Evidence for the Chylomicron Origin of Lipids Accumulating in Diabetic Eruptive Xanthomas: a Correlative Lipid Biochemical, Histochemical, and Electron Microscopic Study

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ABSTRACT Plasma lipoprotein alterations in nine insulin-dependent diabetics with hyperlipemia have been related to the lipid accumulating in eruptive xanthomas evolving in these patients. Histochemical and electron microscopic examination of xanthomas have been correlated with the lipid analyses in order to obtain additional evidence regarding the lipoprotein origin of lipids accumulating in the lesions. Both analytical and morphologic evidence suggested that circulating chylomicrons significantly contribute to the xanthoma lipids. All the patients had large quantities of circulating triglyceride-rich chylomicrons which carried approximately 70% of the triglyceride found in the plasma. The fatty acid pattern of chylomicron and xanthoma triglycerides were similar. Triglyceride constituted the major lipid found in the xanthomas when they were sampled during their eruption. These findings, taken in conjunction with histochemical and electron microscopic evidence of chylomicron particles in the dermal capillary walls, support the theory that blood lipoproteins, and particularly chylomicrons, permeated the vascular walls and the triglycerides carried by these lipoproteins apparently accumulated in tissue macrophages and perithelial cells which evolved into foam cells. Initiation of appropriate therapy resulted in clearance of the chylomicronemia and a concomitant resolution of the xanthomas as reflected by a decrease in total xanthoma lipid. Sequential studies of resolving xanthomas in five patients revealed that xanthoma triglyceride was mobilized more rapidly than cholesterol, resulting in a redistribution of the xanthoma lipids, so that the resolving lesions were cholesterol rich. Consistent with this change in lipid composition, correlative electron microscopy revealed loss of amorphous material from many of the foam cell vacuoles.

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INTRODUCTION

An understanding of the process by which lipids accumulate in foam cells of the dermis to form xanthomas has significance because the development of these lesions may reflect analogous pathologic reactions leading to the formation of atherosclerotic plaques (1). The lipids found in xanthomas are thought to be derived from circulating plasma lipoproteins (2). This assumption is based on the well known clinical observations that xanthomas may appear and regress with the rise and fall in plasma lipids (3) as well as radioisotopic tracer studies which have demonstrated that plasma lipoproteins find their way into the cutaneous lesions (4).

Analytical studies, however, have revealed significant differences between the lipid composition of xanthomata and the plasma lipids and lipoproteins (5–8). Indeed, cholesterol is the major lipid found in various forms of xanthomas regardless of whether hypercholesterolemia or hypertriglyceridemia is present (5–8). These dissimilarities are even greater when the fatty acid content of certain lipid classes in the circulation are compared with those in xanthomas (5–8). Thus, while certain clinical and metabolic observations support the popular theory of the plasma origin of some xanthoma lipids (4, 9), analytical studies apparently do not substantiate this widely held view.

In contrast to the past analytical investigations, the present study made use of eruptive xanthomas developing in patients with insulin-dependent diabetes mellitus. Analyses of xanthomas in these patients offer certain advantages over the investigations previously cited. First, the lesions may be analyzed early in their evolution as they appear in association with distressing complaints related to uncontrolled diabetes and marked hyperlipemia. Second, the xanthomas are readily studied during their resolution since they regress rapidly in conjunction with dietary and insulin therapy. Third, these xanthomas typically develop in the presence of an elevation in primarily one type of lipoprotein, the chylomicrons (10, 11), so that the xanthoma lipids should resemble specifically chylomicron lipids if circulating lipoproteins are involved in the pathogenesis of the lesions. Chylomicrons are easily and specifically isolated for lipid analysis (12) and they are of large enough size to be visualized by electron microscopy (13).

Accordingly, in the present investigation lipid analytical findings are correlated with histochemical and ultrastructural morphologic information in order to obtain additional evidence regarding the plasma origin of xanthoma lipids, as well as to examine certain interrelationships between circulating lipoproteins and dermal connective tissue. This investigation provides both analytical and morphologic evidence that chylomicrons significantly contribute to the lipid accumulating in diabetic eruptive xanthomas and it further demonstrates a close correlation between ultrastructural and compositional alterations within the xanthomas as lipid is mobilized from resolving lesions.

METHODS

Clinical procedures

Nine patients with diabetic lipemia and eruptive xanthomas were studied. Six to eight xanthomas were initially excised from each patient under local xylocaine anesthesia within a day of hospitalization, prior to the initiation of therapy. The xanthomas were utilized for lipid analytical, histochemical, and electron microscopic examinations. Xanthomas were also studied in an identical manner in five patients at several points in time during the resolution of the lesions, while the patients received fat-free diets and (or) insulin therapy.

At the time of each biopsy, fasting plasma samples for determination of total plasma and chylomicron lipid distribution and fatty acid composition were obtained.

Postheparin lipolytic activity (PHLA) was measured by the method of Fredrickson, Ono, and Davis (14) after intravenous administration of heparin (380 U per square meter of body surface area).

Prior to hospitalization, all the patients were ingesting normal (40% fat) diets ad lib., although several had decreased their dietary intake for several days because of abdominal pain.

Laboratory methods

Procedures on xanthomas. Four to five of the six to eight xanthomas removed at each sampling were rinsed in ice-cold isotonic saline at which time adherent adipose tissue was carefully cleaned away under observation with a dissecting microscope. The xanthomas were then blotted dry, weighed, homogenized in a tissue grinder, and the lipids extracted by the method of Folch, Lees, and Sloane Stanley (15). One of the remaining xanthomas was fixed in 10% formalin and frozen sections were stained for neutral lipid with Oil Red O (16), for phospholipid with Baker's acid hematin (17) and for acid phosphatase (18). The remaining two additional xanthomas which were biopsied with a high-speed rotary 2 mm punch drill were prepared for electron microscopy as previously described (19). These specimens were examined with an RCA-EMU 2C electron microscope after staining with 3% uranyl acetate (10–30 min) and lead citrate (3–5 min). In several cases, chylomicrons isolated from the plasma by polyvinylpyrrolidone flocculation (20) were fixed in osmium tetroxide and *s*-collidine buffer (19) and after embedding in epoxy resin and sectioning on an LKB Ultramicrotome, they were also examined by electron microscopy.

Procedures on plasma and chylomicron separation. Chylomicrons were isolated by polyvinylpyrrolidone (PVP) flocculation (20) from plasma samples of venous blood collected in ethylenediaminetetraacetate (EDTA) tubes after an overnight fast. Columns of 3% PVP were used rather than 0–5% gradients because of the relative ease of preparation and the demonstration that both methods provide similar separations (12). Recovery of the chylomicrons ("dietary" or "primary particles") was accomplished quantitatively with a tube slicer. Total plasma and chylomicron lipids were then extracted and washed by the method of Folch et al. (15) and the plasma lipids and chylomicron composition were calculated.

Lipid separations and quantitation. Free and esterified cholesterol (21), free fatty acids (22), triglycerides (23), and phospholipids (24) were determined in the xanthoma, plasma, and chylomicron lipids after separation by thin-layer silicic acid chromatography as described by Parker, Rauda, and Morrison (25). The per cent composition by weight of each major lipid class was calculated after the following corrections were made: (*a*) lipid phosphorus (P) $\times$ 25 = phospholipid; (*b*) total sterol in cholesteryl ester $\times$ 1.5 = total cholesteryl ester (assuming average molecular weight of fatty acid = 280); (*c*) total free fatty acids in microequivalents $\times$ 280 = total micrograms of free fatty acids.

The fatty acid composition of each of the xanthoma, plasma, and chylomicron classes was determined by methods previously described (26, 27). This includes scraping the separated lipid classes off thin-layer plates directly into 20-ml reflux tubes and transmethylating by the method of Ways, Reed, and Hanahan (28) except that the methyl esters were extracted three times in 2 ml of redistilled hexane. The hexane extract was washed twice with 15 ml of glass-distilled water, dried under nitrogen, and redissolved in a suitable volume of hexane. In the case of cholesteryl esters, after methylation, the resulting fatty acid methyl esters and free cholesterol were separated by thin-layer chromatography and the methyl esters were eluted from the silica gel with 15 ml of redistilled ether.

Gas-liquid chromatography was performed on a Barber-Colman Model 5000 apparatus (Barber-Colman, Chroma-

TABLE I

Clinical Findings in Diabetics with Eruptive Xanthomas at the Time of Hospitalization

Patient	Sex	Age	Duration of diabetes	Duration of xanthoma	FBS	HCO_3^-	PHLA*	Comments
		yr		*wk*	*mg/100 ml*	*mmoles/liter*	*μEq FFA/ml per min*	
1	F	48	14 yr	2	242	24	0.16/0.24	
2	M	38	1 yr	4	404	14	0.18/0.58	Pancreatitis‡
3	M	39	1 wk	4	310	—	—	
4	M	40	1–2 yr	12	298	20	0.28/—	
5	M	22	1–2 months	8	306	19	0.25/0.59	
6	M	33	2 yr	2	324	23	0.29/—	
7	F	42	1 month	3	500	22	0.24/0.35	
8	M	61	2 wk	2	280	30	0.41/0.36	Severe coronary disease
9	M	39	1½ yr	2	370	17	0.12/0.48	Pancreatitis‡

* Values cited are peak (10 min PHLA) pre/post diabetic therapy.
‡ ↑ Amylase.

tography Products Div., Rockford, Ill.) equipped with a hydrogen flame detector, utilizing glass columns 6 ft × 5 mm I.D. packed with 19 g of 12% ethylene glycol succinate polyester on 60-80 mesh Chromosorb W (Applied Science Labs., Inc., State College, Pa.). A column temperature of 190°C was maintained with a nitrogen carrier gas flow of 175 ml/min at the outlet. Peaks were identified by comparison with standards, with the aid of logarithmic plots of relative retention time *versus* chain lengths, or degrees of unsaturation, and with the use of Apiezon columns (Apiezon L, 12% on Anakrom AB, 70/80 mesh, Analabs, Inc., North Haven, Conn.) before and after hydrogenation of representative samples. Relative mass distributions were calculated from the major peaks (<16:0, 16:0, 16:1, 17:0–17:1, 18:0, 18:1, 18:2, 18:3, 20:4, and >20:0[1]) by triangulation. Qualitative results with NIH fatty acid standards A to D agreed with the stated composition data with an error of less than 15% (29).

RESULTS

The clinical and laboratory findings in the nine diabetics are summarized in Table I. Only five patients had known diabetes prior to hospitalization, although all were symptomatic for periods varying from weeks to months. Xanthomas had been noted for 2–12 wk and in most cases, new xanthomas were still evolving when they initially sought medical help. All subjects demonstrated fasting hyperglycemia and mild, if any, degrees of ketoacidosis, similar to previous findings in diabetic lipemia (10, 11). The postheparin lipolytic activity (PHLA) was either less than normal (cases 1, 2, and 9) or at the lower limits of normal (cases 4 through 7) in every patient except one (case 8). The cutaneous lesions, hyperlipemia, and symptoms in all patients were reversed by insulin administration. Following exogenous insulin therapy, the PHLA activity increased in five of the six cases where this measurement was performed (11).

The distribution of the major lipid classes isolated from the eruptive xanthomas of the nine patients when they were initially hospitalized are shown in Table II. The total lipid content of the xanthomas ranged between 0.6 and 10.6% of wet weight. In each case the predominant lipid was triglyceride, representing an average of 44% of the total xanthoma lipid, although the absolute quantities of triglyceride varied from 3.0 to 58.0 μg/mg wet weight of tissue. Total cholesterol accounted for about 24% of the xanthoma lipids with an average of 58% of the total in the esterified form. Phospholipids and free fatty acids averaged 20 and 11% of the total lipid content, respectively.

Triglyceride also was the major plasma lipid in each case (Table III). Approximately 70% (mean) of the total plasma triglyceride was carried in the chylomicron fraction. Less marked increases were observed in plasma free and esterified cholesterol lipids which were primarily carried in nonchylomicron lipoproteins.

The relative percentages (moles per 100 moles) of the fatty acids found in the triglycerides accumulating in the xanthomas appeared to approximate closely the triglyceride fatty acids of plasma and chylomicrons (Table IV).

However, the fatty acid distribution in xanthoma cholesteryl ester, free fatty acids, and phospholipids differed from those found in their corresponding plasma and chylomicron lipid fractions (Table V). Xanthoma cholesteryl esters contained less percentages of linoleic acid (18:2) and more oleic acid (18:1) and long-chain

[1] Shorthand designation for fatty acids identified by chain length and number of double bonds.

TABLE II

Distribution of Major Lipids in Diabetic Eruptive Xanthomas

Patient	% Total lipid in wet wt xanthoma	Triglycerides		Cholesteryl esters		Free cholesterol		Free fatty acid		Phospholipid	
		μg/mg*	%‡	μg/mg*	%‡	μg/mg*	%‡	μg/mg*	%‡	μg/mg*	%‡
1	4.0%	18.3	41	8.8	18	8.0	17	1.7	4	8.2	20
2	1.9%	9.0	47	2.2	11	2.0	11	3.4	17	2.5	14
3	0.6%	3.1	49	0.9	14	0.4	6	1.3	20	0.1	11
4	10.6%	58.0	54	22.0	20	13.0	12	1.2	1	11.5	13
5	1.8%	8.0	45	2.2	13	1.0	7	1.3	7	5.0	28
6	1.6%	6.2	40	1.6	10	1.2	8	1.6	10	5.0	32
7	7.5%	35.9	48	9.3	12	8.2	11	10.7	15	10.6	14
8	1.2%	4.5	39	2.1	16	0.7	6	1.7	12	3.4	27
9	2.9%	9.0	33	5.1	17	4.5	15	2.0	7	8.5	28
Mean ±SD	3.5 ±3%	18 ±14	44 ±6	6.3 ±5	14 ±5	4.3 ±12	10 ±6	3 ±2	11 ±6	7 ±2	20 ±7

* μg of lipid per mg wet weight of tissue.

‡ Per cent of total identified lipid found in the xanthoma.

fatty acids (> 20:0) than the plasma and chylomicrons in each patient studied. Free fatty acids in the xanthomas consistently displayed relatively more palmitoleic acid (16:1) and stearic acid (18:0) than the plasma (Table V). Finally, fatty acids in xanthoma phospholipid showed relatively less linoleic acid than either plasma or chylomicrons (Table V). Therefore, although xanthoma triglyceride fatty acids were similar to the fatty acid distribution found in the plasma and chylomicron triglycerides, the fatty acid patterns of other lipids in the xanthomas displayed variations from the corresponding lipids in the circulation.

Oil Red O staining of the xanthomas of all nine patients revealed myriads of lipid droplets in the walls of many dermal capillaries found throughout the evolving xanthomatous lesions (Fig. 1). It can be seen in Fig. 1 that the dermal vessels were precisely outlined by various sized lipid-staining droplets. It is not possible to determine with the light microscope whether the lipid droplets around the vascular channels are within the cells

TABLE III

Distribution of Major Lipids in Plasma and Chylomicrons

Patient	Total lipid	Triglycerides		Cholesteryl esters		Free cholesterol		Free fatty acid, plasma	Phospholipids plasma
		Plasma	% as chylo*	Plasma	% as chylo*	Plasma	% as chylo*		
	mg/100 ml	mg/100 ml		mg/100 ml		mg/100 ml		mg/100 ml	mg/100 ml
1	4177	2574	—	707	—	279	—	42	575
2	9530	7790	60	520	18	330	26	180	710
3	3260	1660	83	590	11	250	21	160	600
4	9368	6450	49	1452	10	650	15	54	762
5	6060	4780	89	450	27	230	26	200	400
6	3280	2580	97	250	30	110	35	60	280
7	3005	2521	50	101	17	63	19	80	240
8	3254	1744	61	796	5	228	12	18	468
9	6960	4596	60	739	—	522	—	63	1059
Mean ±SD			70 ±16‡		17 ±7‡		22 ±7‡		

* Per cent of the plasma lipid class found in chylomicron fraction of lipoproteins.

‡ Average and standard deviations of per cent of the plasma lipid found in the chylomicrons.

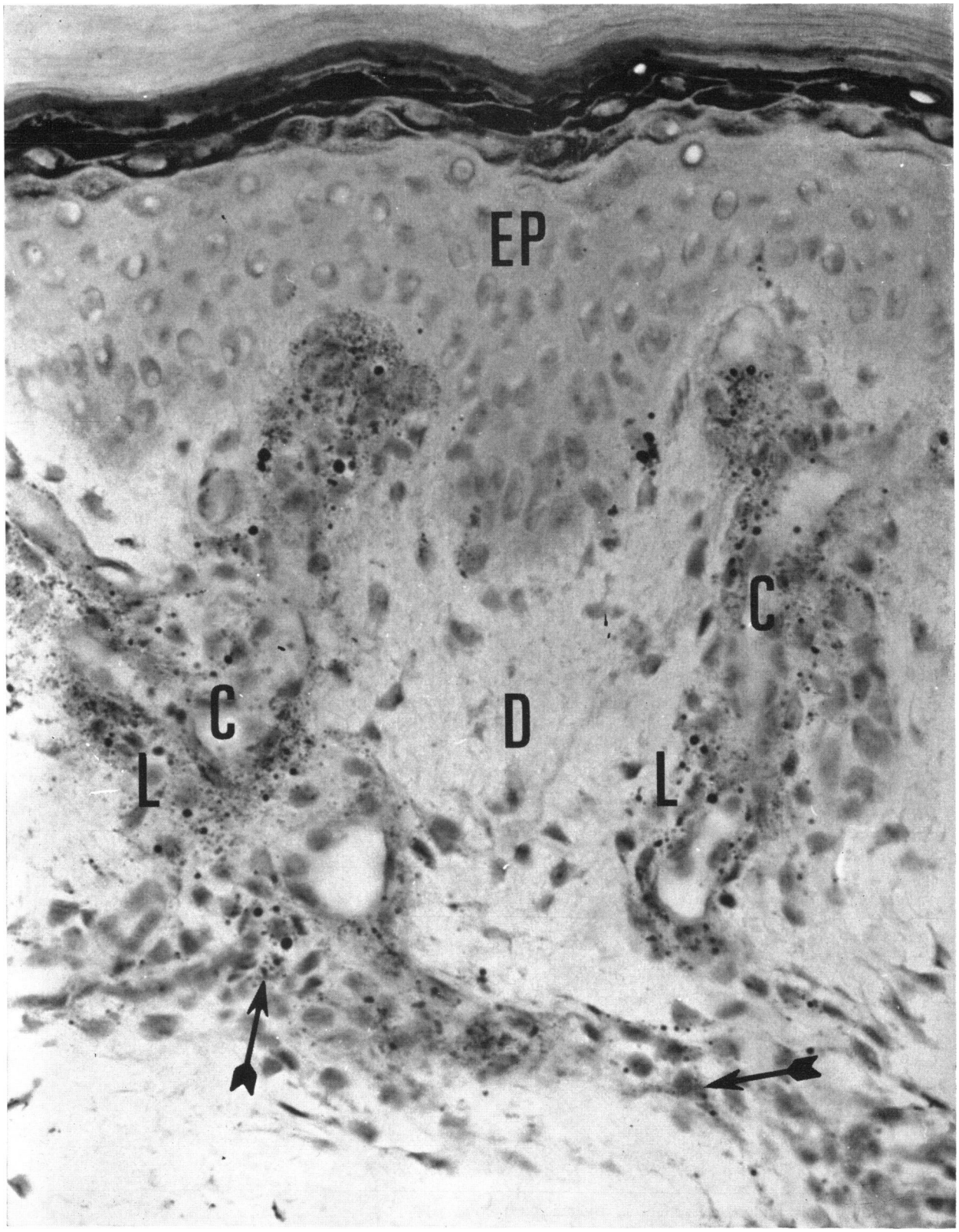
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TABLE IV

*Triglyceride Fatty Acid Composition of Xanthomas, Plasma, and Chylomicrons**

	% fatty acids									
	<16:0	16:0	16:1	17:0–17:1	18:0	18:1	18:2	18:3	20:4	>20:0
Xanthomas	3 ±1	24 ±3	4 ±1	Trace	8 ±1	41 ±4	12 ±3	2 ±0	2 ±1	3 ±1
Plasma	3 ±1	26 ±1	3 ±2	1 ±0	8 ±3	40 ±5	12 ±3	1 ±1	1 ±1	1 ±1
Chylomicrons‡	3 ±1	26 ±1	3 ±2	1 ±0	8 ±1	41 ±3	11 ±4	1 ±1	2 ±0	1 ±2

* Expressed as the mean ±SD of moles per 100 moles of each fatty acid or groups of fatty acids of the nine diabetic patients studied.

‡ Mean ±SD for eight of the nine patients. Chylomicrons were not analyzed in patient 1.

TABLE V

*Fatty Acid Composition of Cholesteryl Esters, Free Fatty Acids, and Phospholipids of Xanthomas, Plasma, and Chylomicrons from Nine Diabetic Patients**

		Fatty acids									
		<16:0	16:0	16:1	17:0–17:1	18:0	18:1	18:2	18:3	20:4	>20:0
Xanthomas	CE	3 ±2	20 ±6	5 ±1	1 ±1	7 ±3	34 ±6	14 ±4	2 ±1	3 ±3	11 ±4
	FFA	3 ±4	33 ±9	22 ±1	2 ±1	32 ±7	11 ±4	2 ±1	3 ±4	Trace	9 ±6
	PL	4 ±3	27 ±7	1 ±1	2 ±1	21 ±3	18 ±3	12 ±4	1 ±0	7 ±5	8 ±4
Plasma	CE	2 ±1	18 ±4	4 ±2	Trace	3 ±1	26 ±4	40 ±6	Trace	4 ±3	3 ±2
	FFA	4 ±1	31 ±7	3 ±1	2 ±1	15 ±1	24 ±7	8 ±5	7 ±1	1 ±1	7 ±2
	PL	1 ±1	33 ±4	Trace	1 ±0	14 ±3	15 ±2	20 ±5	Trace	9 ±5	8 ±4
Chylomicrons‡	CE	4 ±6	23 ±7	4 ±1	Trace	5 ±2	23 ±5	30 ±8	1 ±2	3 ±2	5 ±1
	PL	1 ±2	24 ±7	2 ±1	1 ±1	20 ±8	24 ±6	15 ±4	1 ±1	3 ±4	7 ±2

* Mean ±SD of moles per 100 moles of each fatty acid or groups of fatty acids of nine diabetic patients studied.

‡ Mean ±SD for eight of the nine patients.

of the vessels or in an extracellular position. However, foam cells adjacent to the vessels contain lipid within their cytoplasm (arrows). The foam cells reacted positively with Baker's acid hematin stain for phospholipid and acid phosphatase (not illustrated).

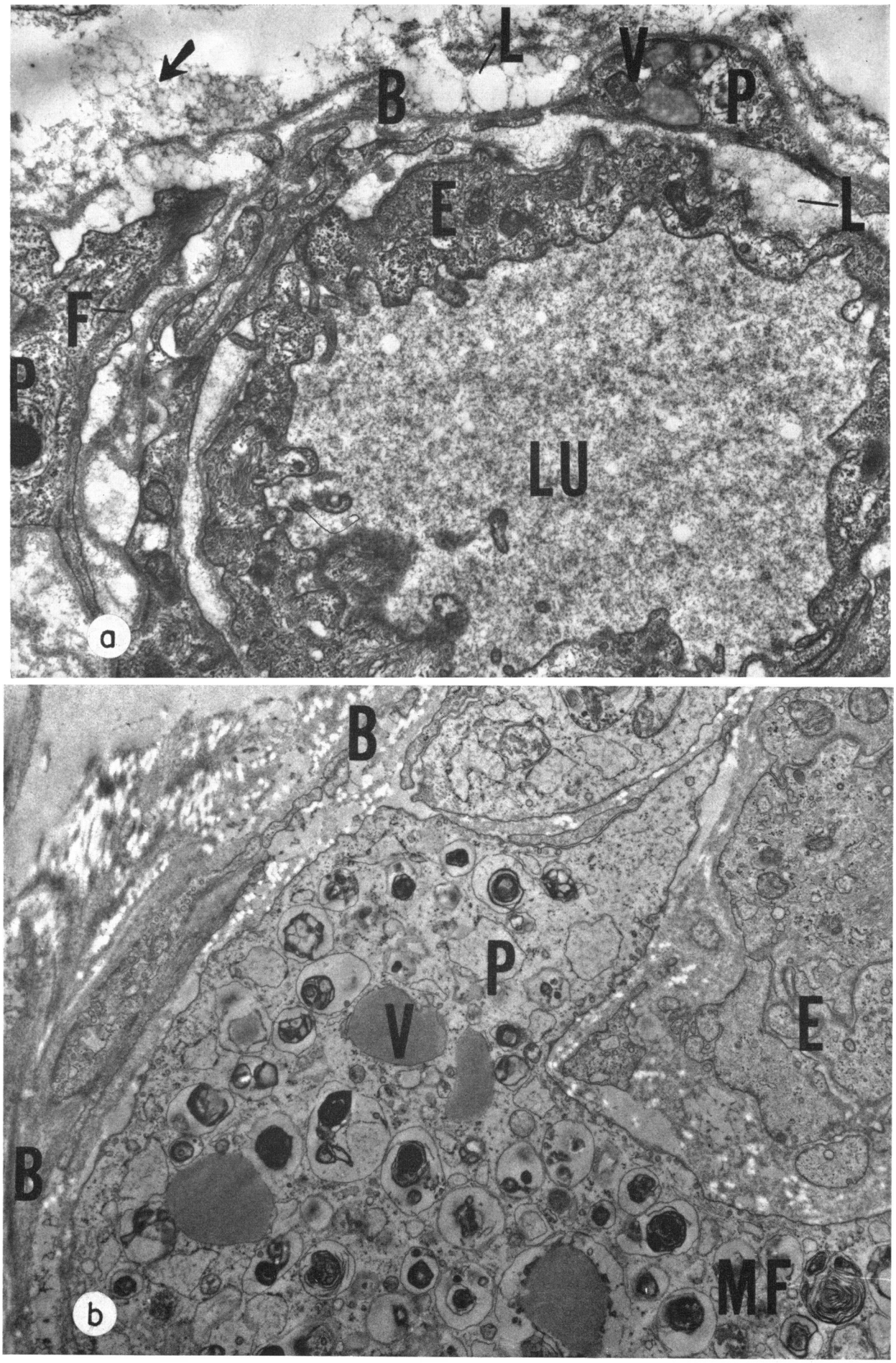
Electron microscopic examination of the dermal capillaries in the evolving diabetic xanthomas of all nine patients revealed aggregations of extracellular droplets both within the basal lamina of the capillary wall and out in the perivascular space, often in close proximity to the perivascular foam cells (Figs. 2 A and 3). At higher magnifications (Fig. 3) it is more easily seen that the droplets showed considerable variation in their size, ranging from 200–300 A to 5000 A in diameter. The average diameter, however, of the majority of the droplets was 2000 ±1000 A. The droplets were circular in cross section, often with an increase in density in their central region and an indistinct narrow band of granular material at the periphery. The droplets in the capillary walls closely resembled chylomicrons isolated from the circulation when these were examined by electron microscopy (inset Fig. 3).

Droplets were not observed within the endothelial cell cytoplasm or in the intercellular spaces between the endothelial cells.

No consistent thickening of the basal lamina of these blood vessels was evident whereas such a change has been reported in the muscle capillaries of diabetics (30).

Perithelial cells, a structural component of the capillary wall, are identified as elongated cells adjacent to and partially surrounding the endothelium (Fig. 2 A). The basal lamina of the capillary wall totally invests the

FIGURE 1 Oil Red O–stained section of a diabetic eruptive xanthoma. Pictured is the epidermis (*EP*) overlying a portion of a xanthoma. Several capillaries (*C*) are clearly discerned in the upper dermis (*D*) by virtue of the numerous lipid droplets (*L*) in close association with the vascular walls. It is not possible to discern if the droplets in close proximity to the vascular channels are within cells or in an extracellular position. However, some perivascular foam cells contain lipid-staining material within them (arrows). Patient 3. × 400.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perithelial cells and their cytoplasm contains a variable, but sparser, quantity of filaments than smooth muscle cells found in arterioles, venules, and larger vessels. Vacuolization of the cytoplasm of many of the capillary perithelial cells was commonly observed (Fig. 2 A and B) and was often sufficiently marked (Fig. 2 B) to give the cell the appearance of a foam cell by light microscopy. Electron microscopy of such cells revealed that some of the vacuoles contained a moderately dense homogeneous material while others were seen to enclose myelin forms (Fig. 2 B). Examination of dermal capillary perithelial cells from normal individuals failed to reveal such vacuoles.

Although some of the foam cells accumulating in the xanthomas were pericytes, the majority of cells with vacuolated cytoplasm were identified as tissue macrophages. Figs. 4 and 5 show portions of these macrophage foam cells for comparison with the vacuolated perithelial cells. The macrophages could be distinguished from perithelial cells by their characteristic finger-like microvilli on the cell surface and their lack of surrounding basement membrane (31) (Fig. 5). The macrophages contained numerous vacuoles within which homogeneous material was found. It is assumed that this material is lipid or lipid residue and accounts for the intense staining of these cells with Oil Red O. Some of the vacuoles contained myelin forms which may represent the source for the positive reaction these cells displayed with Baker's phospholipid stain. Large numbers of lysosomes were also dispersed throughout the cytoplasm between the lipid vacuoles (Figs. 4 and 5) and this probably accounts for the strong reaction these cells showed when stained for acid phosphatase activity.

Dietary fat restriction and insulin therapy cleared the chylomicronemia and the eruptive lesions resolved in all of the cases. The effects of diabetic treatment on the plasma, chylomicron, and xanthoma lipids of four patients are presented in Table VI. The response in the fifth patient (No. 5) is diagrammatically detailed in Fig. 6. Xanthoma analyses were performed in patient 5 on three occasions (Fig. 6). First, on hospitalization, approximately 2 months after the xanthomas initially appeared, at a time when the patient was ingesting an ad lib. diet containing approximately 40% fat, the plasma triglycerides were at a maximum with 89% carried in the chylomicrons (Table III and Fig. 6). The major xanthoma lipid at this initial sampling was triglyceride, representing 45% of the xanthoma lipid, while cholesterol (free and esterified) accounted for only 20% of the total lipid. After 4 days on a fat-free diet (17th day) plasma triglycerides fell precipitously. No chylomicrons were found in the fasting plasma samples. After 12 days on the no-fat diet, the xanthomas flattened visibly and their total lipid content declined to about half of the value found in initial biopsies. This decrease was primarily due to the loss of triglyceride with very little decline in either free or esterified cholesterol. This resulted in a redistribution of the xanthoma lipids such that total cholesterol represented 31% of xanthoma lipid, while triglyceride only accounted for 25% (Fig. 6). Insulin therapy, in addition to dietary manipulation, raised PHLA (Table I), brought plasma triglycerides to normal, and almost completed the resolution of the xanthomas at the time of final sampling with a further decline in xanthoma total lipid to one-fourth of the original content. Although all xanthoma lipids further decreased in absolute amounts, the triglyceride fraction again displayed the greatest change so that total cholesterol represented 30% and triglyceride only 11% of the total.

The results of similar studies in four additional patients (Nos. 6–9) are outlined in Table VI. In each case, the resolving xanthomas displayed a proportionately greater loss of triglyceride than free and esterified cholesterol, resulting in a change in the lipid composition from triglyceride-rich erupting xanthomas during the height of the chylomicronemia, to cholesterol-laden resolving xanthomas after the chylomicronemia had cleared.

FIGURE 2*a* (top) Electron micrograph of a portion of a dermal capillary cut in cross section is pictured. Profiles of several endothelial cells (*E*) outline the lumen (*LU*) of this vessel. Elongate perithelial cells (*P*) partially surround the endothelial cells. The perithelial cells can be identified by their close proximity to the endothelium, by the fact that they are invested by the basal lamina (*B*) of the capillary and because they contain sparse quantities of filaments (*F*). Some of the perithelial cells have vacuoles (*V*) containing dense homogeneous material within their cytoplasm. The contents within these vacuoles is thought to be lipid since such cells appear to stain with Oil Red O as shown in Fig. 1. Numerous extracellular lipid droplets (*L*) are present in the wall of this capillary, within the basal lamina between endothelial and perithelial cells, and out in the perivascular space (arrow) in comparable positions to those observed in frozen sections stained with Oil Red O as shown in Fig. 1. In several places the basal lamina appeared to be lifted away from the perithelial cells by the accumulation of these lipid droplets. Patient 2. × 16,680. (*2b*) (bottom) An electron micrograph depicting the profiles of parts of several endothelial cells (*E*) and a perithelial cell (*P*) containing many vacuoles (*V*), some enclosing homogeneous dense lipid material and myelin forms (*MF*). The lumen of the vessel is not pictured in this micrograph. The highly vacuolated foam cell can be identified as a perithelial cell because it is totally invested by the capillary basal lamina (*B*). Only a few filaments are seen in this cell. Patient 8. × 13,948.

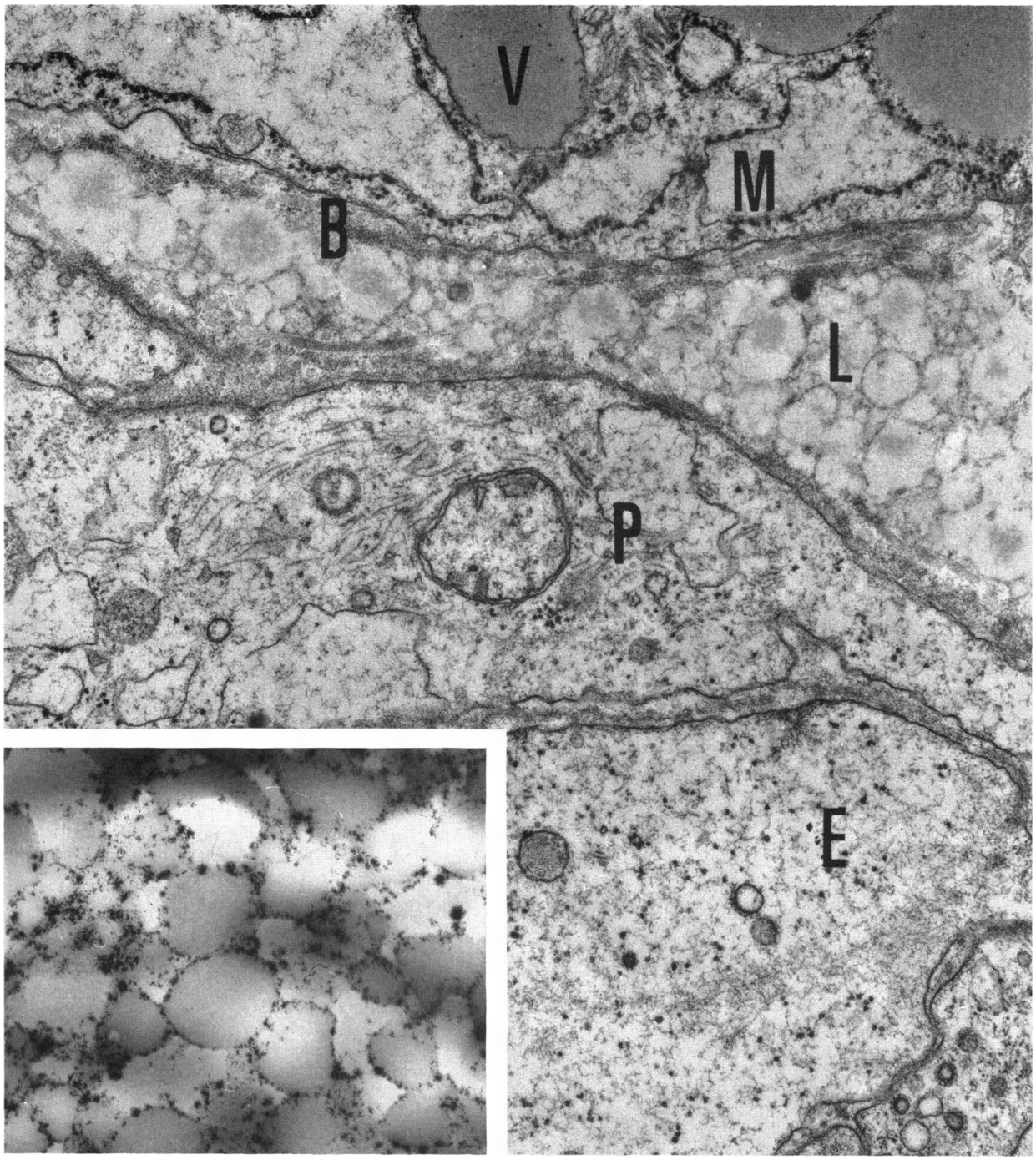

FIGURE 3 Electron micrographs comparing lipid droplets in the capillary wall with chylomicrons found in the circulation (inset). A small portion of a dermal capillary wall, including a perithelial cell (*P*) and endothelial cell (*E*), are shown. Nearby a portion of a macrophage foam cell (*M*) with several lipid-containing vacuoles (*V*) appears. Between the perithelial cell and the macrophage, within the capillary basal lamina (*B*), numerous droplets (*L*) are seen. These droplets are circular in cross section with an average diameter of 2000 ±1000 A. They are of a similar size and appearance as the chylomicrons isolated from the patient's circulation (inset). Circulating chylomicrons were isolated by PVP flocculation, fixed in osmium tetroxide–*s*-collidine buffer, embedded in epoxy resin, and sectioned on an LKB ultramicrotome. Patient 4. Large micrograph × 44,965; inset × 44,340.

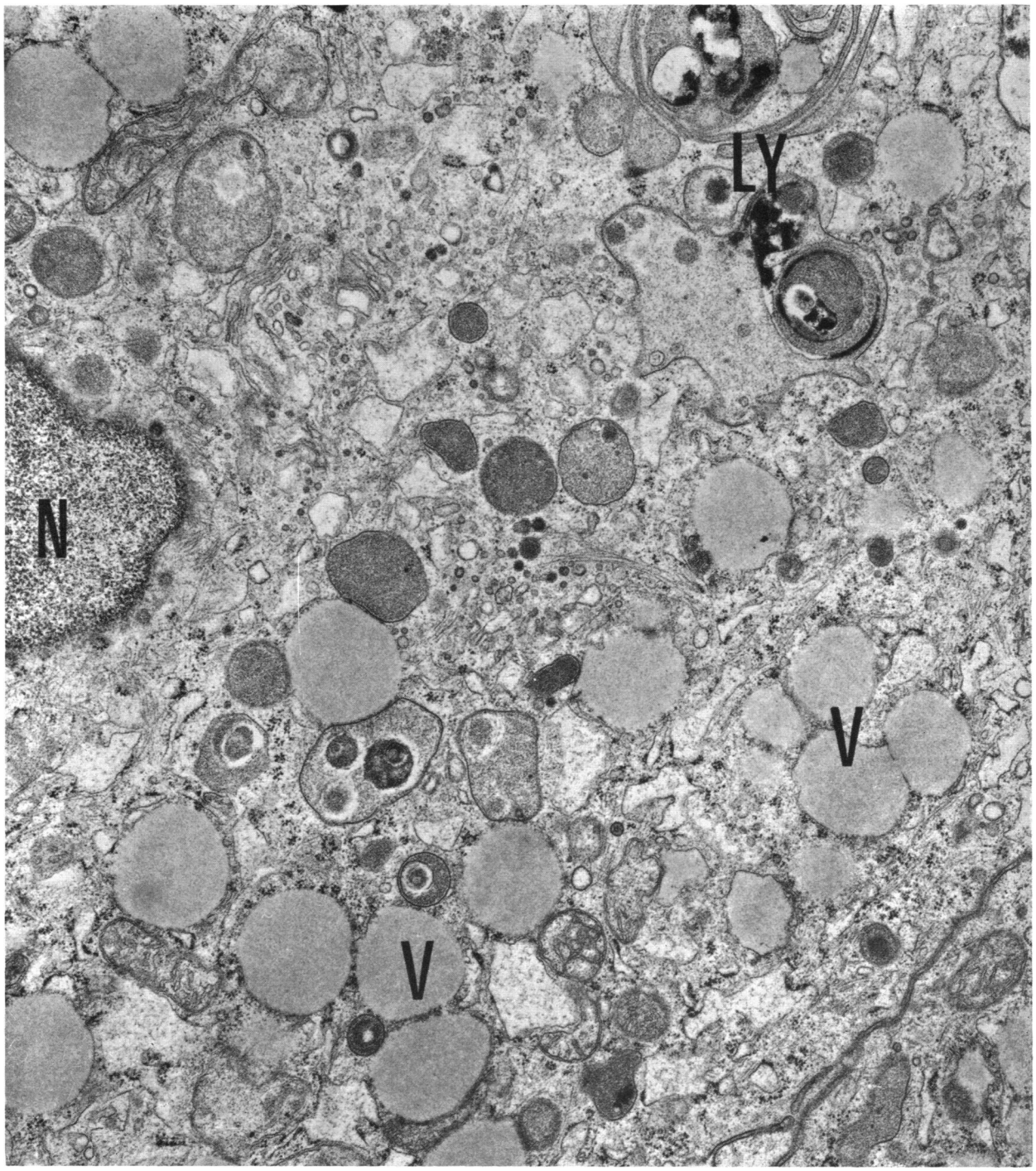

FIGURE 4 Electron micrograph of macrophage foam cell in an erupting xanthoma. The small portion of the foam cell depicted in this micrograph displays numerous distended vacuoles (*V*) containing moderately dense homogeneous material. This cell also has many lysosomes (*LY*) in the cytoplasm, often found in close proximity to the vacuoles. Nucleus of the cell (*N*). Patient 5 when first hospitalized. No therapy. × 22,104.

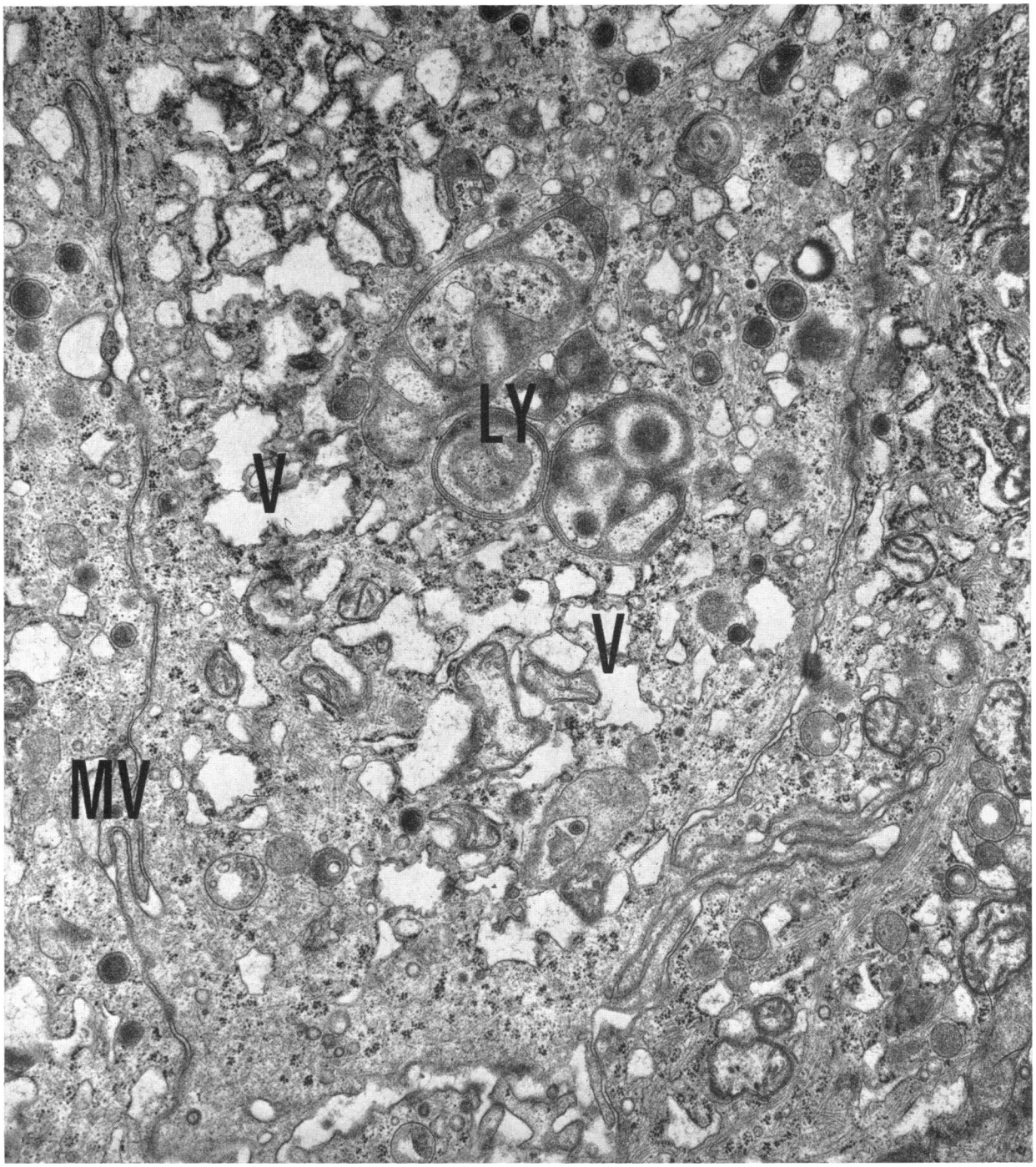

FIGURE 5 Electron micrograph of macrophage foam cells in resolving xanthoma. Portions of threee foam cells are pictured. The boundaries of these cells are seen as the tightly apposed plasma membranes running vertically on the right and left hand portion of the micrograph. The cell plasma membranes are often thrown into elongate microvilli (*MV*). In contrast to perithelial foam cells no basal lamina invests macrophages that form foam cells. This was the third biopsy taken from patient 5 after the patient was on no-fat diet and insulin therapy for several weeks and the xanthomas were resolving (see Fig. 6). Note that the vacuoles (*V*) in these foam cells appear to have lost the homogeneous material of moderate density commonly seen in foam cells of evolving xanthomas. Further, there appears to be a folding and an apparent collapse of the limiting vacuolar membranes since many appear to have a scalloped border. Lysosomes (*LY*). ×22,104.

TABLE VI

Analysis of Xanthomas, Plasma, and Chylomicrons during Therapy of Diabetes and Resolution of Xanthomas

Patient and therapy used	Xanthoma					Plasma					Chylomicron, % of plasma TG found in chylomicron
	TG	CE	FC	FFA	PL	TG	CE	FC	FFA	PL	
	μg/mg wet wt tissue					*mg/100 ml*					
No. 6											
Initial biopsy	6.2 (40)*	1.6 (10)*	1.2 (8)*	1.6 (10)*	5.0 (32)*	2580	250	150	60	280	97
After no fat +Insulin, 3 wk	1.8 (36)	0.6 (12)	0.5 (10)	1.1 (22)	1.0 (20)	170	270	50	20	180	None
No. 7											
Initial biopsy	35.9 (48)	9.3 (12)	8.2 (11)	10.7 (15)	10.6 (14)	2521	567	338	80	247	50
1 wk low fat diet	21.0 (50)	7.9 (19)	2.8 (7)	2.1 (5)	7.6 (19)	775	720	175	29	245	None
Low fat, 3 wk +Insulin, 1 wk	6.0 (40)	2.5 (17)	1.5 (11)	1.0 (6)	3.3 (23)	—	—	—	—	—	None (clear plasma)
No. 8											
Initial biopsy	4.5 (39)	2.1 (16)	0.7 (6)	1.6 (12)	3.4 (27)	1211	796	228	20	468	59
9 days fat free	0.3 (32)	0.2 (30)	0.01 (2)	0.1 (18)	0.1 (18)	346	279	80	60	195	None
No. 9											
Initial biopsy	9.2 (33)	5.1 (17)	4.6 (15)	2.0 (7)	8.4 (28)	4595	739	522	63	1058	62
No fat-11 days +Insulin	3.3 (23)	3.2 (22)	1.9 (13)	2.1 (14)	4.4 (28)	197	190	59	25	149	None

TG = triglyceride; CE = cholesteryl ester; FC = free cholesterol; FFA = free fatty acid; PL = plasma lipids.
* Per cent of total identified lipids in xanthoma.

The fatty acid patterns of plasma and xanthoma triglyceride and cholesteryl esters were not altered by the periods of dietary manipulation and insulin therapy. Thus the similarities in triglyceride fatty acids and dissimilarities in the cholesteryl esters of the tissue and plasma noted in the biopsies taken before therapy persisted following therapy.

Electron microscopic examination of the resolving xanthomas in all five patients revealed that the lipid vacuoles in many of the macrophage foam cells appeared to have lost the homogeneous material of moderate density frequently found in the cytoplasmic vacuoles and, in addition, there was a folding and apparent collapse of the limiting vacuolar membranes (Fig. 5). No extracellular droplets were seen in the capillary basal lamina of these resolving lesions which were so prominent in the eruptive xanthomas.

DISCUSSION

The present study on diabetic eruptive xanthomas examined evolving lesions so that early biochemical and ultrastructural events leading to xanthoma formation could be correlated. Results of both analytical and electron microscopic observations provide new evidence that lipoproteins, specifically the triglyceride-rich chylomicrons, contribute significantly to the lipids, particularly triglycerides, which accumulated in these eruptive xanthomas. In addition, sequential analyses and ultrastructural observations on resolving xanthomas demonstrate that lipid was mobilized from the foam cells and that triglyceride was most readily removed after the chylomicronemia cleared. These observations on the early alterations in xanthoma formation, as well as changes occurring concomitant with their resolution, deserve further comment.

The lipid composition of diabetic eruptive xanthomas. The types and amounts of lipid found in xanthomas depend upon a series of complex metabolic reactions. Of fundamental importance is probably the deposition of lipids from the circulation, but xanthomas are far from being inert deposits of plasma lipids. Indeed, certain deposited lipids are apparently mobilized back into circulation, others are transesterified or catabolized, and some lipids are added to the xanthoma by local synthesis (5, 9). Little actually is known regarding these

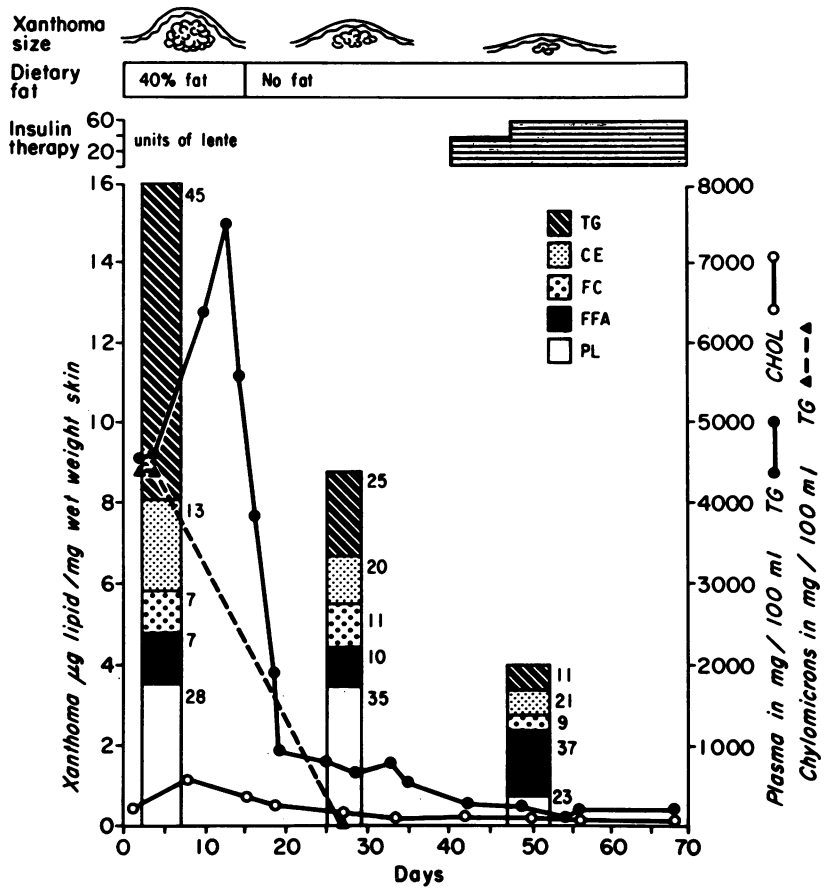

FIGURE 6 Response of patient 5 to dietary and insulin therapy. Xanthoma lipid distribution was determined on three occasions (depicted as three bars on the diagram), initially on hospitalization, next after 12 days on a no-fat diet, and last while on this diet and isulin therapy. The absolute quantities of each kind of lipid (μg/mg wet weight) may be read on the left ordinate. The per cent that each lipid contributed to total xanthoma lipid is given in the figures to the right of each bar. Plasma and chylomicron triglycerides and cholesterol are read on the right ordinate. Note that as the plasma lipids and chylomicrons decreased with therapy, the xanthomas decreased in size and concomitantly their total lipid content decreased. The major decrease was in the triglyceride moiety so that xanthomas sampled during the height of the chylomicronemia were triglyceride-rich while those studied during resolution were relatively rich in cholesterol.

metabolic events, but the present studies provide new information on some aspects of these reactions.

First, when these lesions were sampled early in their evolution, they contained large amounts of triglycerides with fatty acid patterns similar to circulating triglyceride-rich chylomicrons. Thus the composition of diabetic xanthomas differed significantly from tendinous, tuberous, and planar xanthomas found in association with various other disorders of lipid transport in which cholesterol (particularly esterified cholesterol) has been reported to be the major accumulating lipid (5–8, 32). These latter forms of xanthoma developed in the presence of increased concentrations of beta and (or) pre-beta lipoproteins which result in hypercholesterolemia and variable degrees of hypertriglyceridemia (33). Thus, in general terms, the lipids contained in diabetic eruptive xanthomas reflect the predominance of the triglyceride-rich chylomicrons in the circulation while other forms of xanthomata examined to date appear to mirror abnormal accumulations of cholesterol containing lipoproteins in the plasma.

Although the similarity between the fatty acid patterns of chylomicron and diabetic eruptive xanthoma triglycerides suggests that the former contributed directly to the latter, no such resemblance was apparent when the fatty acid distribution of the other major xanthoma lipids

were compared. For example, cholesteryl esters, which represent 14% of the lipid found in diabetic xanthomas, displayed increased relative amounts of cholesterol oleate and decreased quantities of cholesterol linoleate when compared with the cholesterol esters found in the circulation. If these lipids were primarily derived from the bloodstream, as some investigators have suggested (9), it might be expected that the fatty acid pattern in the xanthomas and circulating lipoproteins would resemble one another, particularly in lesions studied early in their development, as was done in the present study. Therefore, the cholesteryl esters accumulating in the foam cells of diabetic xanthomas, if they do originate from the plasma, might come from nonchylomicron lipoproteins which may have a different fatty acid content than the chylomicrons. In fact, the chylomicrons in the patients studied seemed to carry less cholesteryl linoleate than the lipoproteins contributing to the total plasma cholesteryl esters (Table V). The bulk of the esterified cholesterol in the plasma was carried by nonchylomicron lipoproteins (Table III), but these plasma cholesteryl esters differed even more markedly from the xanthoma sterol esters, suggesting that the cholesteryl esters found in the lesions undergo modification *in situ*. This could involve one or more processes, including selective uptake of certain esters, hydrolysis of other esters accumulating in the lesions, or simply esterification of free cholesterol. Whatever the mechanism responsible for the cholesteryl ester fatty acid pattern in diabetic xanthomas, it would seem to be a universal phenomenon, since all types of xanthomas studied to date (5–7, 32) have displayed the same cholesteryl ester fatty acid pattern. The striking resemblance between the fatty acid composition of cholesteryl esters in human xanthomata and the fatty streaks of human aorta (34) suggests that similar biochemical reactions may mediate cholesteryl ester accumulation in both kinds of lesions.

The present investigations also provide new insights into the mobilization of various lipids from the eruptive lesions following clearing of the chylomicronemia. Triglycerides appeared to be mobilized more readily than free and esterified cholesterol, resulting in the progressive transition from eruptive lesions with a predominance of triglyceride to cholesterol-rich resolving xanthomas. Obviously, the rates of removal and deposition of various lipids will affect the precise chemical composition of xanthomas. These findings stress the potential hazards of relating xanthoma lipid composition to plasma lipids when studying patients receiving various forms of therapy for their lipoprotein disorders or in patients whose duration of the xanthomas is unknown. Whether such a process might also be in operation in atheromas and thus, in part, explain the accumulation of cholesterol in preference to triglyceride in vascular lesions is only conjectural at this time.

The ultrastructural morphology of diabetic eruptive xanthomas. The accumulation of droplets in the basal lamina of the dermal capillary walls, as well as their occurrence in the perivascular space, is taken as ultrastructural evidence of lipoproteins permeating the vascular walls. The droplets are thought to contain lipid because they were observed in analogous positions to droplets in the capillary walls which stain with Oil Red O in frozen sections (Fig. 1). In this regard, Cornog, Fitts, and Kuo (35) observed Oil Red O staining lipid droplets in and around gingival capillary walls of carbohydrate-induced hyperglyceridemic patients whose plasma contained increased concentrations of triglyceride-rich lipoproteins. In the patients studied by Cornog et al., severe hyperlipemia seemed to correlate best with the presence of this stainable pericapillary lipid.

Specifically, the droplets in the present study are believed to be large lipoproteins, probably chylomicrons, because their ultrastructural appearance and size are similar to the chylomicrons isolated from the circulation of the diabetic patients in this study (inset Fig. 3). However, not all of the droplets in the vessel walls are necessarily chylomicrons since the smaller droplets (200–1000 A) may correspond to pre-beta lipoproteins (36). Indeed, there is a spectrum of light-scattering lipoproteins found in the circulation in these diabetic patients varying in size but with probably a similar basic structure which makes it impossible to precisely identify chylomicrons by electron microscopic criteria alone.

Studies in newborn rats with hyperlipemia following feeding (37, 38), as well as experimentally induced xanthomas in cholesterol-fed rabbits (39, 40), have revealed lipoprotein droplets with ultrastructural characteristics similar to those seen in these human eruptive lesions.

This study on human xanthomas provides electron microscopic evidence supporting the theory of the plasma lipoprotein origin of xanthoma lipids. Of course, such ultrastructural observations cannot provide definitive evidence as to which direction the lipid particles were moving, but correlation of the lipid analytical findings with these pathologic observations suggest that the major movement of lipid was into the skin.

Electron microscopic evidence in this study indicates that the foam cells found in diabetic xanthomas originate from two distinct cell types—perithelial cells and tissue macrophages; the latter had previously been assumed to be the only cell accumulating lipid in these lesions (41), but the present study suggests that pericytes of the dermal capillary wall also participate in this process.

Although it is impossible to precisely identify by electron microscopy what is contained within the vary-

ing number of vacuoles observed in the perithelial cells (Fig. 2 A and B), it seems reasonable to assume these vacuoles represent intracellular sites of accumulated lipid, since cells in comparable positions appeared to stain with Oil Red O (Fig. 1). Many of the vacuoles contained electron-dense material while others appeared empty possibly reflecting variations in the kinds and amounts of lipids in the vacuoles, as well as differences in the solubility of these lipids in the organic solvents required for tissue fixation. Therefore it is not surprising that the ultrastructural appearance of the chylomicrons in the capillary wall was not precisely like the lipid material found within the perithelial cell vacuoles even though the lipid analytical data support the contention that foam cells in these xanthomas accumulate triglyceride primarily derived from chylomicrons. Indeed it has not been possible with the static techniques of electron microscopy to discern how chylomicrons or chylomicron lipids were taken up by these cells.

In electron microscopic investigations of rabbit xanthomas, in which it was possible to sequentially sample the lesions during their development, perithelial cells also were observed to evolve into foam cells (39). The perithelial foam cells in rabbit xanthomas are ultrastructurally indistinguishable from the perithelial foam cells observed in diabetic xanthomas. This postulated progression of pericytes into foam cells is of more than casual interest since similar morphologic changes occur in the intimal smooth cells found in both human (42–44) and rabbit (45) atherosclerotic plaques. These findings suggest that perithelial cells might be analogous to smooth-muscle cells of large blood vessels and that both cell types may react similarly to the accumulation of lipids.

The majority of the foam cells found within the xanthomas were macrophages with the same structural features as foam cells observed in tuberous (46) and tendinous xanthomas (47). The macrophage foam cells in diabetic xanthomas apparently accumulate large quantities of triglyceride within cytoplasmic vacuoles. But as in the case of perithelial cells these studies have not determined how these cells take up and accumulate this lipid. As the xanthomas resolve with a decrease in the triglyceride content, these events can be correlated with electron microscopic observations which display a loss of the homogeneous, moderately electron-dense material from the vacuoles with apparent collapse and folding of the limiting vacuolar membranes (Fig. 5).

It is known that in high concentrations phospholipids tend to form myelin figures when found in tissue (48, 49). In diabetic xanthomas, phospholipids comprised approximately 20% of the total lipids and the presence of myelin figures along with strong histochemical staining for phospholipid in the macrophage foam cells suggest that these cells contain considerable amounts of the accumulating polar lipid. The in vitro metabolic studies on human xanthomas by Wilson (9) and the work of Day, Fidge, and Wilkinson (50) on isolated animal macrophages suggest that phospholipids are synthesized by the macrophage foam cells, perhaps in response to the influx of other lipids such as triglycerides and cholesterol.

The present study has suggested that diabetic xanthomas may offer a convenient model for the study of lipoprotein transport across vascular walls and interaction of these lipids with cellular elements in the dermis. Further, xanthomas may be useful for studies on the mechanisms by which various lipids found accumulating within foam cells are mobilized. Investigation of these various events might also provide some insight into analogous mechanisms postulated to occur in atheromatous plaques.

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