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Phospholipid Synthesis by Foam Cells in Human Atheroma[1]

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In a previous paper (Day and Wahlqvist, 1969) it has been shown by autoradiography that the phospholipid synthesis which occurs in the rabbit atherosclerotic intima incubated *in vitro* takes place predominantly in the foam cells present. In the present paper these studies have been extended to the human atherosclerotic lesion and the localization of phospholipid synthesis by the foam cells in the intima has been investigated by autoradiography using ^{14}C-labeled choline and ^{3}H-labeled oleic acid as precursors.

MATERIALS AND METHODS

Choline chloride (methyl-^{14}C) of specific activity 32 mCi/m*M* and oleic acid-9-10-^{3}H of specific activity 3020 mCi/m*M* were obtained from the Radiochemical Centre, Amersham, U.K., and the purity of each isotope checked as described previously (Day and Wahlqvist, 1969).

The abdominal aorta used was obtained from a male renal transplant donor, aged 20 years, who died of head injuries. Fatty-streak lesions (WHO Grade I, American Heart Association Grading Committee (McGill *et al.*, 1968) Grade 2) were present. The femoral artery was obtained at the time of midthigh amputation, from a 74-year-old female with peripheral vascular disease; the atherosclerotic lesion was complicated by an organized thrombus with a further superadded foam cell lesion (WHO Grade III, American Heart Association Grading Committee Grade 5–6).

A portion of the abdominal aorta was incubated in 5 ml 50:50 Hanks': normal human serum containing 1.62×10^6 cpm of ^{14}C-labeled choline, the femoral artery in 10 ml of the same medium containing 3.20×10^6 cpm ^{14}C-labeled choline. Following incubation at 37°C for 2 hours, the arterial specimen was washed thoroughly in saline and then fixed in 1% calcium chloride in 4% formal saline for 4 days. This was followed by a further wash in running water for 24 hours. A representative piece of tissue was then reserved for autoradiography. The remaining tissue was used for radioassay, lesion being dissected from normal and intima stripped from media. Lipids were extracted with chloroform:methanol 2:1 by the method of Folch *et al.* (1957). The amount of nonlipid ^{14}C-labeled choline was assessed by counting the upper phase of the Folch wash, together with the residue as described previously (Day and Wahlqvist, 1969).

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TABLE I
PERCENTAGE DISTRIBUTION OF LIPID ^{14}C-CHOLINE IN HUMAN ARTERIES

Vessel	Lesion type	% Conversion of medium ^{14}C-choline to phospholipid	Lysolecithin	Sphingomyelin	Lecithin	Other lipids
		Intima				
Abdominal aorta	Normal	2.30	4.0	8.4	82.1	6.0
	Fatty streak	0.10	8.2	10.1	75.1	6.8
Femoral	Normal	0.17	3.0	2.3	92.4	2.5
	Complicated (organized thrombus)	1.69	3.0	4.6	91.4	1.1
		Media				
Abdominal aorta		13.1	8.9	4.7	82.1	4.5
Femoral	Normal	0.16	2.7	1.8	94.3	1.2
	Complicated (organized thrombus)	1.17	4.1	4.0	91.5	0.5

Sections of 6 μ were cut, without prior embedding, on an International Cryostat Model CTI, and mounted with Kodak AR10 stripping film. Following development, the autoradiographs were stained through the film with Sudan IV and hematoxylin.

In the case of the abdominal aorta a segment was also incubated with 34.4×10^6 cpm of oleic acid-9-10-^{3}H complexed to the normal human serum albumin of the incubation medium. The medium consisted of 0.5 ml sodium oleate-9-10-^{3}H, and 5 ml of 50:50 Hanks':normal human serum. After incubation at 37°C for 3 hours the artery was thoroughly washed in 0.9% sodium chloride solution and reincubated in nonlabeled medium for 1 hour to reduce the amount of free ^{3}H-labeled oleic acid. Six-μ sections were cut and mounted on each end of glass slides and one end dipped into acetone at 4°C for 25 minutes. Enough slides were set up to compare the effect of acetone extraction on the distribution of ^{3}H-labeled oleic acid among arterial lipids with the distribution in the control. Otherwise autoradiographs were prepared as above.

Distribution of ^{14}C-labeled choline among phospholipids and of ^{3}H-labeled oleic acid among neutral lipids was determined by thin-layer chromatography as previously described (Day and Wahlqvist, 1969). Counting was performed in a Packard Tricarb Spectrometer.

RESULTS

^{14}C-labeled choline was taken up and incorporated into phospholipid by both intima and media and by normal and atherosclerotic portions of the two arteries studied (Table I). Up to 13% of the choline present in the incubation medium was taken up and incorporated into phospholipid by the various portions of the

artery. Since the area of artery involved varied considerably no significance can can be attached to the relative uptake and incorporation in the lesion compared with the normal artery; the differences indicated in Table I reflect by and large the difference in relative size of the lesion. The individual phospholipids labeled in the various portions of the two arteries studied is also given in Table I. Most of the choline has been incorporated into lecithin with smaller amounts of label incorporated into the other choline containing phospholipids, sphingomyelin, and lysolecithin. Very little label appears in the noncholine containing phospholipids. No obvious differences in labeling pattern of the phospholipids occur between the media, intima, or the two types of lesion studied although insufficient data are presented to assess less than gross differences.

The localization of the ^{14}C-labeled phospholipid formation in the artery to foam cells present is shown in Figs. 1–5. More than 90% of the ^{14}C-labeled choline present in the arteries used for autoradiography was present as phospholipid so that localization of phospholipid formation in the artery could be determined from localization of the radioactivity present.

FIG. 1. Autoradiograph of an early fatty streak in the abdominal aorta of a 20-year-old man. Incubated with ^{14}C-labeled choline *in vitro*. The intima (I) is uppermost. In the region of the intimo-medial junction a sudanophilic round mononuclear (F) has localized ^{14}C-choline containing phospholipids. Hematoxylin and Sudan IV. Exposure time 7 days.

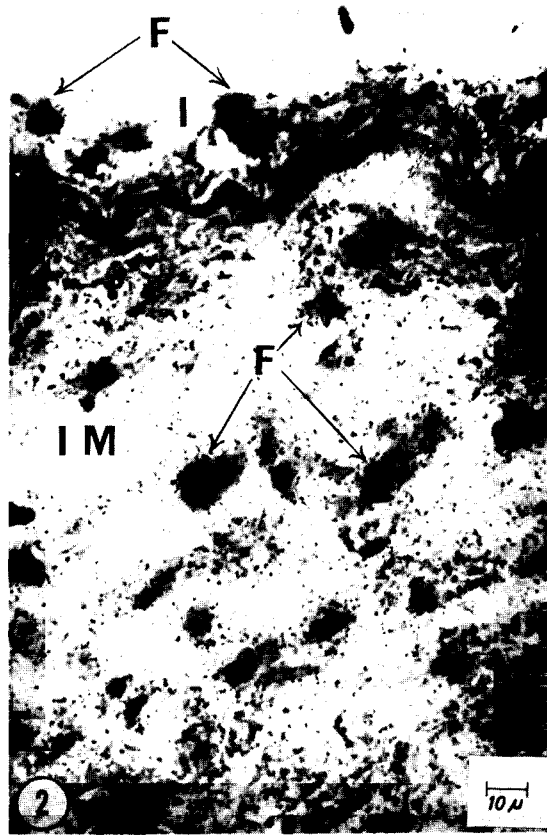

FIG. 2. Autoradiograph of a lesion from the same vessel as Fig. 1. Sudanophilic cells (F) in the intima (I) and disorganized intimo-medial junction region (IM) have in their vicinity a concentration of silver grains representing ^{14}C-choline containing phospholipids. Hematoxylin and Sudan IV. Exposure time 19 days.

Localization to foam cells in both the simple fatty-streak lesion present in the aorta and in the complicated lesion in the femoral artery is confirmed by the more quantitative information given in Table II. Grain counts over various areas carried out in both cases studied show clear localization of ^{14}C to the foam cells present. The other cell types present in the intima and the general distribution of radioactivity in the media indicate the uptake and incorporation of ^{14}C-labeled choline into phospholipid by the foam cells present.

Localization of phospholipid formation was also studied in portions of one of the arteries following incubation with ^{3}H-labeled oleic acid. Significant uptake of oleic acid and its incorporation into phospholipid and cholesterol ester by both lesion and normal intimae occurred as previously demonstrated (Wahlqvist *et al.*, 1969). Some of the sections cut, however, were exposed to acetone extraction in order to remove lipids other than phospholipid and so study specifically the localization of phospholipid formation in the artery. The distribution of label in control and in acetone-treated sections is shown in Table III. Most

of the other ^{3}H-labeled lipids present have been removed by the acetone leaving most of the radioactivity present as phospholipid. It was not possible, however, to remove all of the remaining lipid. Figures 6 and 7 show autoradiographs from control and acetone-extracted sections of the atherosclerotic artery incu-

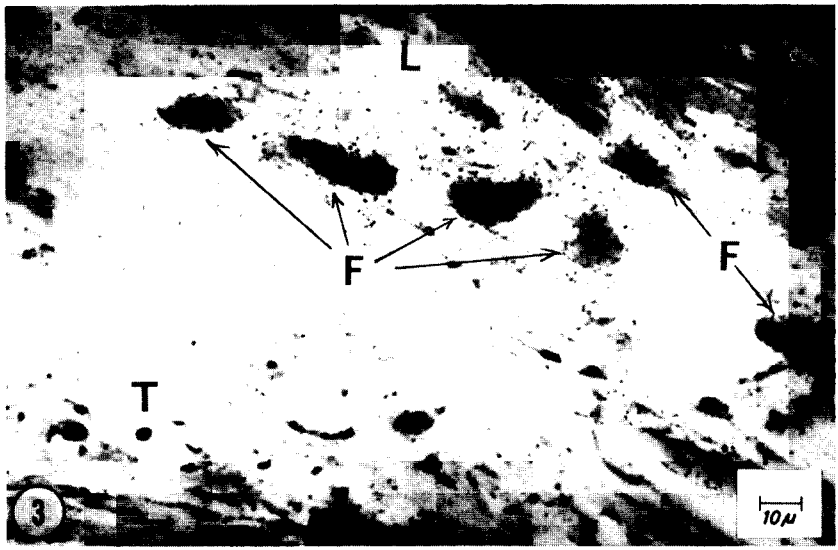

FIG. 3. Autoradiograph of an atherosclerotic lesion complicated by an organized thrombus (T) and a super-added foam cell lesion (L) in the femoral artery from a 74-year-old female. Foam cells (F) have localized phospholipids formed from the ^{14}C-labeled choline with which the artery was incubated. Hematoxylin and Sudan IV. Exposure time 19 days.

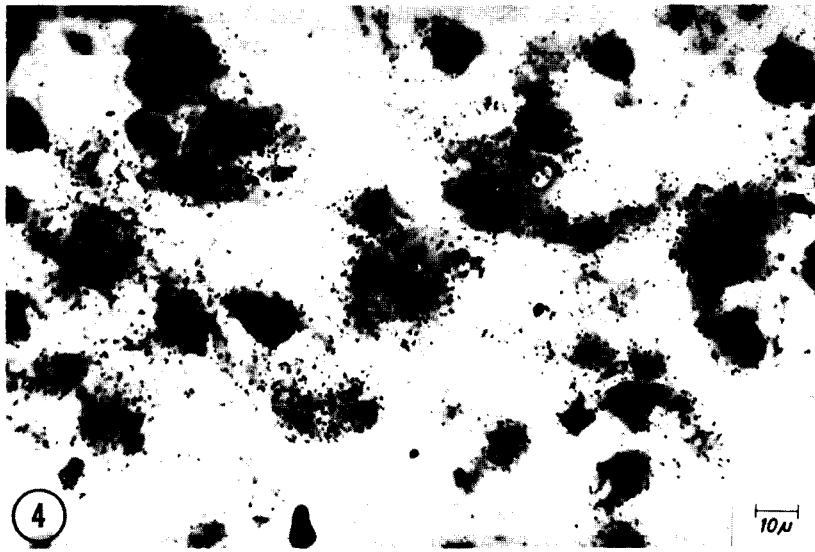

FIG. 4. Autoradiograph of another part of the lesion shown in Fig. 3, showing the localization of ^{14}C-labeled lipid in foam cells. Hematoxylin and Sudan IV. Exposure time 20 days.

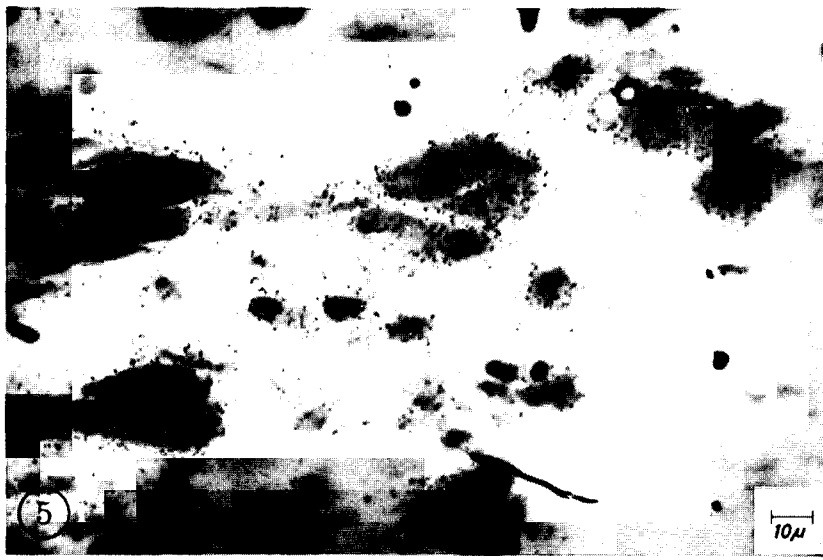

FIG. 5. Autoradiograph of the atherosclerotic intima of the femoral artery showing concentration of silver grains in the region of variously-shaped foam cells. The vacuoles with no overlying silver grains in the cell in the upper right hand corner are presumably artifacts. Hematoxylin and Sudan IV. Exposure time 20 days.

TABLE II

GRAIN COUNTS (no. grains/100 μ^2) ON AUTORADIOGRAPHS PREPARED FROM HUMAN ATHEROSCLEROTIC LESIONS INCUBATED WITH ^{14}C-LABELED CHOLINE[a]

Lesion	Intima				Media
	Foam cells	Nonsudanophilic round mononuclears	Spindle-shaped cells	Extracellular	
Complicated	14.8	1.5	0.3	0.2	0.1
Fatty streak	14.1	3.0	1.6	2.0	1.6

[a] 6000 μ^2 counted.

TABLE III

PERCENTAGE DISTRIBUTION OF ^{3}H-LABELED OLEIC ACID AMONG HUMAN ARTERIAL LIPIDS OF AUTORADIOGRAPH SECTIONS

	Phospholipid	Diglyceride	Fatty acid	Triglyceride	Cholesterol ester
Control	18.6	9.6	59.9	5.7	5.9
Acetone-extracted	50.7	20.3	25.0	3.9	0.0

bated with ^{3}H-labeled oleic acid. The ^{3}H-labeled lipid is present mainly in the foam cells and this data is confirmed by grain counts as shown in Table IV. Localization of ^{3}H has taken place in the foam cells scattered through the lesion, with little activity in other cells or the normal intima or media.

DISCUSSION

Atherosclerotic human arteries investigated *in vivo* (Zilversmit, *et al.*, 1961) or *in vitro* (Chobanian and Hollander, 1966) have been shown to incorporate ^{32}P-labeled phosphate into phospholipid. ^{14}C-labeled acetate and fatty acid have also been incorporated *in vitro* into phospholipid in atherosclerotic human arteries (Maggi, 1964; Parker *et al.*, 1964; Chobanian and Hollander, 1966; Wahlqvist *et al.*, 1969). Human umbilical artery also will take up and incorporate ^{14}C-labeled linoleic acid and labeled lysolecithin into phospholipid (Stein *et al.*, 1963; Eisenberg *et al.* 1967).

The study reported here indicates that ^{14}C-labeled choline is incorporated into phospholipid, predominantly into lecithin, in normal and atherosclerotic human intimae and media. In these short-term incubations steady-state conditions are presumably present so that *de novo* synthesis of phospholipid has not

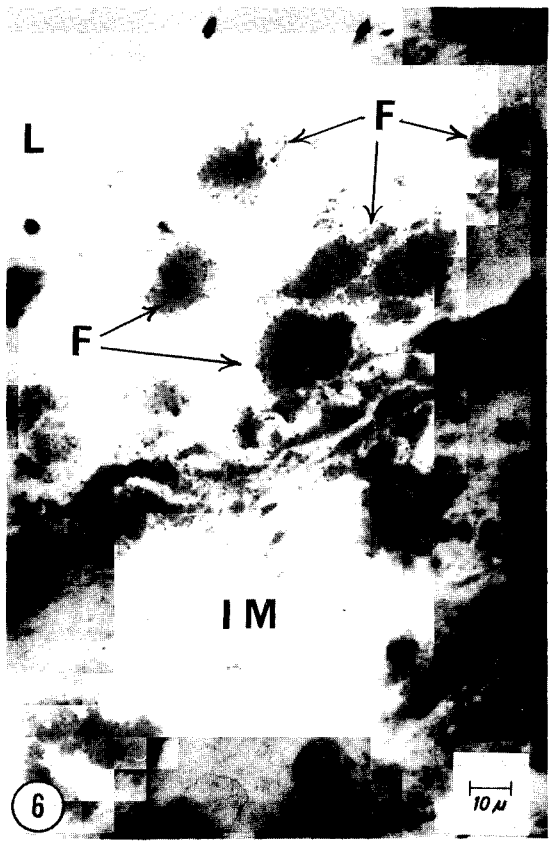

FIG. 6. Autoradiograph of the same lesion in Fig. 1, this time incubated with ^{3}H-labeled oleic acid. Localization of ^{3}H-labeled oleic acid and its metabolic derivatives, phospholipid, triglyceride, and cholesterol ester to intimal foam cells (F) is apparent. The luminal border (L) is uppermost and the region of the intimo-medial junction is denoted IM. Hematoxylin and Sudan IV. Exposure time 30 days.

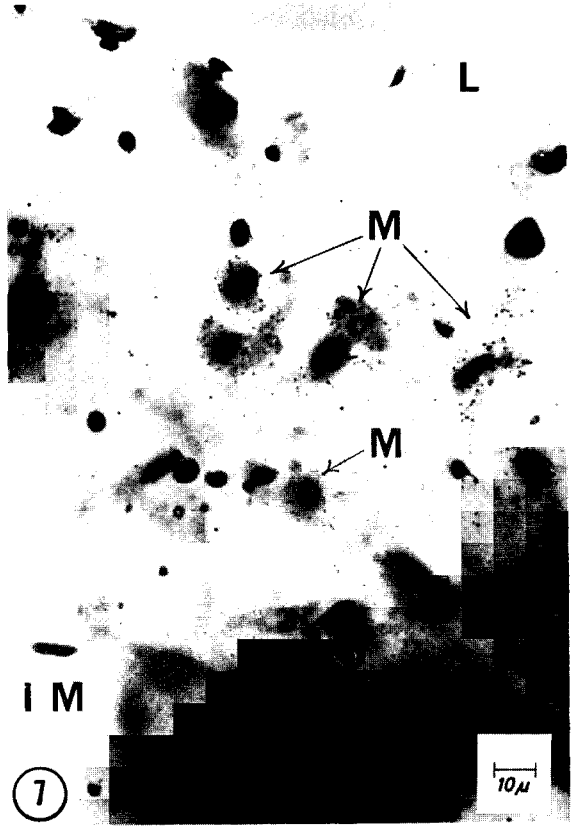

FIG. 7. An adjacent lesion to that shown in Fig. 6, but extracted with cold acetone so that most of the ^{3}H-labeled lipid is phospholipid. Localization of silver grains to intimal mononuclear cells (M) is evident. The luminal border (L) is uppermost and the region of the intimo-medial junction is denoted IM. Hematoxylin and Sudan IV. Exposure time 30 days.

TABLE IV

GRAIN COUNTS (no. grains/100 μ^2) ON AUTORADIOGRAPHS PREPARED FROM HUMAN FATTY STREAK LESION INCUBATED WITH ^{3}H-LABELED OLEIC ACID

	Intima				Media
	Foam cells	Nonsudanophilic mononuclears	Spindle-shaped cells	Extracellular	
Control	13.2	2.7	2.9	2.7	0.6
Acetone-extracted	7.6[a]		2.2	1,6	0.6

[a] Where the section has been acetone extracted, it is not possible to distinguish sudanophilic from nonsudanophilic cells.

specifically been examined. Enzymic exchange, as discussed for oleic acid incorporation into cholesterol ester in rabbit atherosclerotic lesions (Day and Wahlqvist, 1968) is a possibility. However, Zilversmit (1961) has provided evidence that the phospholipid of the human atherosclerotic lesion arises *in situ*.

It is likely, therefore, that the factors associated with the incorporation of ^{14}C-labeled choline into phospholipid are relevant to phospholipid accumulation in the human artery.

The present work, however, was concerned mainly with localization of phospholipid synthesis in human arteries. The localization of ^{14}C-labeled oleic acid uptake and incorporation into, mainly phospholipid, but also triglyceride and cholesterol ester, in foam cells in human atherosclerotic lesions has been reported by Wahlqvist *et al.* (1969). In the present paper ^{14}C-labeled choline has been used as a specific phospholipid precursor and autoradiographic studies show that choline containing phospholipid formation, chiefly that of lecithin, is localized to the foam cells. The acetone extraction studies using ^{3}H-labeled oleic acid have confirmed this observation. When the relative amount of phospholipid is increased in adjacent sections by the removal of free fatty acid and cholesterol ester, the localization to intimal foam cells as demonstrated by autoradiography is still evident.

The general conclusion appears to be, therefore, that the human atherosclerotic artery is capable of forming phospholipid and that this formation takes place essentially in foam cells.

SUMMARY

Localization of the synthesis of phospholipids in the human atherosclerotic lesion has been investigated *in vitro*. ^{14}C-labeled choline is incorporated principally into lecithin and autoradiographs demonstrate that, in fatty-streak and complicated atherosclerotic lesions, the label is predominately in foam-cell areas. When ^{3}H-labeled oleic acid is used as precursor, incorporation into phospholipid, triglyceride, and cholesterol ester is found and localization of label in the region of foam cells is apparent in autoradiographs. Further, localization to foam cells is preserved when the ^{3}H-labeled lipid other than phospholipid is removed by acetone extraction. It is concluded that foam cells are chiefly responsible for phospholipid synthesis in human atherosclerotic lesions.

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