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INCORPORATION OF DIFFERENT FATTY ACIDS INTO COMBINED LIPIDS IN RABBIT ATHEROSCLEROTIC LESIONS

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SUMMARY

^{14}C-labelled palmitic, oleic and linoleic acids have been incubated *in vitro* with aortas obtained from cholesterol-fed rabbits and the relative incorporation of each of these fatty acids into cholesterol ester, phospholipid and triglyceride has been compared using gas–liquid radiochromatography. Incorporation of oleic acid into cholesterol ester is greater than that of either linoleic or palmitic acid. The amount of the individual fatty acids incorporated into triglyceride and into phospholipid, however, is roughly the same. Calculation of pool sizes indicates that the incorporation of palmitic, oleic and linoleic acid into the lipid fractions parallels the chemical amount of fatty acid in the respective lipid fraction. Differential esterification in the atherosclerotic arterial wall may, therefore, account for some of the accumulation of lipid in the lesion.

Removal of the three fatty acids esterified in cholesterol ester, phospholipid and triglyceride has also been investigated in similar experiments. Intimal cholesterol ester, phospholipid and triglyceride were first labelled with palmitic, oleic and linoleic acid and then their respective specific activities followed over a subsequent incubation period. While both phospholipid and triglyceride were removed more readily than were the cholesterol esters as a group, there was no evidence to support a differential removal of different fatty acid esters within any of these groups in the atherosclerotic lesion.

Key words: *Atherosclerosis – Cholesterol ester – Cholesterol-fed rabbit – Fatty acid, saturated, monounsaturated, polyunsaturated – Phospholipid – Rabbit aorta – Triglyceride*

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INTRODUCTION

Various workers have examined the incorporation of [^{14}C]fatty acids into arterial lipids in both the normal artery[1–4] and in atherosclerotic lesions[5–8] in man and experimental animals. In the normal artery, most of the fatty acid is incorporated into phospholipid with little cholesterol ester formation, whereas in the atherosclerotic intima more fatty acid is incorporated into cholesterol ester. These studies have suggested that the esterification of fatty acid in the arterial wall may contribute to the accumulation of cholesterol ester in the atherosclerotic lesion. The fact that the fatty acid composition of the cholesterol ester of the arterial fatty streak lesion differs from that of the serum and the normal intima[9–12] would support this concept. BOWYER *et al.*[13] have recently compared the esterification of different fatty acids in the arterial wall by perfusing atherosclerotic rabbit arteries with different [^{14}C]fatty acids and determining the relative uptake and incorporation of these fatty acids into cholesterol ester in the artery. Under these circumstances it was shown that [^{14}C]oleic acid was taken up and incorporated into cholesterol ester more readily than was palmitic, stearic or linoleic acids. These observations did not distinguish, however, between the uptake and the esterification of the fatty acid since the intimal [^{14}C]fatty acid pool was not measured. Studies of the relative incorporation of fatty acid into other lipid moieties have not been reported. In the present paper, therefore, the incorporation of different [^{14}C]fatty acids in the atherosclerotic intima into cholesterol ester and into phospholipid and triglyceride are reported. In addition, these observations have been extended to compare the subsequent removal of the different fatty acids esterified as cholesterol ester, phospholipid or triglyceride.

MATERIALS AND METHODS

[^{14}C]fatty acids

[1-^{14}C]Palmitic acid, specific activity 55.2 mCi/m*M*, [1-^{14}C]oleic acid specific activity 57.8 mCi/m*M*, and [1-^{14}C]linoleic acid 52.9 mCi/m*M* were obtained from the Radiochemical Centre, Amersham, Great Britain. Dissolved in 0.05 *N* sodium hydroxide, they were added to the serum of the various incubation media to form fatty acid albumin complexes. Radiochemical purity was checked by thin-layer chromatography and by gas–liquid radiochromatography as described below.

Experimental procedure

Atherosclerotic thoracic aortas were obtained from male New Zealand white rabbits fed daily 1 g cholesterol and 3 ml peanut oil in 100 g rabbit chow for 4–5 months. Three series of experiments were carried out. In the first series the uptake and incorporation of either [^{14}C]linoleic or [^{14}C]palmitic acid into combined lipid in the atherosclerotic aorta was observed. The aortas were divided longitudinally into halves and each half incubated for 4 h at 37°C in 10 ml of Hank's solution–normal rabbit serum (50:50, v/v) containing either [1-^{14}Ci]palmitic acid (12.2 μCi) or [1-^{14}C]linoleic acid (17.4 μCi). As the thoracic aortas used were extensively involved with athero-

sclerosis, it was possible to obtain comparable portions (either halves or in later experiments thirds) by dividing the aorta longitudinally. After incubation the tissues were washed in 0.9% sodium chloride solution, the intima was stripped from the underlying media, ground in a mortar and its lipids extracted by the method of FOLCH *et al.*[14].

In the second series of experiments atherosclerotic thoracic aortic halves obtained from cholesterol-fed rabbits were incubated for 4 h at 37°C in 10 ml of Hank's solution–normal rabbit serum (50:50, v/v), containing a mixture of [1-^{14}C]-palmitic acid (13.3 μCi), [1-^{14}C]oleic acid (4.3 μCi) and [1-^{14}C]linoleic acid (5.1 μCi). Lipid extracts of the intima were prepared as before.

In the third series of experiments the removal of labelled lipid from the atherosclerotic lesion was studied. Atherosclerotic thoracic aortas obtained from cholesterol-fed rabbits were divided longitudinally into thirds and each third incubated in a medium containing a mixture of [^{14}C]palmitic (23.9 μCi), oleic (7.8 μCi) and linoleic (9.2 μCi) acids as described above. After an initial 2 h incubation the aortic thirds were removed from the medium and washed in 0.9% sodium chloride solution. One-third was taken as the "0 h" specimen and its intimal lipids extracted. The other two-thirds were re-incubated in 5 ml non-labelled Hank's solution–normal serum (50:50, v/v) for further periods of 2 and 4 h respectively. The intima was separated from the media and intimal lipid extracts prepared as before.

Thin-layer chromatography

Lipid extracts 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. Spots were either scraped into counting vials for direct counting in a Packard Tricarb Spectrometer Model 3375, using the dioxane water scintillator of SNYDER[15], or were methyl esterified for gas–liquid radiochromatography as described below.

Individual phospholipids were separated by thin-layer chromatography according to the method of SKIPSKI *et al.*[16] after initial separation of the total phospholipid by thin-layer chromatography as described above and its elution by the method of ARVIDSON[17]. Scrapes of individual phospholipids were counted directly by the method of SNYDER.

Gas–liquid radiochromatography

Methyl esters of the non-esterified fatty acids present in the intimal lipid extracts were prepared using diazomethane with heptadecanoic acid added as an internal standard. Diazomethane, prepared by the method of DE BOER AND BACKER[18], was collected in ether and added to the solvent-free total lipid in a molar ratio of at least 5:1. Ether and excess diazomethane were removed under a stream of nitrogen. The methyl esters formed were then separated from the other lipids present by thin-layer chromatography on Silica Gel G using diethyl ether–acetic acid–*n*-hexane (30:2:180,

v/v/v) as developing solvent and eluted with 3 · 6–10 ml washings of light petroleum (40–60°C). The selectivity of the procedure for non-esterified fatty acid was checked by processing heptadecanoic acid with various combined lipids; only 17:0 peaks appeared on gas–liquid chromatograms.

Methyl esters of the fatty acids of cholesterol esters, triglycerides and phospholipids were prepared by heating at 65°C with 5% sulphuric acid in methanol in sealed ampoules, and extracted with light petroleum.

Separation of methyl esters was carried out 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 one of the streams combusted to $^{14}CO_2$; its ^{14}C content was monitored as an integrated write-out with a Pye Radiochromatoggraphy Unit. Specific activities were expressed as counts/min of ^{14}C/mμmole fatty acid using methyl [1-^{14}C]palmitate of known specific activity (35.2 counts/min/mμmole). The coefficient of variation ($n = 9$) of the methyl [^{14}C]palmitate was 6.3% for the second series (combined uptake) and 7.2% for the third series (removal) of experiments. The standard gave rise to about 700 integrated counts in 1 min at a flow rate of 15 ml/min. Background was approximately 20 counts/min. In most cases samples gave rise to more than 200 integrated counts/min and in no case less than 80. Duplicate determinations were routine.

RESULTS

The uptake and incorporation into intimal lipid of either [^{14}C]palmitic acid or [^{14}C]linoleic acid in rabbit atherosclerotic aortas is shown for two experiments in Table 1. Up to 3.9% of the [^{14}C]fatty acids added to the incubation medium were taken up and most of this incorporated into combined lipid in the intima. The amounts of each fatty acid taken up were of the same order, although it is not strictly valid to compare uptake on this basis since the specific activity of the two fatty acids in the

TABLE 1

UPTAKE AND INCORPORATION INTO COMBINED LIPID OF [1-^{14}C]PALMITIC ACID AND [1-^{14}C]LINOLEIC ACID BY RABBIT ATHEROSCLEROTIC INTIMA[a]

Experiment	*Fatty acid*	*% uptake from incubation medium*	*% ^{14}C initially present in medium incorporated into combined lipid*[b]
1	palmitic	3.9	2.5
	linoleic	3.3	2.9
2	palmitic	2.1	1.3
	linoleic	2.0	1.8

[a] In each experiment data relate to aortic halves — one half being incubated with [^{14}C]palmitic and the other with [^{14}C]linoleic acid.

[b] This value represents the total ^{14}C present in the intimal lipid extract minus the free fatty acid fraction.

TABLE 2

PERCENTAGE DISTRIBUTION OF [1-^{14}C]PALMITIC ACID AND [1-^{14}C]LINOLEIC ACID AMONG LIPID FRACTIONS OF RABBIT ATHEROSCLEROTIC AORTIC INTIMA[a]

Experiment	*Fatty acid*	*Phospho-lipid*	*Diglyceride*	*Free fatty acid*	*Triglyceride*	*Cholesterol ester*
1	palmitic	41.8	3.8	32.1	11.2	11.2
	linoleic	49.2	3.8	13.7	13.7	19.7
2	palmitic	41.6	3.6	38.4	8.7	7.7
	linoleic	49.0	4.3	14.4	10.8	21.5

[a] In each experiment aortic halves were incubated with either [^{14}C]palmitic or linoleic acid.

TABLE 3

PERCENTAGE DISTRIBUTION OF [1-^{14}C]PALMITIC ACID AND [1-^{14}C]LINOLEIC ACID AMONG INDIVIDUAL PHOSPHOLIPIDS OF RABBIT ATHEROSCLEROTIC AORTIC INTIMA[a]

Experiment	*Fatty acid*	*Lyso-lecithin*	*Sphingo-myelin*	*Lecithin*	*Phospha-tidyl inositol/ phosphatidyl serine*	*Phospha-tidyl ethanol-amine*	*Solvent front*
1	palmitic	0.5	8.0	72.7	6.0	5.7	7.0
	linoleic	0.3	1.5	74.2	7.7	6.1	10.2
2	palmitic	0.6	6.9	75.7	5.5	4.8	6.4
	linoleic	0.3	0.5	72.3	10.2	6.3	10.4

[a] In each experiment aortic halves were incubated with either [^{14}C]palmitic or linoleic acid.

incubation medium may have differed. The distribution of the labelled fatty acid among the intimal lipids is shown in Table 2. In both experiments palmitic and linoleic acids were incorporated into phospholipid and, to a lesser extent, into cholesterol ester and triglyceride. However, relatively more cholesterol ester was formed from linoleic acid than from palmitic acid. Fractionation of the labelled phospholipid formed into individual phospholipids indicated predominant labelling of lecithin with both fatty acids (Table 3). However, there is more incorporation of palmitic acid than of linoleic acid into sphingomyelin. In these experiments, phospholipid, triglyceride, and cholesterol ester fatty acids were methyl esterified and the [^{14}C]fatty acid observed by gas–liquid radiochromatography. In this way it was possible to determine that no change in chain length or saturation of the linoleic or palmitic acid had occurred. A previous experiment had established the same stability for oleic acid. It was therefore valid to assume that in the subsequent experiments where [^{14}C]-palmitic, oleic and linoleic acids were mixed that no interconversion had occurred, so that the metabolism of individual fatty acids could be compared in the one artery.

The uptake and incorporation of [^{14}C]palmitic, oleic and linoleic acids into intimal lipids for the second series of experiments is given in Table 4. This data has been

obtained following separation by gas–liquid radiochromatography of the [^{14}C]fatty acids in the various lipid fractions and is expressed in relation to the counts/min of the respective labelled fatty acid present in the incubation medium. The amount taken up (2–4.6%) is similar to that observed in the first experimental series where fatty acids were incubated separately; again most of the fatty acid taken up is

TABLE 4

UPTAKE AND INCORPORATION INTO COMBINED LIPID OF [^{14}C]FATTY ACIDS BY ATHEROSCLEROTIC RABBIT INTIMA[a,b]

Fatty acid	*% uptake from incubation medium*	*% ^{14}C initially present in incubation medium incorporated into combined lipid*[c]
Palmitate	2.03 ± 0.44	1.71 ± 0.32
Oleic	3.37 ± 0.90	2.70 ± 0.75
Linoleic	4.63 ± 1.15	3.86 ± 1.10

[a] Aortic halves were incubated with a mixture of the [^{14}C]fatty acids.
[b] Means and standard errors of means for three experiments are given.
[c] This value represents the total ^{14}C present in the intimal lipid extract minus the free fatty acid fraction.

TABLE 5

SPECIFIC ACTIVITIES OF LIPID FRACTIONS IN RABBIT ATHEROSCLEROTIC INTIMA FOLLOWING INCUBATION WITH [^{14}C]FATTY ACIDS[a,b]

	Free fatty acid	*Phospholipid*	*Triglyceride*	*Cholesterol ester*
Palmitic	1024 ± 157	67 ± 18	73 ± 33	14 ± 1
Oleic	366 ± 62	43 ± 11	29 ± 12	5 ± 1
Linoleic	1182 ± 313	107 ± 13	67 ± 33	19 ± 3

[a] Specific activities are expressed as counts/min ^{14}C/mμmole fatty acid.
[b] Means and standard errors of means of three experiments are given.

TABLE 6

INCORPORATION OF [^{14}C]FATTY ACIDS INTO LIPID FRACTIONS OF RABBIT ATHEROSCLEROTIC INTIMAS AND POOL SIZES OF LIPID FRACTIONS[a]

	Incorporation (mμmoles/g dry defatted weight)			*Pool size (μmoles fatty acid)*		
	phospholipid	*triglyceride*	*cholesterol ester*	*phospholipid*	*triglyceride*	*cholesterol ester*
Palmitic	4843 ± 1171	1277 ± 341	1423 ± 387	3.49 ± 1.39	0.85 ± 0.20	4.34 ± 1.56
Oleic	5243 ± 1196	1645 ± 399	7370 ± 1854	2.10 ± 0.85	0.96 ± 0.18	21.77 ± 7.96
Linoleic	4530 ± 2194	790 ± 360	3237 ± 1984	1.90 ± 0.85	0.55 ± 0.08	4.76 ± 1.93

[a] Means and standard errors of means for three experiments are given.

incorporated into combined lipid. The specific activity of the palmitic, oleic or linoleic acid in the intimal phospholipid, triglyceride or cholesterol ester is given in Table 5. This data has been obtained from gas–liquid chromatography. The methylation of the free fatty acids in the intimal pool by diazomethane has enabled the specific activity of the three fatty acids present in this pool to be determined by gas chromatography and these specific activities are also shown in Table 5. The specific activity of the free fatty acids in the intimal pool has been used to calculate the incorporation of palmitic, oleic or linoleic acids into each of the three lipid fractions investigated. Thus, the μmoles incorporated into cholesterol ester, phospholipid or triglyceride is given by the counts/min incorporated, divided by the specific activity of the intimal fatty acid and this data is given in Table 6. Since this data is based on the specific activity of free fatty acids in the intima, any differences associated with different uptake of the three fatty acids can be excluded and direct comparison of their incorporation into the three lipid fractions can be made. More cholesterol oleate was formed than any other lipid although it can be seen that the variance is relatively large. Approximately equal amounts of the three fatty acids were incorporated into phospholipid and rather smaller amounts of the three fatty acids into triglyceride. The incorporation of the respective fatty acid into its corresponding lipid fraction, however, was closely related to the amount of that lipid fraction present as can be seen from the data given for pool sizes in the second part of Table 6. These pool sizes have been derived from the specific activity of the respective fractions and the total amount of radioactivity present. Considerably more cholesterol oleate was present in the intima than any of the other 8 pools determined. The incorporation of the respective fatty acid into cholesterol ester, phospholipid and triglyceride, relative to the pool size for the individual experiments, has been calculated and is shown in Table 7. Within each lipid group the amount of each fatty acid incorporated into that group, in relation to the amount of that fatty acid present in the group, was almost the same. The amount of incorporation, therefore, was directly related to the chemical content of the intima.

The removal of individual fatty acids incorporated into cholesterol ester, phospholipid, and triglyceride was studied in the third series of experiments and this data is given in Fig. 1. The specific activity of the different fractions at zero time (*i.e.* after the preliminary 2 h incubation to effect labelling of the lipid fractions) is taken

TABLE 7

AMOUNT (mμmoles) OF INDIVIDUAL FATTY ACIDS INCORPORATED/μmole FATTY ACID IN LIPID FRACTIONS[a]

	Phospholipid	*Triglyceride*	*Cholesterol ester*
Palmitic	66 ± 15	86 ± 52	14 ± 2
Oleic	118 ± 19	99 ± 60	15 ± 2
Linoleic	101 ± 22	90 ± 64	18 ± 2

[a] Means and standard errors of means for three experiments are given.

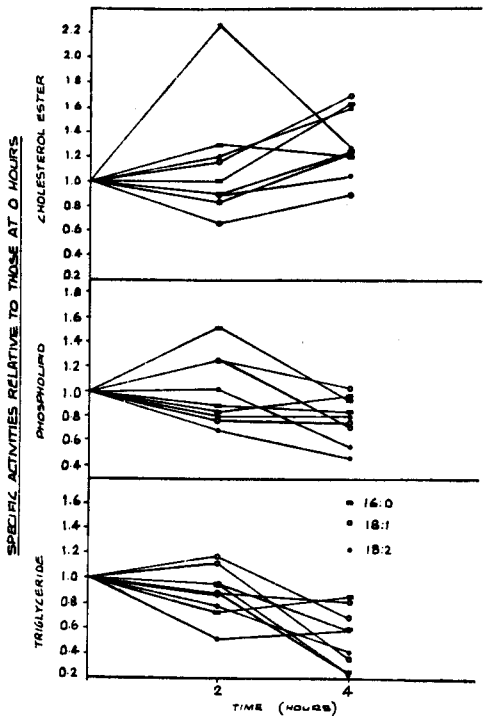

Fig. 1. Removal of [^{14}C]palmitic, oleic and linoleic acids incorporated by preliminary incubation into lipid fractions of rabbit atherosclerotic intimas. The relative specific activities of the respective fatty acids in the three lipid fractions are plotted in relation to time for the 4-h period following the initial labelling incubation. Data for the individual experiments are shown and in each case the 0-, 2- and 4-h figures were obtained from aortic thirds of the same artery.

as unity and the change in specific activity of the three fatty acids of the lipid fractions are expressed relative to their specific activity at zero time. Fig. 1 gives this data for three experiments. In general, there was a reduction with time in the specific activity of the three fatty acids in both phospholipid and triglyceride, so that 4 h after removal of the radioactive medium the specific activity in all but one case was below unity. The fall in relative activity was more apparent in the triglyceride group than in the phospholipid group. In the cholesterol ester group, however, the relative specific activity continued to rise in the three experiments and in all but one case was above one after 4 h. Comparison of the three fatty acids within each lipid group did not indicate any difference between them in their relative removal rates. So that, while triglyceride and phospholipid appeared to be removed more readily than cholesterol ester, the individual esters within each group showed no difference in relative removal rates.

DISCUSSION

The experiments described in the first series were carried out for two main reasons. In the first place they provided a background upon which to plan the

experiments containing mixtures of labelled fatty acids described in the second series. They also provided an opportunity to verify the fact that the palmitic and linoleic acids remained unchanged in the various lipid fractions in which they were incorporated. That this was so for the [1-^{14}C]oleic acid had already been shown in previous work using this precursor[7]. If these three precursors failed to retain their identity then incubation of the three fatty acids together as was carried out in the second series of experiments would not have provided a valid means of comparing their separate behaviours. In addition, however, opportunity was taken in these preliminary experiments to investigate the incorporation of linoleic and of palmitic acids into the individual phospholipids. In previous work[7] it had been shown that oleic acid taken up and incorporated into phospholipid in the atherosclerotic rabbit aorta was incorporated predominantly into lecithin. It is interesting to note that where palmitic acid was used as precursor that a greater percentage of this precursor was incorporated into sphingomyelin than was the case with either the linoleic acid used in the present work or with oleic acid as reported previously. Palmitic acid is the major fatty acid present in the sphingomyelin of the rabbit atherosclerotic aorta[19], whereas smaller proportions of both linoleic and oleic acid are present in this fraction.

The data presented in the second series of experiments indicates that the amount of oleic acid incorporated into the cholesterol ester of the atherosclerotic arterial intima is higher than that of either palmitic or linoleic acid. This observation is in agreement with the conclusions reported by BOWYER *et al.*[13]. The approach adopted in the present work, however, has two advantages over that described by the latter workers. Firstly, a comparison of the incorporation of the three fatty acids incubated together has been made possible by gas–liquid radiochromatography and, secondly, the incorporation of oleic, palmitic and linoleic acids into cholesterol ester and into the other lipid fractions was based on the measurement of the specific activity of the free fatty acids in the intima rather than that in the incubation or perfusion medium. We are therefore dealing with a precursor pool of fatty acid which is much nearer the immediate precursor pool than is the case where esterification comparisons are based on amounts in the incubation medium. It is not only possible but probable that the uptake of the fatty acids will vary independently of their subsequent esterification in the wall.

The finding that the amount of cholesterol oleate formed is greater than the amount of either cholesterol palmitate or cholesterol linoleate corresponds to the known composition of cholesterol esters in such lesions[9–12,19]. However, the amount of the respective [^{14}C]fatty acid incorporated in each lipid fraction closely parallels the amount of that fatty acid present in the particular fraction. The possibility emerges, therefore, that the larger amount of oleic acid incorporated into cholesterol oleate does not indicate a greater synthesis of this fraction, but is merely a reflection of a slower fractional turnover of this large pool. Unfortunately, no data concerning the incorporation of either palmitic or linoleic acid into cholesterol ester in relation to time is available, although in a previous study[7] it has been shown that the incorporation of oleic acid into cholesterol ester is approximately linear with time over the 4-h incuba-

tion period. Nevertheless, since the intimal free fatty acid specific activity is considerably in excess of that of the corresponding cholesterol ester specific activity it would seem unlikely that the latter fractions were nearing isotopic equilibrium. However, it must be admitted that there may be a more active pool of cholesterol ester in the arterial wall. The foam cell fraction of the arterial wall, for example, has been shown to be such an active pool[20]. One other reason, however, which would suggest that the increased formation of cholesterol oleate cannot be explained by a large slowly turning over pool is provided by the data of the removal experiments reported in the third series. Where the fatty acid is removed from the incubation medium there is some residual free fatty acid in the intima that continues to be incorporated into cholesterol ester. This is also potentially contributed to by breakdown of phospholipid and triglyceride. Under these circumstances, with an intimal free fatty acid specific activity that is presumably falling, the specific activity of cholesterol palmitate and cholesterol linoleate continues to rise over the subsequent 4-h period and does not show any difference in behaviour to that shown by cholesterol oleate. It would seem likely, therefore, that isotopic equilibrium has not been reached for cholesterol palmitate or cholesterol linoleate and that their behaviour resembles that shown in previous work for cholesterol oleate. The greater incorporation of oleic acid into cholesterol oleate presumably represents a real increase in its synthesis, therefore, rather than an artifact due to exchange with a large pool. The implications are therefore that the greater amount of cholesterol oleate present in the atherosclerotic lesion is a consequence of the preferential esterification of oleic acid in the formation of this cholesterol ester. There are other possibilities that might account in part for the large amount of cholesterol oleate in the atherosclerotic lesion. In previous work from this laboratory[21] the differential entry of cholesterol esters into the atherosclerotic aorta in the cholesterol-fed rabbit has been investigated, and it has been demonstrated that, under these circumstances, monounsaturated cholesterol esters enter and accumulate at a greater rate than do poly- or saturated cholesterol esters. A further possibility that could account for the preferential accumulation of cholesterol oleate in the atherosclerotic lesion is that the removal of the cholesterol esters from the atherosclerotic lesion depends on their fatty acid moiety and this point is taken up below.

In the present work, the incorporation of different fatty acids into phospholipid and into triglyceride has also been investigated. It is apparent from the data presented in Table 6 that the amounts of both palmitic and linoleic acid incorporated into phospholipid are greater than those into cholesterol ester and certainly greater than those into triglyceride. With regard to oleic acid, the amount incorporated into phospholipid exceeds that incorporated into triglyceride although, as has been discussed above, the amount of oleic acid incorporated into cholesterol ester exceeds that of all other fractions. The accumulation of these fractions in the atherosclerotic lesion, however, may be influenced not only by the esterification reaction, but also by removal.

BOWYER *et al.*[13] have claimed that cholesterol oleate is removed less readily from

atherosclerotic lesions than are other cholesterol esters and relate this retention to the slower hydrolysis of cholesterol ester in the arterial lesion. In the latter work[13,22] the cholesterol esters were presented to the artery in ethanol solution and differences in hydrolysis and subsequent removal under these circumstances could well be accounted for by physico-chemical factors. ABDULLA *et al.*[23] have investigated the differential removal of cholesterol esters from subcutaneous lipid implants and have shown that cholesterol arachidonate and linolenate were more rapidly removed from this situation than other cholesterol esters. Unfortunately, the removal of cholesterol arachidonate or linolenate was not studied in the present work, but ABDULLA *et al.*[23] reported that cholesterol linoleate was removed more slowly than cholesterol oleate. In the present work, the cholesterol esters were labelled endogenously so that the removal of the fatty acids in this fraction provided a closer assessment of the removal of different cholesterol esters under physiological circumstances. We recognise, however, that these experiments are subject to several limitations. The free fatty acid could not be removed completely from the intima and up to 30% of the [^{14}C]lipid present in the intima at the beginning of the removal period was still in the free fatty acid form and, of course, the intimal [^{14}C]free fatty acid pool would be contributed to by the breakdown of each of the lipid fractions during the removal period. However, if one accepts that it has been possible to investigate predominantly the removal process, the data with regard to removal of the three fractions can be taken as valid. It was apparent from these experiments that there were no differences within each lipid fraction on the basis of their fatty acid content. However, the removal of cholesterol esters as a group was slower than was the case with either phospholipids or triglycerides.

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