Reprint from

Atherosclerosis:
Proceedings of the Second International Symposium

Edited by
Richard J. Jones

(Springer-Verlag New York · Heidelberg · Berlin 1970.
Printed in Germany—Not for sale)

LOCALIZATION OF LIPID SYNTHESIS BY FOAM CELLS IN ATHEROMATOUS LESIONS

ALLAN J. DAY and MARK L. WAHLQUIST

References to the literature appearing in the text are available in a comprehensive bibliography which may be obtained at cost from the Chicago Heart Association, 22 West Madison Street, Chicago, Illinois 60603, U.S.A.

LOCALIZATION OF LIPID SYNTHESIS BY FOAM CELLS IN ATHEROMATOUS LESIONS*

ALLAN J. DAY and MARK L. WAHLQVIST**

* Data presented herein is derived from Day and Wahlqvist [379] and Wahlqvist and Day [1513], and is published with the permission of Academic Press.

** This work was supported by grants from the National Heart Foundation of Australia, National Health and Medical Research Council of Australia and U.S.P.H.S. Grant No. R05-TW00318. Technical assistance by Mrs. G. M. Neill, Misses J. Dare and A. Lawrence is also gratefully acknowledged.

The development of the atherosclerotic lesion is characterized by the accumulation in the intima of increasing amounts of lipid, in particular, of cholesteryl ester and of phospholipid. The origin of this lipid and its role in the pathogenesis of atherosclerosis, however, is still the subject of intensive investigation in various laboratories. Much of the phospholipid and possibly the fatty acid in experimental and human atherosclerotic lesions arises by synthesis *in situ* [1093, 1592]. The cholesterol, on the other hand, appears to originate from the serum by infiltration [162]. In early lesions most of the lipid is present in the foam cells scattered throughout the intima, and the suggestion has been made that these foam cells contribute to the synthesis of lipid in the atherosclerotic arterial wall. This possibility has been investigated in our laboratory, initially using normal macrophages as a model system [371] and more recently, using either foam cells isolated from atherosclerotic lesions or atherosclerotic arteries incubated *in vitro* with ^{14}C-labeled oleic acid. Foam cells isolated from experimental atherosclerotic lesions take up and incorporate ^{32}P-labeled phosphate, ^{14}C-labeled acetate, and ^{14}C-labeled oleic acid into phospholipid and cholesteryl ester [376, 377, 380]. Autoradiographic studies published recently have demonstrated that the fatty acid which is incorporated into phospholipid and cholesteryl ester in the atherosclerotic arterial wall is localized to intimal foam cells [378, 1514].

Herein the question of phospholipid synthesis in the atherosclerotic artery is investigated and data presented indicating that most of such synthesis is localized to the foam cells.

METHODS

Atherosclerotic aortas obtained from rabbits fed 1% cholesterol and 3% peanut oil in the diet for one to four months were used. Human arteries were obtained at surgery or from renal transplant donors. The arteries were incubated *in vitro* in 50:50 Hank's solution: rabbit/human serum containing either ^{14}C-labeled choline or ^{3}H-labeled oleic acid. Following incubation and suitable washing and fixing, portions of the intima were extracted for radioassay and separated into lipid components. The remainder was sectioned and examined by autoradiography using Kodak AR10 stripping film.

RESULTS AND DISCUSSION

Experimental Rabbit Atherosclerosis. Up to 11.3% of the ^{14}C-labeled choline present in the medium was taken up by the atherosclerotic rabbit intima and incorporated into phospholipid. Incorporation of the choline into phospholipid in the intima was linear over the four hour period studied and separation of individual phospholipids by thin layer chromatography indicated that most of the choline (80–90% of the total) had been incorporated into lecithin with lesser amounts into the other choline containing phospholipids, sphingomyelin (1.2–5% of the total) and lysolecithin (6.4–13.4% of the total).

In order to confirm that most of the ^{14}C-labeled choline in the artery was present as lipid, radioassay of the protein precipitate and of the Folch wash

Table 1. *Grain counts (No. grains/100 μ^2)* [a] *in autoradiographs prepared from rabbit aortas incubated in vitro with ^{14}C-labeled choline*

Period rabbit cholesterol fed (months)	Incubation time (hours)	Intima		Media
		foam cells	extracellular	
1	4	8.3	5.4	6.2
2	4	8.3	4.5	3.2
3	4	10.7	6.7	9.9
3	1	6.5	1.1	2.0
3	3	11.3	4.6	5.9
3	4	9.6	3.2	5.4
4	0.25	7.3	2.2	14.5
4	0.5	5.9	1.9	7.3

[a] At least 6,000 μ^2 assessed for each feature.

was carried out. Following the standard fixation and washing procedures used, it was found that 94–98% of the ^{14}C-labeled choline present in the artery was present in the lipid extract, so that localization of ^{14}C by autoradiography was indicative of localization of phospholipid synthesis. Autoradiographs indicated that while some of the ^{14}C-labeled phospholipid synthesized was scattered throughout the arterial wall, most of it appeared in the foam cells. Grain counts (Table 1) showing this localization give a quantitative picture of these observations.

When ^{3}H-labeled oleic acid was incubated with atherosclerotic rabbit aortas, most of the oleic acid taken up was incorporated into phospholipid and cholesteryl ester as has been previously shown [378]. Following extraction of the sections with cold acetone most of the ^{3}H-labeled lipid, with the exception of the phospholipid, was removed, so that in the sections presented for autoradiography 88.9% of the ^{3}H was present as phospholipid. This ^{3}H-labeled phospholipid was present almost entirely in foam cells (Fig. 1) so that it can be concluded that the incorporation of ^{3}H-labeled oleic acid into phospholipid which takes place in the atherosclerotic arterial wall occurs predominantly in the foam cells.

Human Atherosclerosis. Similar findings were observed in human fatty streak lesions. ^{14}C-labeled choline was taken up and incorporated primarily into lecithin

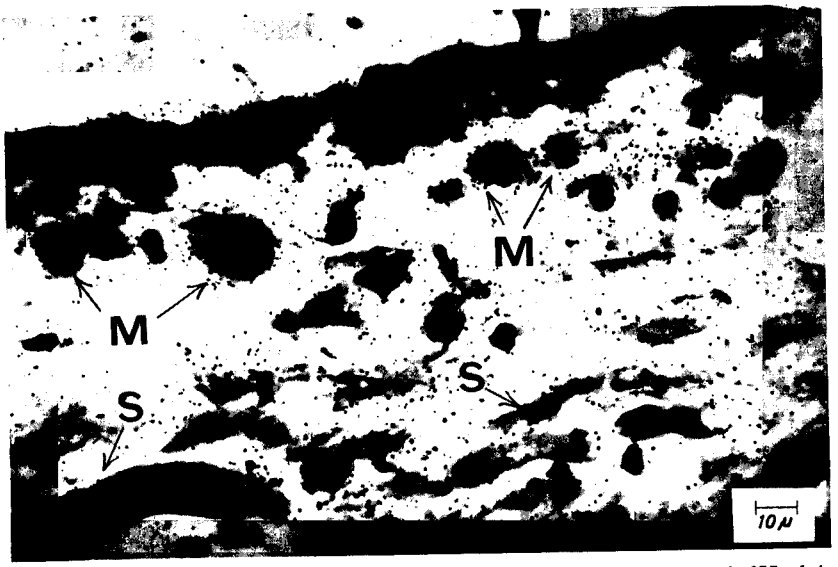

Fig. 1. Autoradiograph of a rabbit atherosclerotic lesion incubated with ^{3}H-oleic acid followed by acetone extraction of the sections. There is localization to superficial round mononuclears (*M*) with little label in spindle-shaped cells (*S*). Hematoxylin and Sudan IV

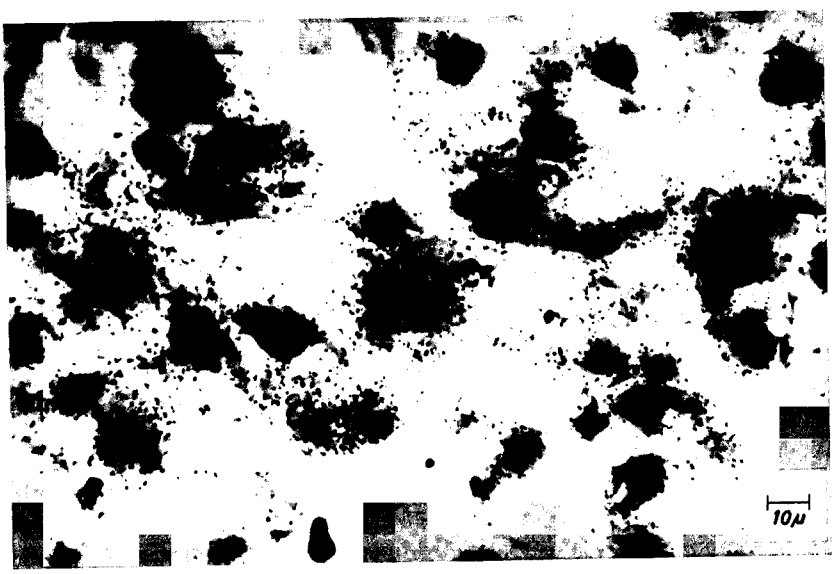

Fig. 2. Autoradiograph of a human atherosclerotic lesion incubated with ^{14}C-choline. Hematoxylin and Sudan IV

by the intimal lesions. Autoradiography showed clear localization of such incorporation to the foam cells of the atherosclerotic lesions (Fig. 2). Quantitative assessment by grain counting indicated that the localization was rather more clear cut than was the case with the rabbit lesions (Table 2). When ^{14}C-labeled

oleic acid was used as a precursor followed by acetone extraction of the sections prepared, incorporation of the fatty acid into phospholipid could be observed by autoradiography as a foam cell phenomenon.

Table 2. *Grain counts (No. grains/100 μ^2) in autoradiographs prepared from human atherosclerotic lesions incubated with ^{14}C-labeled choline*[a]

Lesion	Intima				Media
	foam cells	non-sudanophilic round mononuclears	spindle-shaped cells	extra-cellular	
Complicated	14.8	1.5	0.3	0.2	0.1
Fatty streak	14.1	3.0	1.6	2.0	1.6

[a] 6,000 μ^2 counted.

It can be concluded, therefore, that in both human and rabbit atherosclerotic lesions the incorporation of precursors into phospholipid *in vitro* takes place predominantly in the foam cells present. Clearly then, synthesis and metabolism of lipid by these cells influences the composition and development of the atherosclerotic lesion.