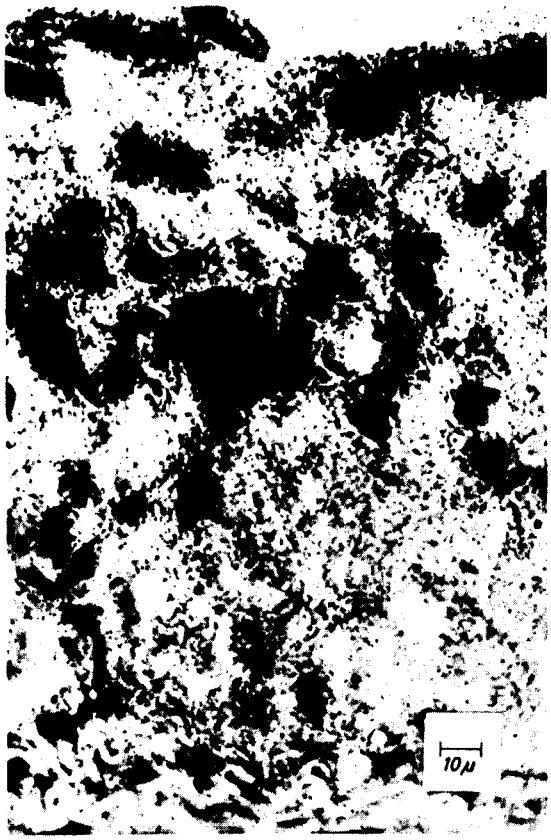

FIGURE 3

Radioautograph of an advanced atherosclerotic lesion with much extracellular lipid. Uptake and metabolism of ^{14}C-labeled oleic acid has occurred in the foam cells. Aorta from rabbit aged 21 weeks and cholesterol-fed for 15 weeks. Hematoxylin and Sudan IV. Exposure time 38 days.

throughout the intima is apparent, and there is little evidence of label in other areas of the thickened intima or in the adjacent media. The foam cells labeled are mainly in the more superficial portion of the intima with little localization at the intimo-medial junction.

Discussion

It has been shown that oleic acid is taken up by both normal and atherosclerotic rabbit intimas and incorporated into various lipids. In the normal intima, fatty acid was incorporated mainly into phospholipid and triglyceride as reported previously by Stein et al. (2, 3). The diversion of oleic acid to cholesterol ester in the atherosclerotic intima, however, is in marked contrast to events in the normal. Whether such incorporation indicates hydrolysis and reesterification of cholesterol ester by the intima, however, cannot be asserted. It is possible that enzymic exchange of ^{14}C-labeled oleic acid for the fatty acids of the cholesterol ester present in the wall is being brought about. Whatever the mechanism, however, the process of rearrangement of the fatty acid pattern of the cholesterol ester is clearly occurring more in the atherosclerotic vessel than in the normal.

The incorporation of ^{14}C-labeled oleic acid into cholesterol ester in the atherosclerotic intima was approximately linear over the 4-hour incubation period studied. If the normal intima behaved in the same way with respect to time, the relative fractional turnover of the phospholipid and cholesterol ester fatty acid pools in the intima can be estimated. The specific activity of the phospholipid fatty acid after incubation for 4 hours was roughly of the same order in the atherosclerotic as that in the normal. The small cholesterol ester fatty acid pool in the normal intima had a specific activity considerably higher than that of the atherosclerotic intima, so that although the total amount of fatty acid incorporated per unit time into cholesterol ester in the atherosclerotic intima was many times that in the normal, the fractional turnover rate was much less.

The pathway for incorporation of fatty acid into cholesterol ester in the atherosclerotic intima is not known. The data presented here may indicate either direct esterification of endogenous cholesterol with fatty acid entering or enzymic exchange with rearrangement of the large cholesterol ester fatty acid pool in the intima. One other possible mechanism may be the acyl transferase mechanism of Glomset (23) which accounts for cholesterol esterification in the serum. In the present experiments the ^{14}C-labeled oleic acid is incorporated predominantly into lecithin, and the transferase mechanism is therefore a possibility. The fact that phospholipid fatty acid has a higher specific activity relative to the

cholesterol ester fatty acid indicates that this transferase mechanism of cholesterol ester formation cannot be excluded in either the normal or atherosclerotic intima. The specific activity of phospholipid relative to cholesterol ester is considerably higher in the atherosclerotic intima, however. Whether this can be interpreted to indicate transferase enzyme activity or simply a reflection of an inert cholesterol ester pool cannot be asserted without further evidence.

The incorporation of ^{14}C-labeled oleic acid into individual phospholipids is similar in the normal and atherosclerotic artery. This is in contrast to previous in-vitro studies (21) in which it has been shown that after 4 hours of incubation in vitro ^{32}P-labeled phosphate is incorporated predominantly into phosphatidyl inositol in the normal artery and into lecithin in the atherosclerotic artery. In the present experiments, oleic acid is incorporated predominantly into lecithin by both normal and atherosclerotic intimas. In the normal artery, fatty acid may be incorporated into phospholipid by the Lands pathway, lysolecithin serving as a fatty acid acceptor (24, 25). It is therefore likely that some, possibly most, of the fatty acid in the present experiments is taken up and incorporated directly into phospholipid via the lysolecithin pathway. In this case, the major phospholipid labeled would be lecithin as observed. For atherosclerotic arteries, however, more information is required before the importance of this pathway can be determined.

In the arteries examined by radioautography, the possibility that some of the radiographically demonstrable material was not lipid was excluded by determining, in some of the experiments, the ^{14}C present in both the Folch wash and the tissue residue following lipid extraction of the ^{14}C-labeled intima. More than 97% of the ^{14}C present in the intima was recovered in the lipid extract. Phospholipid and cholesterol ester present in approximately equal amounts together accounted for over 70% of this lipid ^{14}C present in the intima. Despite the high amount of combined oleic acid in the intima as a whole, however, the presence of up to 30% free oleic acid in some intimas presented the possibility that the ^{14}C localized over foam cells may represent predominantly uptake of oleic acid and have no implications with respect to localization of synthesis of phospholipid and cholesterol ester from this oleic acid. This possibilty can be dismissed on experimental evidence. It has been shown (Day and Tume, unpublished observations) that isolated foam cells incubated in vitro take up and incorporate oleic acid into both phospholipid and cholesterol ester, little free fatty acid remaining unchanged. Further, it has been shown that foam cells isolated from atherosclerotic intimas incubated in vitro with ^{14}C-labeled oleic acid, as for the present experiments, contain between 85% and 90% of their ^{14}C-labeled lipid as cholesterol ester and phospholipid, about 1% only being present as free fatty acid (Day, unpublished observations). Further, in the latter experiments, the specific activity of oleic acid in both cholesterol ester and phospholipid in the isolated foam cells was seven to eight times that in the original intima, so that the ^{14}C-labeled cholesterol ester and phospholipid present in the foam cells are not likely to be synthesized elsewhere and transferred preformed to the foam cell.

In the present experiments, oleic acid was used as a precursor for cholesterol ester and phospholipid synthesis so that radioautographic examination could be meaningful, that is, with no nonlipid ^{14}C and little ^{14}C lipid precursor present. However, it has been shown previously that ^{14}C-labeled acetate is incorporated into fatty acids by both atherosclerotic rabbit intima and by isolated foam cells (13), so that the provision of fatty acid, by synthesis in the foam cell, for incorporation into cholesterol ester and phospholipid presents no problem.

When the above information is considered with the radioautographic evidence of localization of ^{14}C by the intimal foam cell, it can be reasonably concluded that, in the

atherosclerotic lesion, most of the incorporation of fatty acid into phospholipid and cholesterol ester takes place in the foam cells present. This possibility has been suggested by indirect evidence in human lesions (14, 15) and by studies using isolated rabbit foam cells (13, 16), but the present data provides direct evidence for this possibility.

The lack of localization of the ^{14}C in the medial cells is striking. Since aortas were incubated suspended freely in the incubation medium and some areas of media covered only by a thin layer of normal intima, this difference is presumed not to be accounted for by reduced access of labeled oleic acid to the medial cells. Clearly uptake and synthesis of lipid is a particular property of the intimal foam cells so that if these arise from smooth muscle cells (26, 27), the metabolism of the differentiated cells in the media is quite different from those synthesizing lipid in the intima. Further, there is little ^{14}C localized at the intimo-medial junction. There is evidence that cells in this area are predominantly smooth muscle cells, and again little synthesis can be attributed to them under the circumstances of the present experiments. However, identification of the more typical foam cells in the lesion is not possible at the light microscope level so that the foam cells which take up the label in the more superficial portions of the intima may represent either smooth muscle or macrophage foam cells, or both.

Although localization of ^{14}C-labeled oleic acid is related to its uptake and conversion to, principally, phospholipid and cholesterol ester by intimal foam cells, there may be other factors which encourage its retention within the cells. The low rate of hydrolysis of cholesterol oleate relative to other cholesterol esters shown by atherosclerotic intima (11) and the slow catabolism of unsaturated fatty acids by this tissue (28) could both be foam cell phenomena and partly explain the localization of label.

Foam cells synthesize polyunsaturated fatty acids from ^{14}C-labeled acetate (13) which may facilitate removal of cholesterol esters formed from such fatty acids more than removal of the cholesterol oleate investigated in the present work. However, the role of foam cells in this and other atherosclerotic processes, and their relevance in terms of accumulation and removal of lipid in the atherosclerotic lesion must await further experimental assessment.

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