Fatty Acid Accumulation and Abnormal Lipid Deposition in Peripheral and Border Zones of Experimental Myocardial Infarcts

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Twenty-eight dogs with acute anterior myocardial infarcts due to proximal occlusion of the left anterior descending coronary artery (LAD) were studied at various periods following the occlusion to determine: (a) the time course and location of abnormal lipid accumulation after infarction, (b) the degree of muscle-cell injury associated with increased lipid deposition, and (c) whether uptake of fatty acid from the circulating fat pool contributes to lipid accumulation in certain myocardial regions. The findings show that myocardial lipid accumulation begins as early as 6 hr after proximal LAD occlusion. The increased lipid deposition occurs as nonmembrane-bound lipid droplets in muscle cells with and without ultrastructural evidence of irreversible injury. Analysis of tissue uptake of intravenously injected [^{14}C] oleic acid conjugated with albumin revealed relatively selective concentration of label in the peripheral and border regions of the infarct, but occasionally even the central subendocardial portion of the infarct concentrated the fatty acid. Thin-layer chromotography showed that most of the label was associated with the triglyceride fraction when the radiolabeled fatty acid was injected 6 or 24 hr after LAD occlusion. These myocardial cellular and topographical alterations will have to be considered when labeled fatty acids are used for imaging acute myocardial infarcts and/or if attempts are made to identify myocardial fat-laden cells scintigraphically.

J Nucl Med 19: 276–283, 1978

During our studies of experimental myocardial infarction, we have observed, in border-zone regions of canine myocardial infarcts, the presence of prominent, fine vacuolization of muscle cells, suggesting the accumulation of lipid droplets in these cells (*1*). These findings are consistent with the observations made over 20 years ago by Wartman and associates, who demonstrated that neutral lipid droplets, stained with oil-red-O, were present in "viable" myocardial fibers within and around an area of experimental myocardial infarction (*2*). They believed that this material accumulated only in reversibly damaged myocardial cells and were uncertain as to whether the "fat" came from circulating plasma lipids or from the internal metabolism of these cells (*2*). Since that time fatty acids have been implicated in the genesis of cardiac arrhythmias and myocardial depression in patients and experimental animals with acute myocardial infarcts (*3–5*). It has also been suggested that decreased fatty acid uptake occurs in regions of acutely ischemic myocardium, and efforts

Received July 12, 1977; revision accepted Sept. 23, 1977.

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have been made to use radioactively labeled fatty acids for the scintigraphic detection of myocardial infarcts as filling defects (*6–9*). Despite these observations, detailed information is lacking regarding the precise myocardial locations of decreased fat uptake and/or of increased fat deposition after myocardial infarction. Accordingly we have attempted to characterize regional myocardial fat uptake in experimental animals at various times after permanent coronary-artery ligation. Specifically we wished to identify: (a) the topographic distribution of increased myocardial lipid deposition after myocardial infarction, (b) the source of the lipid material, (c) the subcellular location of the lipid material, and (d) the degree of myocardial muscle-cell injury associated with fat accumulation.

MATERIALS AND METHODS

Twenty-eight adult mongrel dogs were anesthetized with pentobarbital (30 mg/kg) and their chests opened under sterile conditions. The proximal left anterior descending coronary artery (LAD) was permanently ligated. A left atrial catheter was inserted and its connection exteriorized. The dogs' chests were closed and they were allowed to awaken.

Preparation of radiolabeled materials. Dog albumin (fraction V), [^{14}C] oleic acid (54.5 mCi/cc), and [^{3}H] glycerol (200 mCi/cc) were obtained commercially. The canine albumin-[^{14}C] oleate complex was prepared by the method of Van Harkin et al. (*10*).

Very low-density lipoprotein (VLDL) for iodination was prepared from the plasma of donor dogs fed a 40% sucrose diet for 1 wk before exsanguination. The blood was collected using EDTA anticoagulant (1 mg/10 ml blood) and the VLDL was isolated by ultracentrifugation at plasma density and then washed twice at salt density 1.006, as previously described for human VLDL (*11*). The VLDL was iodinated using the iodine monochloride method previously described (*11*). The labeling efficiency was 4%; 32% of the radioactivity was bound to lipid.

To prepare VLDL labeled with [^{3}H] glycerol and [^{14}C] oleic acid in the triglyceride moiety, [^{3}H] glycerol (5 mCi) and canine [^{14}C] oleate-albumin (1 mCi) were simultaneously injected intravenously into a fasting donor dog. The fast was continued and 6 hr later the animal was exsanguinated and the VLDL was isolated, as described above. The C-14/H-3 ratio on VLDL was 0.11. This material was then administered to recipient dogs, as described below.

In initial experiments, nine dogs with proximal ligation of the LAD were studied after 6 hr. Three of the dogs received 1 μCi of [^{125}I] VLDL (3 ng VLDL), three dogs received 0.5 μCi [^{14}C] oleate-albumin [0.31 μmole free fatty acid (FFA) and 15 mg albumin], and three dogs received dual-labeled VLDL containing 0.02 μCi [^{14}C] oleate and 0.35 μCi [^{3}H] glycerol in the triglyceride moiety. The material was diluted in 10 ml of saline and injected directly into the left-atrial catheter, and the dogs were killed 24 hr later. In these studies only the [^{14}C] oleate-albumin produced measurable counts in the myocardium 24 hr after its administration. Accordingly, the subsequent experiments were performed with only the [^{14}C] oleate-albumin complex.

Experiments were also performed in an attempt to identify the time course for fat accumulation in the various regions of the heart after infarction. For examination by optical and electron microscopy, myocardial tissue was obtained 5–6 hr (2 dogs), 24 hr (2 dogs), and 48 hr (2 dogs) after LAD occlusion. For optical microscopy, histologic sections of formalin-fixed, paraffin-embedded blocks were stained with hematoxylin and eosin or the von Kossa method for calcium salts, and frozen sections of formalin-fixed blocks were stained with oil-red-O for neutral lipids. Small samples of tissue also were immersed in a solution of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 *M* cacodylate buffer (pH 7.4); rinsed in cacodylate-buffered sucrose; post-fixed in 1% osmium tetroxide in veronal acetate buffer (pH 7.4); dehydrated through a graded series of alcohols and propylene oxide; and embedded in an Epon-Araldite mixture. One-micron sections were stained with toluidine blue. Thin sections were stained with uranyl acetate and lead citrate and examined in an electron microscope.

In nine additional dogs, [^{14}C] oleic acid (8 million cpm in 10 ml of saline) was injected into the

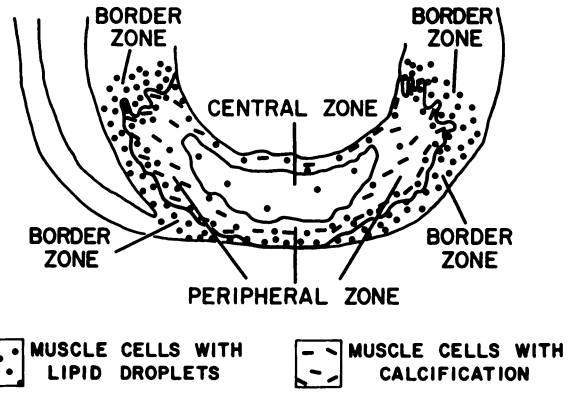

FIG. 1. Typical distribution of lipid accumulation in an acute transmural myocardial infarct. Lipid droplets occur predominantly in border-zone muscle cells that do not show histological evidence of necrosis. Lipid deposits also occur in some necrotic muscle cells at infarct's periphery, and occasionally in necrotic muscle cells at center of infarct. Infarct periphery also contains necrotic muscle cells at center of infarct. Infarct periphery also contains necrotic muscle cells with calcium deposits.

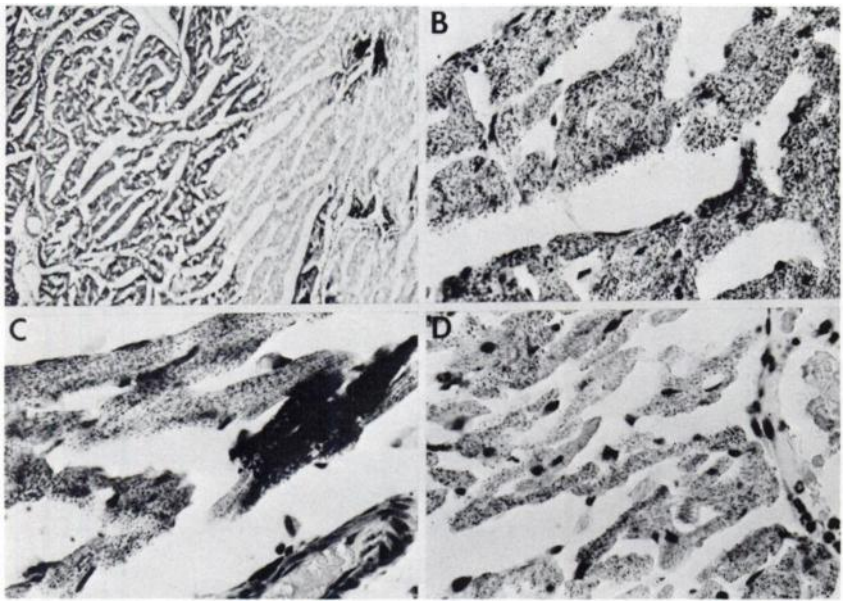

FIG. 2. Light micrographs of formalin-fixed, frozen sections of myocardium stained for fat. (A) Band of lipid-containing cells is present at edge of 48-hour-old infarct, and additional foci of lipid deposition are located deeper within infarct. (B) Muscle cells at edge of the 48-hour-old infarct contain numerous lipid droplets. (C) Muscle cells with lipid droplets are located adjacent to densely stained, heavily calcified muscle cells in periphery of 48-hour-old infarct (D) Muscle cells in region of 5-hour-old infarct contain lipid droplets. Oil-red-O and hematoxylin stains. (A, × 40; B, C, D, × 400% photographic reduction.)

left-atrial catheter 24 hr after proximal LAD occlusion; the dogs were killed 24 hr later and the hearts sectioned according to a previously established protocol (*1*) into (a) normal left ventricle, (b) border zone of the acute myocardial infarct, (c) periphery of the acute myocardial infarct, and (d) central subendocardial region of the area of infarction (Fig. 1). Tissue was removed from each of these areas for subsequent liquid-scintillation counting and for light-microscopic analysis. Each heart served as its own control, since myocardial tissue was removed from both ischemic and nonischemic regions of the left ventricle for scintillation counting and histology.

For well counting, each regional sample was weighed and then extracted twice with 10 ml of (2:1) chloroform-methanol following homogenization. The two 10-ml fractions were then combined and 1 ml of acid water was added according to the method of Folch et al. (*12*). This mixture was vigorously shaken and the phases were then separated by centrifugation. The lower chloroform phase containing lipid was removed and evaporated to dryness in glass counting vials. Aquasol (15 ml) was added and the contents were vigorously stirred with a Vortex mixer. The vials were refrigerated in the dark for 24 hr before being counted in a liquid-scintillation counter. Samples were counted repeatedly until stable count rates were obtained and an internal standard was used to correct for quenching. Radioactivity in these samples was subsequently expressed as cpm per gram of wet tissue.

In order to obtain information regarding cellular incorporation of intravenously injected [^{14}C] oleate-albumin ([^{14}C] FFA-albumin), a qualitative analysis was made of the C-14 content of myocardial lipids in heart tissue from three dogs injected with [^{14}C] FFA-albumin 6 hr after LAD occlusion, two dogs injected at 24 hr, and three injected at 48 hr. The animals in each group were then killed 24 hr after the labeled FFA was administered. These dogs therefore had myocardial infarctions for either 30, 48, or 72 hr before death. The tissue was extracted in chloroform-methanol as described above. Each extract was evaporated to dryness and then resolubilized in 10 ml chloroform-methanol (2:1). The phases were separated by addition of 2 ml 0.1 *M* KCl. The upper phase from each sample was withdrawn, placed in a counting vial, and evapo-

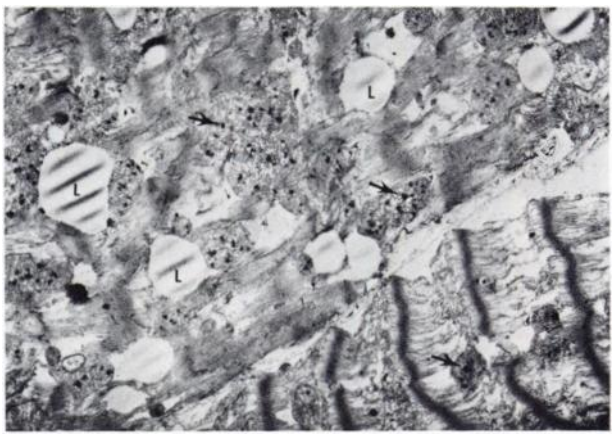

FIG. 3. Electron micrograph of two muscle cells from peripheral zone of a 48-hour-old myocardial infarct. One cell contains numerous lipid droplets (L). Both cells exhibit glycogen depletion, marked myofibrillar disruption, contraction-band formation, and amorphous matrix densities (flocculent densities) in mitochondria (arrows). The latter finding is confirmatory evidence of irreversible injury. (× 15,800% photographic reduction.)

rated to dryness. Aquasol (15 ml) was added to each sample for liquid-scintillation counting.

The lower phase from each sample was placed in a conical tube and evaporated to dryness. The residue was solubilized in 0.5 ml chloroform, spotted on 250-μ-thick silica gel G plates, and developed in (75:25:1) petroleum ether, diethyl ether, and acetic acid. Following identification by iodine vapor, the lipid spots were scraped from the plates directly into counting vials. Aquasol (15 ml) was added to each sample for liquid scintillation counting, and internal standards were used to correct for quenching. This procedure resulted in separation of myocardial lipids into the major lipid classes of phospholipid, fatty acid, triglyceride, and esterified cholesterol fractions. Their identity was confirmed using purified lipid standards: cholesterol, cholesterol oleate, triolein lecithin, and oleic acid. In some instances additional but unidentified bands were observed on the thin-layer plates, but none of these contained significant radioactivity.

RESULTS

The distribution of abnormal lipid accumulation in the myocardial infarcts is shown in Fig. 1. Major localization of oil-red-O-positive neutral lipid droplets occurred in a narrow, irregular band of muscle cells encompassing edges of areas of acute myocardial infarction (Fig. 2). Most muscle cells in this region had intact nuclei and lacked histologic features of necrosis. Some muscle cells containing oil-red-O-positive lipid droplets also were observed deeper within the peripheries of the infarcts in areas of histologically apparent necrosis (Fig. 2). Excess accumulation of lipid droplets was detected in sections obtained 5–6 hr after proximal LAD occlusion, but the amount of lipid accumulation was increased at 24 and 48 hr (Fig. 2).

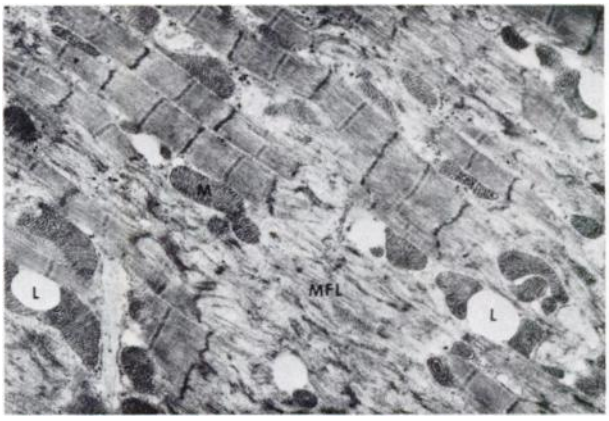

FIG. 4. Muscle cell from border zone of a 48-hour-old infarct. The cell contains lipid droplets (L) and shows evidence of damage, including decreased glycogen content, focal areas of myofibrillar lysis (MFL), and abnormal mitochondria (M). (× 13,900% photographic reduction.)

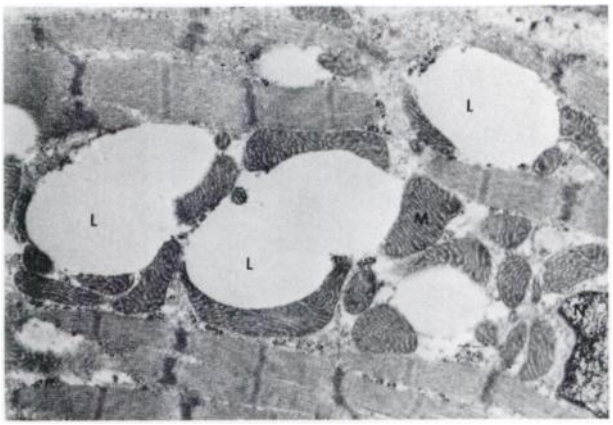

FIG. 5. Muscle cell from border zone of a 48-hour-old infarct. The cell contains numerous, coalescent lipid droplets (L), which are not bounded by membranes. The mitochondria (M) exhibit an unusual, condensed configuration, but are devoid of amorphous matrix (flocculent) densities. Glycogen is decreased; the nucleus (N) is normal. (× 22,500% photographic reduction.)

Electron-microscopic examination showed that the lipid accumulation occurred in the form of nonmembrane-bound lipid droplets. Some muscle cells with lipid droplets showed features suggesting irreversible injury (Fig. 3), including plasma-membrane defects, glycogen depletion, disrupted myofibrils, contraction bands, amorphous matrix (flocculent) densities in mitochondria, and in some instances, calcifications in mitochondria. Border-zone muscle cells with lipid droplets lacked ultrastructural features of irreversible injury, but many of these cells showed evidence of significant damage, including decreased glycogen, focal lysis of myofibrils, and abnormal mitochondria with a pronounced condensed configuration (Figs. 4 and 5). Other border-zone muscle cells with lipid droplets did not show any other alterations (Fig. 6).

In eight of nine dogs given the [^{14}C] oleate-albumin complex 24 hr after acute anterior myocardial infarction, significantly more radioactivity was found in the peripheral and border zones of the infarcts compared with the normal LV. The peak accumulation of the [^{14}C] oleate-albumin complex in these zones was 254 $\pm$ 46.6 (s.e.) % of that found in the normal LV ($p < 0.01$; Fig. 7). The central subendocardial portions of the acute myocardial infarcts showed significantly reduced oleate-albumin uptake, with a peak uptake of 58 $\pm$ 13.6% ($p < 0.01$) of normal LV (Fig. 7). Radioactivity in the most anterior subepicardial region of the infarct was lower than that noted in normal left ventricle, but the degree of reduction was not statistically significant (Fig. 7). In one dog, however, radioactivity in both the periphery and the central subendocardial regions of the infarct exceeded that in

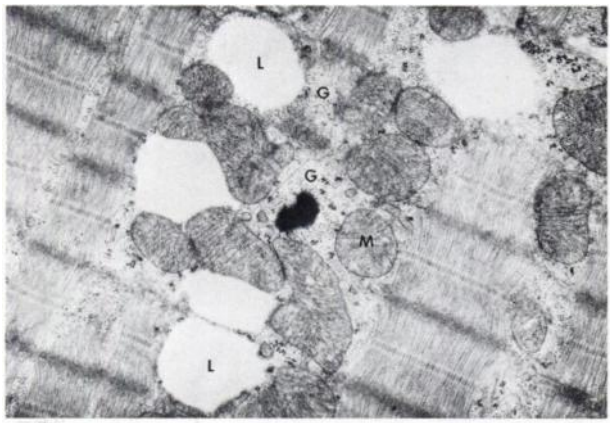

FIG. 6. Muscle cell from border zone of a 48-hour-old infarct. The cell has numerous lipid droplets (L), but exhibits otherwise normal ultrastructure. Note abundant glycogen deposits (G) and normal mitochondria (M). (× 19,000% photographic reduction.)

the normal left-ventricular region, with uptake being 321% greater in the periphery and 156% in the central subendocardial region of the infarct, compared with uptake in normal LV.

In the three dogs injected with FFA 6 hr after LAD occlusion and the two injected after 24 hr, the majority of the labeled material in the periphery and border-zone regions of the infarcts was incorporated into the triglyceride fraction of myocardial lipids (Table 1 and Fig. 8). In three dogs injected 48 hr after infarction, phospholipid contained 55% of the radioactivity in the periphery of the infarcted tissue, whereas FFA contained only 19% and triglyceride contained only 15% (Table 1 and Fig. 8).

DISCUSSION

The normal myocardium relies principally on free fatty acids for its nutrition, both at rest and during exercise (*13*). Indeed, it has been suggested that during rest and exercise the myocardium uses substrates in the following proportions: free fatty acids 65%, glucose 15%, lactate and pyruvate 12%, and amino acids 5% (*14*). Oliver has speculated that ischemic myocardium extracts proportionately more free fatty acid than normal myocardium, and that the proportion of substrates used by ischemic myocardium is approximately 70–75% from free fatty acids, 18–23% from glucose and the remainder presumably from amino acids (*14*). Several investigators have been particularly interested in the possibility that free fatty acids are "toxic" to ischemic myocardium (*5,15*). Thus, it has been demonstrated previously that: (a) elevation of free fatty acids in the plasma of dogs with myocardial ischemia can lead to ventricular arrhythmias without alteration of plasma catecholamine levels, (b) increased free fatty acids increase myocardial oxygen consumption in dogs, and (c) antilipolytic therapy reduces both the increase in oxygen consumption and the increased serum free fatty acids (*3–5,14–16*). Furthermore, Oliver and others have suggested that alteration of the two principle substrates, free fatty acids and glucose, used by the myocardium can influence the extent of myocardial damage during the course of development of acute myocardial ischemia, and that an excess of free fatty acids may extend the area of ischemia (*5,14,15*).

Others have also suggested that there is a reduction in [^{11}C] octanoate and C-11-labeled palmitate in regions of severe myocardial ischemia and infarction, and have used C-11 fatty acids to measure noninvasively the extent of myocardial infarction using positron-emission transaxial tomography (*7–9*).

It has been suggested that by using radionuclide-labeled fatty acids for myocardial imaging one may be able to determine the integrity of myocardial metabolic pathways that might be related to tissue viability (*8,9*).

Thus, whereas there is evidence suggestive that free fatty acids may on the one hand be toxic to ischemic myocardium and on the other might be used diagnostically to image acutely ischemic or infarcted myocardium, there is very little information regarding the exact location of the uptake of fat in the infarct zone and the degree of cellular damage associated with fat accumulation at various periods following experimental myocardial infarc-

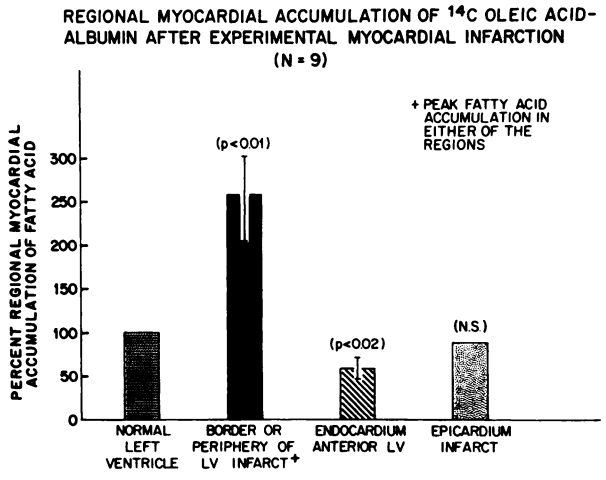

FIG. 7. Diagram illustrating regional myocardial accumulation when [^{14}C] oleate-albumin was injected 24 hr after proximal ligation of left anterior descending coronary artery in dogs and the animals were killed 24 hr later. Note significantly increased accumulation of [^{14}C] oleate-albumin in border and periphery of LV infarct. Site of maximal labeled fatty acid accumulation was chosen from border or periphery of infarct for this analysis. Also note significantly decreased accumulation of [^{14}C] oleate-albumin in central subendocardial region of infarct; however, labeled fatty acid accumulation was not significantly reduced from control values in the most anterior subepicardial portion of infarct.

TABLE 1. DISTRIBUTION OF INJECTED FFA IN LIPID IN MYOCARDIAL TISSUE

	Injection of FFA 6 hr after LAD occlusion				Injection of FFA 24 hr after LAD occlusion			Injection of FFA 48 hr after LAD occlusion			
	Dog 1	Dog 2	Dog 3	Avg*	Dog 4†	Dog 5	Avg*	Dog 6	Dog 7	Dog 8	Avg*
Normal LV tissue											
PL‡	13.6	18.4	17.0	16.3	9.7	30.2	20.0	86.4	81.6	72.4	80.1
FA§	14.2	12.5	11.7	12.8	6.2	7.6	6.9	2.6	4.2	16.8	7.9
TG‖	11.9	11.7	11.6	11.7	23.3	10.7	17.0	4.6	7.0	3.2	4.9
CE¶	9.8	11.4	12.8	11.3	32.6	8.7	20.7	1.9	2.8	0	1.6
Border of LV infarct											
PL	14.8	23.1	11.5	16.5	12.1	19.3	15.7	74.2	75.9	61.2	70.4
FA	11.6	12.6	11.0	11.7	6.4	4.7	5.6	12.9	10.1	20.0	14.3
TG	24.2	21.0	24.1	23.1	23.8	33.3	28.6	6.8	7.4	9.1	7.8
CE	8.1	7.5	7.2	7.6	3.4	4.8	4.1	1.9	0.9	1.5	1.4
Periphery of LV infarct											
PL	6.0	13.4	9.0	9.5	7.1	11.9	9.5	59.6	46.8	57.4	54.6
FA	6.8	14.3	9.5	10.2	7.5	5.1	6.3	16.5	20.0	21.3	19.3
TG	61.3	36.3	48.4	48.7	57.5	45.4	51.4	15.1	18.5	12.4	15.3
CE	4.2	7.6	5.7	5.8	2.3	4.7	3.5	2.0	2.0	1.8	1.9
Anterior subepicardium of LV infarct											
PL	8.3	7.7	17.5	11.2	6.2	8.7	7.5	41.8	60.5	40.8	47.7
FA	8.9	9.5	10.3	9.6	7.4	5.5	6.5	34.3	12.6	27.8	24.9
TG	51.3	48.2	26.8	42.1	64.2	44.5	54.4	15.6	1.7	22.8	13.4
CE	4.5	7.1	9.9	7.2	2.9	—	2.9	1.8	6.4	1.4	3.2
Anterior subendocardium of LV infarct											
PL	8.0	14.8	15.6	12.8	10.1	12.8	11.5	71.7	46.0	69.7	62.5
FA	6.8	13.6	11.6	10.7	9.9	8.2	9.1	1.1	5.3	14.4	6.9
TG	59.0	18.6	20.8	32.8	34.2	14.5	24.3	10.7	15.5	7.5	11.2
CE	3.9	15.7	11.1	10.2	7.3	7.5	7.4	8.4	7.7	0	5.4

* Percentage of counts per minute recovered in the four major lipid classes that were identified. Remainder of radioactivity was uniformly distributed in several unidentified bands, none of which represented a significant percentage of total counts in the organ.

† Dog died approximately 16 hr after FFA was injected.

‡ PL = phospholipid.

§ FA = fatty acid.

‖ TG = triglyceride.

¶ CE = cholesterol ester (free cholesterol was not labeled above background).

tion. The results of the present study demonstrate that increased fatty acid accumulation (either uptake and/or abnormal retention of labeled fatty acid material) is a characteristic feature of the border and peripheral zones of experimental myocardial infarcts, the major location of lipid accumulation being in the border zone.

It is unclear whether FFA-albumin or VLDL-TG serves as the principal source of the fatty acids. In our initial experiments, dual-labeled VLDL-TG failed to produce measurable radioactivity in the myocardial tissue, but the radioactivity in the in vivo preparation may have been too low to yield a positive result. The I-125 VLDL failed to produce radioactivity in the myocardium, suggesting that the lipid accumulation was not due to intracellular entrapment of whole VLDL particles by ischemic cells. One cannot exclude the possibility, however, that whole VLDL particles were taken up by phagocytosis, with rapid digestion of the protein portion and storage of the lipid portion. The intracellular accumulation of I-125 may, therefore, have been missed. We have shown, however, that intravenously injected [^{14}C] oleate-albumin readily concentrates in peripheral infarct regions and that the [^{14}C] oleate-albumin injected at 6 or 24 hr after coronary occlusion accumulates in the triglyceride fraction of infarcted tissue and in the border and peripheral regions of the infarct. These data suggest that the injected [^{14}C] oleic acid is incorporated into the neutral lipid droplets observed microscopically. The data obtained in this study, however, do not, allow us to determine rates of fatty acid uptake by different regions of the infarct tissue.

Accumulation of lipid in the border zone of the infarct supports the thesis that these injured cells

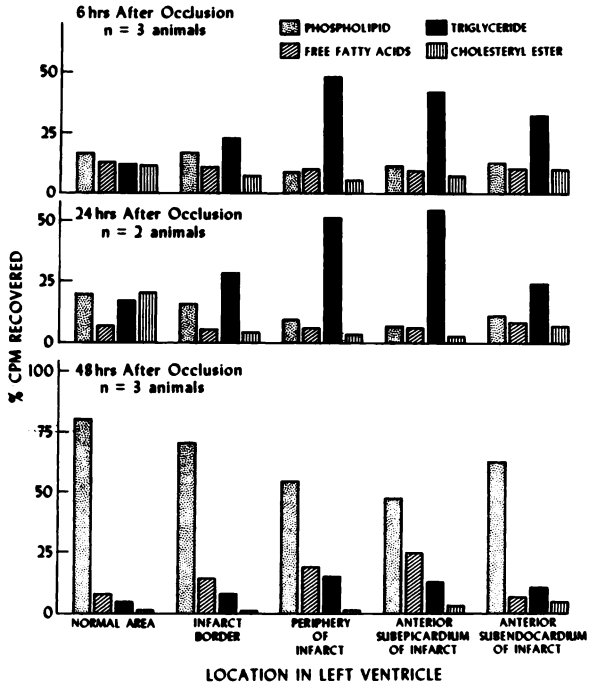

FIG. 8. Distribution of injected free fatty acid in myocardial lipids at various times following coronary artery occlusion, as measured in different portions of left ventricle. Bars indicate mean values listed in Table 1.

continue to take up FFA but fail to oxidize it for contractile energy. Presumably, this increased accumulation of fat after myocardial infarction is a manifestation of the inability of ischemic muscle cells to metabolize fat at normal metabolic rates. Previously, Scheuer and Brachfeld have demonstrated that during acute myocardial ischemia there is a decrease in [^{14}C] palmitate incorporation into tissue free fatty acid and an increase in the triglyceride content in ischemic tissue, which suggested to these investigators that ischemia is associated with a reduced rate of triglyceride metabolism (*17*). Work by Wood et al. has suggested a possible mechanism for the abnormal lipid accumulation, namely impaired mitochondrial metabolism of long-chain fatty acids resulting from reduced levels of carnitine and activity of carnitine palmityl coenzyme A transferase (*18*).

The histologic data obtained in the present study demonstrate that accumulation of fat occurs as early as 6 hr after proximal LAD occlusion and that increasing amounts of fat appear at 24 and 48 hr, thus confirming the earlier observations of Wartman et al. (*2*). These workers also concluded that "neutral fat" appeared in appreciable quantity within the sarcoplasma of fibers partially injured by ischemia, but that probably most of the fibers were only reversibly injured. The precise source of the "neutral fat" accumulating around the area of infarction was not identified (*2*). Our data indicate that one source of the increased myocardial fat observed after infarction is circulating fatty acids, and that the greatest accumulation occurs in border zones adjacent to the area of myocardial necrosis. The data also suggest that accumulation of radioactive FFA occurs in some lipid-laden, irreversibly injured myocardial cells in the periphery of the infarct and occasionally even in its central portion (Figs. 2, 3, and 4). Nevertheless, since most samples contain mixtures of border, peripheral, and central zones (Fig. 1), one cannot be certain as to which cell populations are responsible for uptake and incorporation of label injected several hours or longer after coronary occlusion.

The marked accumulation of fat in border and peripheral zones—occasionally even in the center of the infarcted area—has certain implications as regards attempts to identify regions of myocardial ischemia through scintigraphic filling defects (*7–9*). These techniques are based on the premise that uptake of labeled fatty acids is limited to myocardial cells with normal, or only minimally impaired, metabolism. However, our findings show that structurally damaged or necrotic muscle cells in border and peripheral infarct regions also concentrate fat, in excess of that noted in nonischemic myocardium, 6–24 hr after the onset of experimental myocardial infarction, indicating that those cells in the peripheral and border zone regions of myocardial infarcts cannot be considered metabolically normal. Furthermore, it was of interest to find a different distribution of injected fatty acid in tissue lipid, depending on when the fatty acid was injected after LAD occlusion. When the injection was delayed until 48 hr after LAD occlusion, the majority of the injected material was incorporated into phospholipids rather than triglyceride. Since the alteration in the incorporation of the injected material was noted in both normal and injured left-ventricular tissue (Table 1 and Fig. 8), it appears to be temporally related to the metabolic handling of the injectate rather than to any specific alteration in lipid metabolism in just ischemic tissue.

Further studies are needed to determine the pathophysiological significance of lipid-laden muscle cells in infarcted myocardium. Wit and colleagues have shown that lipid accumulation and altered electrophysiological behavior may be reversible phenomena in Purkinje fibers located in the immediate subendocardium (*19,20*). These workers also have proposed that the lipid-laden, acutely ischemic Purkinje cells are the source of ventricular arrhythmias occurring during the first few days after myocardial infarction (*19,20*). Our study has shown, however, that muscle cells of the contractile myocardium represent the

vast majority of cells with abnormal lipid accumulation following myocardial infarction. We are currently uncertain as to the ultimate fate of the lipid-laden cells that do not exhibit ultrastructural criteria of irreversible damage, in the border zones of acute myocardial infarcts, but this is a matter that needs additional evaluation. Furthermore, the potential role of lipid-laden cells of the contractile myocardium in the genesis of ventricular arrhythmias during myocardial infarction also remains to be elucidated.

ACKNOWLEDGMENTS

This work was supported by NIH Ischemic Heart Disease Specialized Center of Research (SCOR) Grant HL-17669 and NIH Grants HL-17777, HL-15522, and HL-15949.

The authors gratefully acknowledge the expert technical assistance of Judy Ober, Janice McNatt, Curtis Garner, Gary C. Fink, Nancy Arnold, Charlene McCoy, Angela Lewis, G. Michael Funk, and Scott Madison, and the secretarial assistance of Donna Place, Belinda Lambert and Mary Rich.

REFERENCES

1. BUJA LM, PARKEY RW, STOKELY EM, et al: Pathophysiology of technetium-99m stannous pyrophosphate and thallium-201 scintigraphy of acute anterior myocardial infarcts in dogs. *J Clin Invest* 57: 1508–1522, 1976

2. WARTMAN WB, JENNINGS RB, YOKOYAMA HO, et al: Fatty change of the myocardium in early experimental myocardial infarction. *Arch Pathol* 62: 318–323, 1956

3. OLIVER MF, KURIEN VA, GREENWOOD TW: Relation between serum-free-fatty-acids and arrhythmias and death after acute myocardial infarction. *Lancet* 1: 710–714, 1968

4. KURIEN VA, YATES PA, OLIVER MF: The role of free fatty acids in the production of ventricular arrhythmias after acute coronary occlusion. *Europ J Clin Invest* 1: 225–241, 1971

5. MJØS OD, KJEKSHUS JK, LEKVEN J: Importance of free fatty acids as a determinant of myocardial oxygen consumption and myocardial ischemic injury during norepinephrine infusion in dogs. *J Clin Invest* 53: 1290–1299, 1974

6. BONTE FJ, GRAHAM KD, MOORE JG: Experimental myocardial imaging with ^{131}I-labeled oleic acid. *Radiology* 108: 195–196, 1973

7. WEISS ES, HOFFMAN EJ, PHELPS ME, et al: External detection of altered metabolism of ^{11}C-labeled substrates in ischemic myocardium. *Clin Res* 23: 383A, 1975 (abst)

8. WEISS ES, HOFFMAN EJ, PHELPS ME, et al: External detection and visualization of myocardial ischemia with ^{11}C-substrates *in vitro* and *in vivo*. *Circ Res* 39: 24–32, 1976

9. WEISS ES, AHMED SA, WELCH MJ, et al: Quantification of infarction in cross sections of canine myocardium *in vivo* with positron emission transaxial tomography and ^{11}C-Palmitate. *Circulation* 55: 66–73, 1977

10. VAN HARKIN DR, DIXON CW, HEIMBERG M: Hepatic lipid metabolism in experimental diabetes. V. The effect of concentration of oleate on metabolism of triglycerides and on ketogenesis. *J Biol Chem* 244: 2278–2285, 1969

11. BILHEIMER DW, EISENBERG S, LEVY RI: The metabolism of very low density lipoprotein proteins. 1. Preliminary *in vitro* and *in vivo* observations. *Biochim Biophys Acta* 260: 212–221, 1972

12. FOLCH J, LEES M, SLOANE-STANLEY GH: A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 226: 497–509, 1957

13. LASSERS BW, CARLSON LA, KAIJSER L, et al: The nature and control of myocardial substrate metabolism in healthy man. In *Effect of Acute Ischemia on Myocardial Function*, Oliver MF, Julian DG, Donald KW, eds. Edinburgh, Churchill Livingstone, 1972, p 200

14. OLIVER MF: The influence of myocardial metabolism on ischemic damage. *Circulation* 53: (Suppl I): I-167–170, 1976

15. KURIEN VA, OLIVER MF: A metabolic cause for arrhythmias during acute myocardial hypoxia. *Lancet* 1: 813–815, 1970

16. OLIVER MF: The metabolic response to acute myocardial infarction. In *Coronary Care*, Meltzer LA, Dunning AG, eds. Amsterdam, Excerpta Medica, 1973, p 231

17. SCHEUER J, BRACHFELD N: Myocardial uptake and fractional distribution of palmitate-1 C^{14} by the ischemic dog heart. *Metabolism* 15: 945–954, 1966

18. WOOD JM, SORDAHL LA, LEWIS RM, et al: Effect of chronic myocardial ischemia on the activity of carnitine palmitylcoenzyme A transferase of isolated canine heart mitochondria. *Circ Res* 32: 340–347, 1973

19. FRIEDMAN PL, FENOGLIO JJ JR, WIT AL: Time courses for reversal of electrophysiological and ultrastructural abnormalities in subendocardial Purkinje fibers surviving extensive myocardial infarction in dogs. *Circ Res* 36: 127–144, 1975

20. FENOGLIO JJ JR, ALBALA A, SILVA FG, et al: Structural basis of ventricular arrhythmias in human myocardial infarction: A hypothesis. *Human Pathol* 7: 547–563, 1976