THE IDENTIFICATION OF CHYLOMICRA AND LIPOPROTEINS IN TISSUE SECTIONS AND THEIR PASSAGE INTO JEJUNAL LACTEALS

J. R. CASLEY-SMITH, D.Phil.

From the Sir William Dunn School of Pathology, Oxford, England. Dr. Casley-Smith's present address is the Department of Anatomy, University of Adelaide, South Australia

ABSTRACT

The electron microscopic appearances of chylomicra and lipoproteins have been investigated. The particles were isolated from rat chyle by differential flotation in an ultracentrifuge. Various fixing and embedding media were used. The two kinds of particles were then identified in thin sections of the jejunum of rats. The chylomicra had diameters of from 1,000 A to 1 μ; the lipoproteins ranged from 100 to 1,000 A. They were identified by their sizes and their similarities to the isolated particles after the various fixing and embedding procedures. In addition, the relative amounts of the two kinds of particle varied greatly under different dietary conditions. The chylomicra had a thin rim, probably of phospholipid. Section B records the passage of the two kinds of particle into the lacteals in the villi of the jejunum. Both chylomicra and lipoproteins were seen passing through many open junctions. From permeability considerations it would seem that this is the most important route. These open junctions appear to act as "inlet valves" which prevent backflow as the contractions of the villi pump material out of the lacteals. Both chylomicra and lipoproteins were also seen entering the endothelial cells and lying inside them. The lipoproteins entered via "normal" caveolae and were seen in "normal" vesicles ($\sim$ 500 A); the chylomicra necessarily occupied much larger organelles. Both kinds of particles were also seen in caveolae on the luminal surface of the endothelium, but it was impossible to be certain that these were not just particles entering the cells from the lumen. The chylomicra often seemed to be washed out of these caveolae as many large, empty ones were seen on the luminal sides of the cells. Frequently, these caveolae had dark membranes.

Almost all the fat which is absorbed from the small intestine passes to the blood stream via the lymphatics ("lacteals") of the villi (46, 51). There have been only two electron microscopic studies relating to how the fat actually enters these vessels, and these reports do not agree. Palay and Karlin (41) showed that fat particles passed through open intercellular junctions; Ashworth, Stembridge, and Sanders (2) said that they passed through "the lymphatic wall" in which they saw "pores." As the author has been studying the permeability of lymphatic endothelium (7) and as the lacteals are well known to be very permeable, it was decided to reinvestigate this question

During the course of the observations it was noticed that the fat particles in the lacteals showed great variations in their morphology, but that they usually belonged to one of two main groups. Fat occurs in the lymph as both chylomicra and lipoproteins (5, 45, 51). It seemed possible that the two groups of morphologically distinct particles might correspond to these two modes of fat

transport. There has been little electron microscopy of chylomicra or lipoproteins. Kay and Robinson (25) have studied thin sections of chylomicra, isolated from chyle; French (16) and Majno and Palade (28) have seen them in tissue sections; Hayes and Hewitt (23) have examined isolated, shadowed lipoproteins. There have been no comparative studies of these two groups of particles with the electron microscope. Therefore, chylomicra and lipoproteins were isolated and examined after various fixing and embedding procedures. These methods were then used to study and identify the fat particles in the tissues. The variations in the number and size of the particles after different diets also aided in their identification.

Once the chylomicra and lipoproteins were identified as separate particles, it was possible to see whether they differed in their methods of entering the lacteals. Since the two groups of particles differ so much in size it was thought that there might be considerable differences in the paths they took through the lacteal endothelial barrier.

EXPERIMENTAL

The animals used were 2-month-old rats ($\sim$ 160 gm) of an albino strain (Wistar). They were housed under colony conditions, given water *ad libitum* and fed on a standard diet of rat nuts (Oxo Ltd. (London, England) Diet 41; 16.9 per cent protein, 56 per cent carbohydrate, and 3.9 per cent fat). When maize oil (corn oil in the United States of America) or olive oil was administered, the animals were anaesthetised with ether and 1 ml of the oil given via an intragastric tube. This procedure was repeated after 3 hours and the tissues were removed after 2 more hours. Some animals were fed only on a 10 per cent glucose solution for 3 days. Some were starved (but given water *ad libitum*) for 1 day, while others were starved for 3 days. The animals used for these dietary experiments were housed in wire-bottomed cages to prevent them from eating their faeces.

To fix the tissues for electron microscopy a piece of jejunum was isolated between ligatures and gently distended with the fixative. It was then excised and placed in more of the fixative. After fixation, small (1 mm^3) blocks were removed for embedding. This procedure was gentle enough to avoid squeezing the fat out of the lymphatics before it was fixed. The fixatives used were Caulfield's (9) osmium tetroxide solution (only using double his quantity of osmium in order to preserve the fat better), Luft's (26) potassium permanganate, and calcium formol. This last was made by adding calcium acetate to make a 1 per cent solution in 10 per cent neutralized formalin contained in the barbiturate-acetate buffer used for

FIGURES 1 TO 8

Chylomicra and lipoproteins which have been isolated by means of the ultracentrifuge. Figs. 1 to 7 were obtained from the chyle of rats which had been fed olive oil. Fig. 8 shows the lipoproteins present in pig's plasma. All the micrographs have the same magnification ($\times$ 40,000).

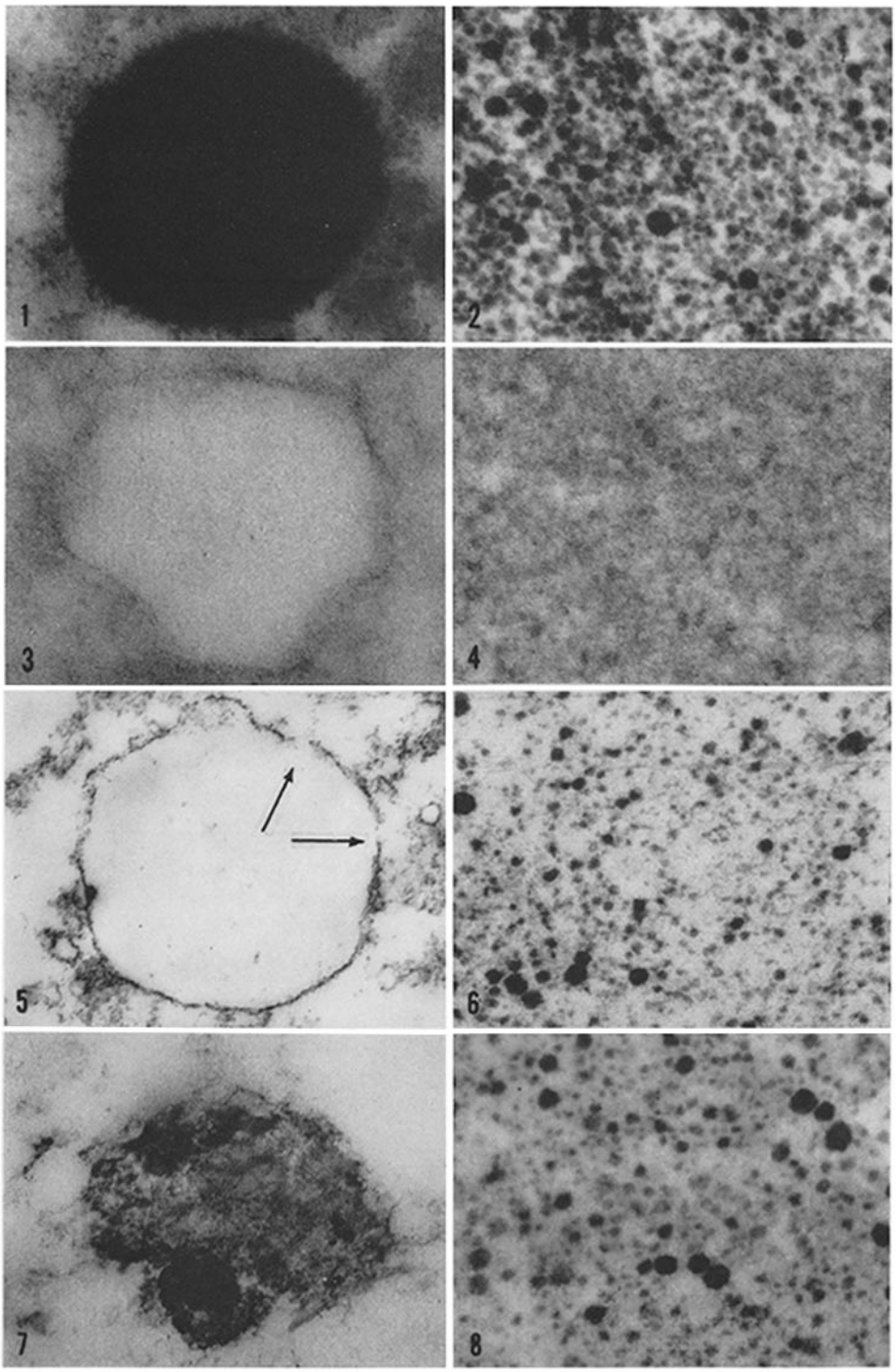
Fig. 1 illustrates a chylomicron fixed with Caulfield's solution and embedded in Durcupan. The whole body is circular and densely black. The lipoproteins which were treated in an identical manner are shown in Fig. 2. It is apparent that they are much smaller (100 to 500 A as compared with about 1 μ). They tend, however, to be agglomerated. The density varies somewhat among the different particles and also in different regions of the same particle. Their outlines are also irregular.

When the chylomicra are fixed with potassium permanganate and embedded in Araldite, only a thin rim of material remains (Fig. 3). This contrasts with the appearance of the lipoproteins (Fig. 4). These are little altered except that the larger ones ($\sim$ 500 A) appear to have denser rims. The dense material surrounding the chylomicron in Fig. 3 would seem to be lipoprotein contamination.

Chylomicra, which were fixed for 2 hours with Caulfield's solution and then embedded in Araldite, have empty centres surrounded by a dark, finely granular rim (Fig. 5). This rim usually shows some breaks (arrows). Surrounding the chylomicron there are many fine dots which represent osmium-triglyceride complexes which have passed out of the ruptured chylomicra. Fig. 6 shows lipoproteins which were treated in the same way. These particles appear to be intact and are very similar to those shown in Figs. 2 and 8.

Fig. 7 shows a chylomicron which was fixed with Caulfield's solution for 48 hours and embedded in Araldite. The central lipid is retained, but the form is distorted.

Lipoproteins from pig plasma (Fig. 8) are very similar to those of rat chyle (Figs. 2 and 6). They were fixed with Caulfield's solution and embedded in Araldite.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Caulfield's solution. The fixatives were used for 2 hours, except that sometimes the osmium tetroxide was used for 2 days to improve the fixation of the fat. The tissues were fixed at 4°C.

The tissues were dehydrated and embedded in Araldite (19) and in Epon (27) by the usual methods. Some blocks were embedded in a water-soluble resin, Durcupan (Fluka, Switzerland). This was described as X 133/2097 (CIBA) by Staübli (47) and has been further developed by Bernhard (1961, personal communication). It was used as suggested in the manufacturer's leaflet except that the tissues were placed directly into the 70 per cent solution, and 20 per cent more accelerator and 10 per cent more plasticiser were used.

Some Araldite blocks were stained by incorporating 0.5 per cent phosphotungstic acid in the dehydrating alcohols. Sections from other Araldite blocks and some of the Epon ones were stained by floating on 2 per cent uranyl acetate for 30 minutes (50). To dissolve the triglycerides some blocks were exposed to the dehydrating alcohols for 12 hours; others were treated with benzene for 30 minutes after dehydrating and before embedding. Sections were cut on a Huxley microtome (Cambridge Instrument Co., Cambridge, England) and examined at 40 or 60 kv in a Philips EM 100B electron microscope. The magnifications were checked with a grating replica and are to within about 10 per cent.

Material was prepared for light microscopy by cutting frozen sections of tissue fixed in calcium formol and embedded in gelatin. These were stained with Sudan IV.

The chylomicra and lipoproteins were obtained by the differential flotation in an ultracentrifuge of chyle from olive oil-fed rats. Some lipoproteins from pig plasma were prepared similarly. The particles were mixed with equal volumes of the various fixatives. They were briefly centrifuged after fixation and the resulting mass usually cohered sufficiently to enable it to be treated as an ordinary block of tissue. Occasionally, additional brief centrifugations were needed during the processing. It is probable that these "lipoproteins" contained both low-density and high-density lipoproteins, together with other proteins.

A—IDENTIFICATION OF CHYLOMICRA AND LIPOPROTEINS

Observations

Isolated Particles

The appearances of chylomicra under various conditions of fixation and embedding are shown in Figs. 1, 3, 5, and 7. They are circular in section with diameters of the order of 1 micron. When embedded in Durcupan (Fig. 1) they appear uniformly black. Permanganate fixation preserves only the peripheries of the particles (Fig. 3). When the chylomicra have been fixed for 2 hours with osmium tetroxide and embedded in Araldite, only the peripheries remain (Fig. 5). These are finely granular and often have breaks. If the fixative is used for 2 days before embedding in Araldite,

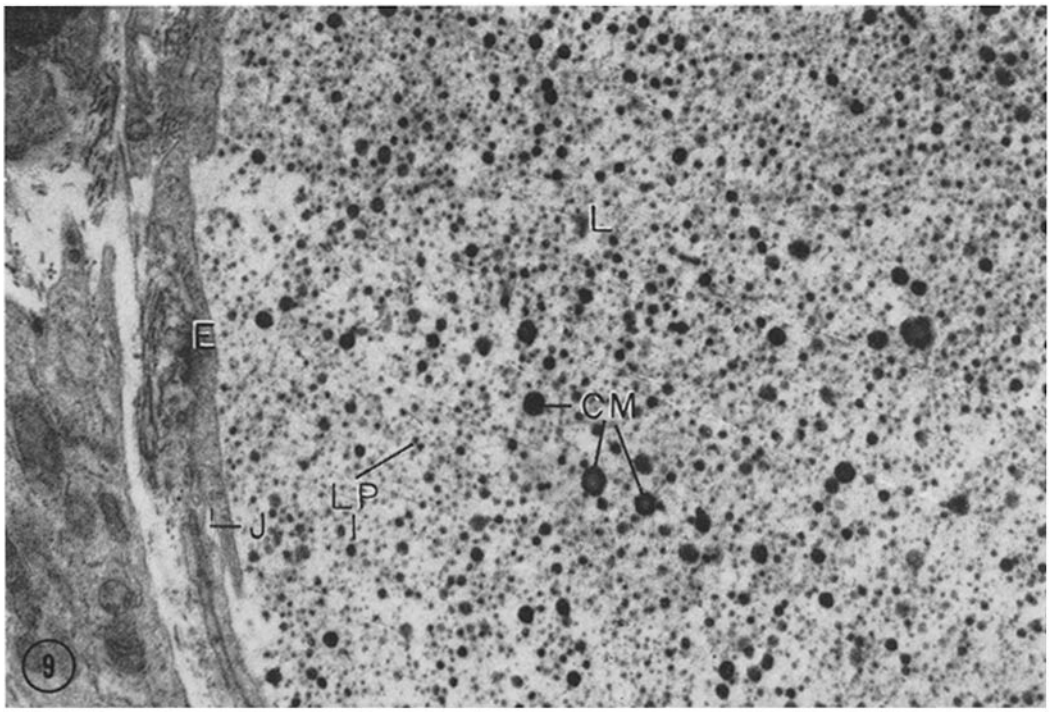
Unless otherwise stated, all the subsequent illustrations are of lacteals in the jejunum of rats. The tissues have been fixed with Caulfield's solution for 2 hours, embedded in Araldite, and the sections mounted unsupported.

FIGURE 9

A lacteal from a rat which has been fed a normal (low fat) diet. In the lumen (*L*) there are many fat particles. Most of these (*LP*) are small and are morphologically similar to the lipoproteins shown in Fig. 6. There are a few larger particles (*CM*). These are smaller than the chylomicra isolated from the chyle of fat-fed rats (Figs. 1, 3, 5, and 7). This difference in size and density is explained in the text, where the larger particles are identified as chylomicra and the small ones as lipoproteins.
The lymphatic endothelium is shown (*E*). It possesses a partly open intercellular junction (*J*). Stained with uranyl acetate. × 10,000.

FIGURE 10

The animal has been fed maize oil. There are many chylomicra (*CM*) in the lumen (*L*). These are larger and much more numerous than those shown in Fig. 9. There are also some lipoproteins (*LP*). These are relatively much fewer than those in Fig. 9, but this may just be due to the increase in the number of the chylomicra. Some chylomicra may be seen in the connective tissue external to the lacteal. Many are concentrated just outside the endothelium (*E*). At some places these large fat particles distort the cells (arrows). Block stained with phosphotungstic acid. × 10,000.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CM
LP
J
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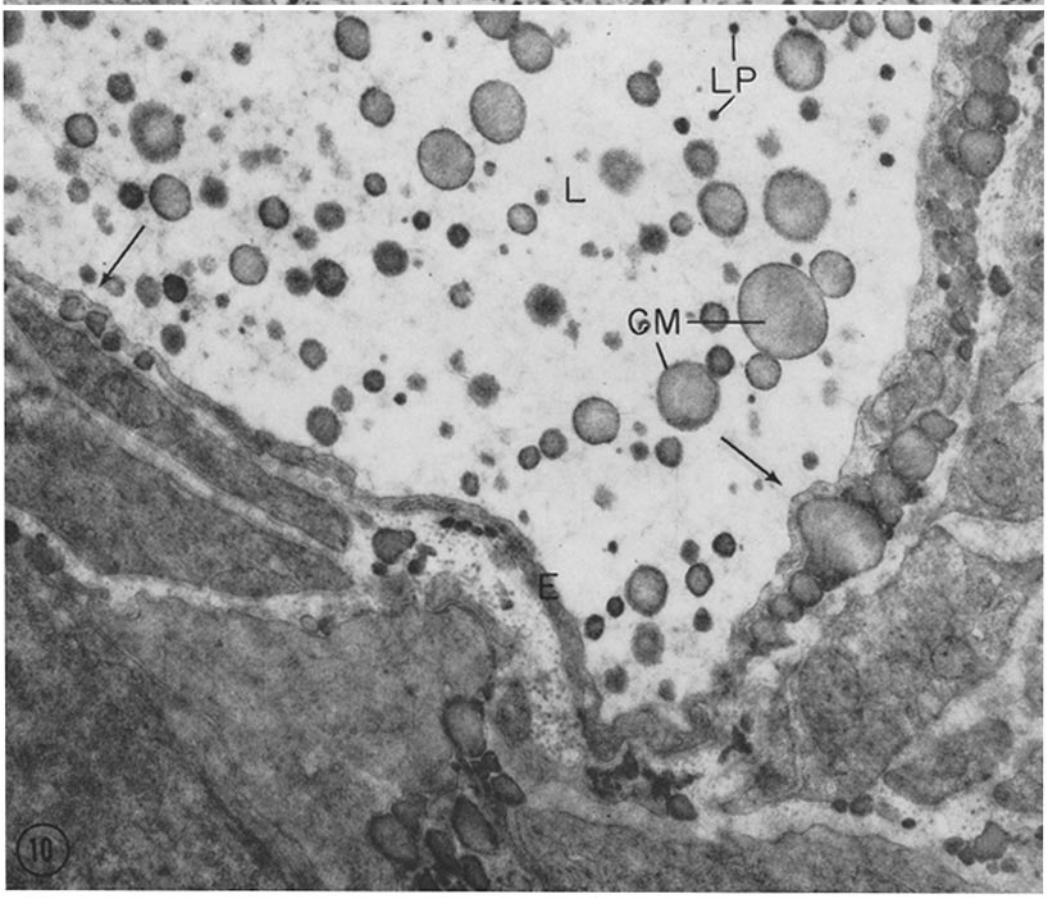
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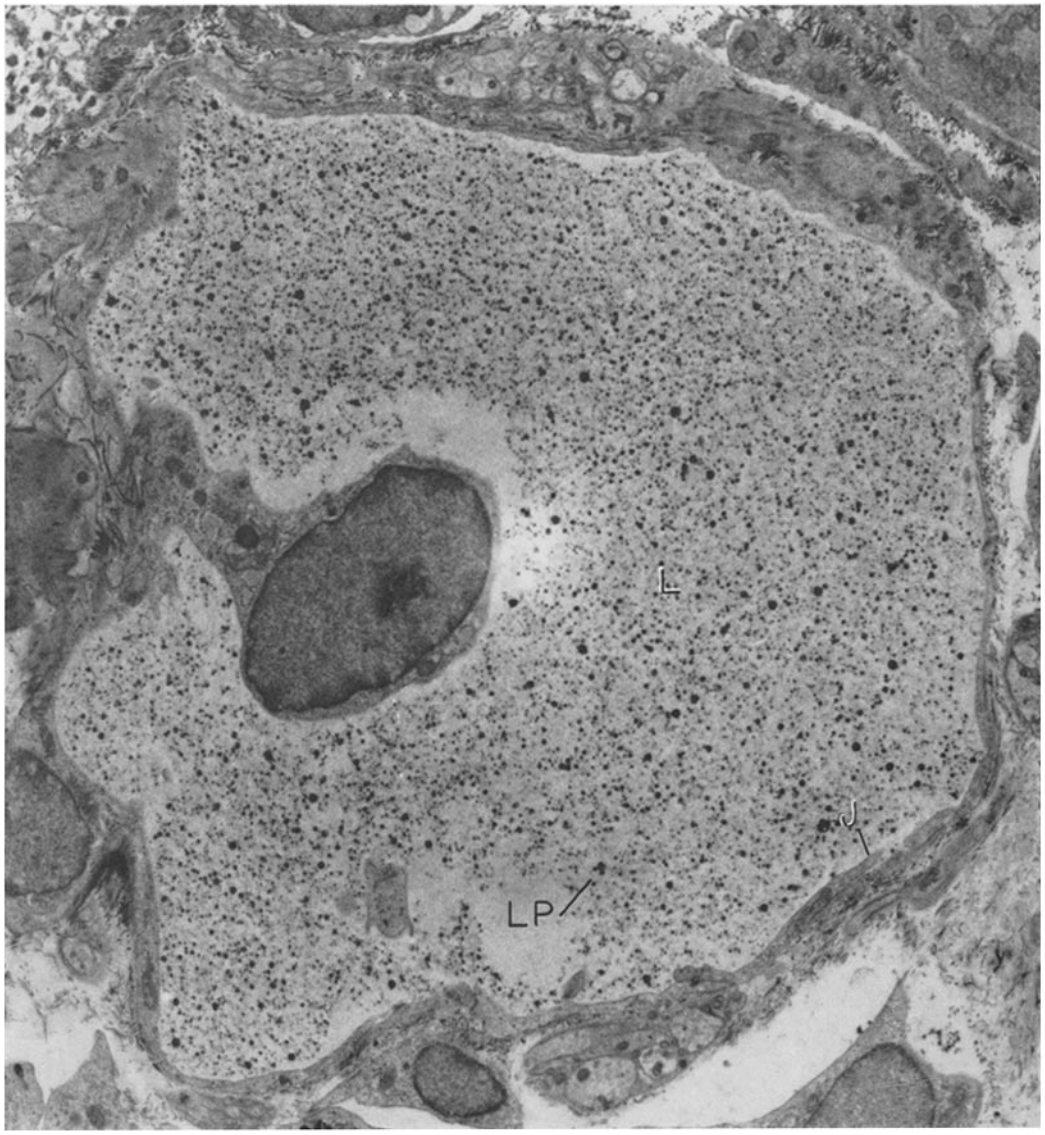


FIGURE 11

The animal has been fed only glucose for 3 days. There are many lipoprotein particles (*LP*) in the lumen (*L*), but almost no chylomicra (*cf.* Fig. 10). A partly open endothelial junction (*J*) contains two lipoprotein particles. It is most unusual to find an endothelial nucleus projecting into the lumen as is shown here. Stained with uranyl acetate. × 6,000.

the central fat is retained, but the particles are much distorted (Fig. 7).

Lipoproteins embedded in Durcupan and Araldite have similar appearances (Figs. 2, 6, and 8). The particles vary in size from about 100 to 1,000 A. These dimensions are larger than those usually given (51) but are similar to those observed when shadowed lipoproteins were used (23). The outlines are very irregular. The densities vary greatly, both among the particles and in different parts of the same one. These variations probably reflect differences in the thicknesses of the particles included in the sections and in their chemical compositions. The lipoproteins from

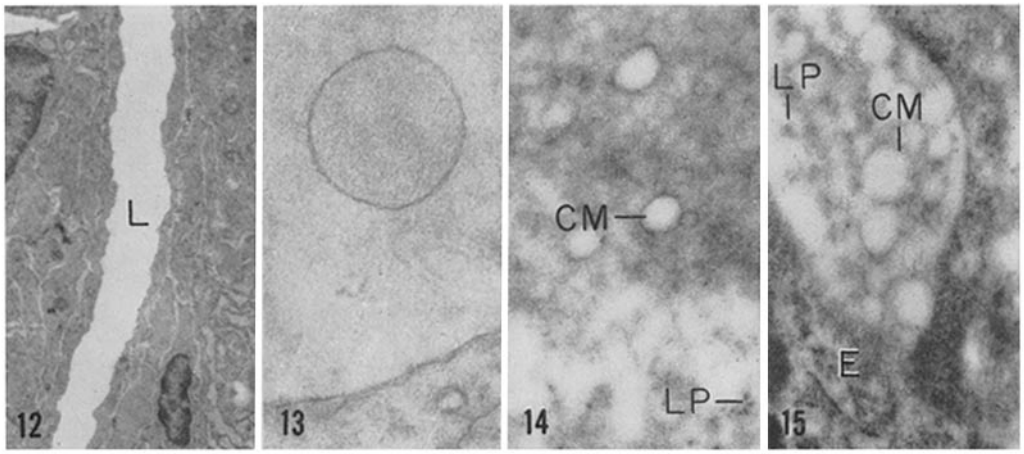


FIGURE 12

The animal has been starved for 3 days. The lumen (*L*) of a lacteal is almost empty and is very narrow (*cf.* Figs. 9 to 11). Stained with uranyl acetate. × 2,500.

FIGURE 13

The animal has been fed olive oil and the tissue embedded in Epon. A chylomicron is shown. It has a central portion of nearly the same density as the empty resin. Around the periphery of the particle is a thin, dense line. Unstained. × 20,000.

FIGURE 14

The animal has been fed maize oil and the tissues were fixed with potassium permanganate. Only the peripheries of the chylomicra (*CM*) remain. Some lipoprotein particles (*LP*) may be identified. Unstained. × 10,000.

FIGURE 15

The animal has been fed maize oil. After fixation with Caulfield's solution, the tissues were dehydrated and placed in benzene for 30 minutes. They were then embedded normally. Only the peripheries of the chylomicra (*CM*) remain and these are faint and blurred, as are the lipoproteins (*LP*). Endothelium, *E*. Unstained. × 15,000.

the pig's plasma (Fig. 8) are very similar to those from the rat chyle (Figs. 2 and 6). When the lipoproteins are fixed with potassium permanganate (Fig. 4) the smaller ones appear little changed. Some of the larger ones (~500 A) show a dark periphery not unlike that of the chylomicra.

PARTICLES IN THE TISSUES

a) NORMAL DIET: The particles in the lacteal lumina constitute two distinct morphological types (Fig. 9). There are a few large particles (0.1 to 1 μ) that have fairly regular outlines and are either uniformly dark or possess wide dark rims and paler centres. They are similar in size to the isolated chylomicra. There are also many small particles (100 A to 0.1 μ) which are irregular in outline and density. These are very similar to the isolated lipoproteins. Some of the smaller particles may be eccentric sections of larger ones. There are too many small particles and too few large ones for more than a few of the small ones to arise in this way.

b) DIFFERENT DIETS: The animals which were fed maize oil show a great increase in the numbers and dimensions of the larger particles (Figs. 10, 16, 17, 19 to 22, 24, 27). The numbers of the smaller particles are relatively diminished. Many investigators (51) have found that the numbers of chylomicra greatly increase after the ingestion of fat, but that those of the lipoproteins

do not rise very much. In addition, Gage and Fish (18) found that the diameters of the chylomicra increase as well as their numbers. Thus, these results are in accord with the tentative identification of the two kinds of particles.

When the animals were fed only glucose for 3 days the picture is quite different. There are many of the small particles, but very few of the large ones distorted but lose their pale centres (*cf.* Fig. 7). The quality of the fixation may vary in the blocks fixed with osmium for 2 hours; sometimes, some of the large particles are quite dark, while others have pale centres (Fig. 24). The material embedded in Durcupan (Fig. 21) shows large, completely dark particles (*cf.* Fig. 1). Thus, it would seem that the fat is poorly fixed after only 2 hours

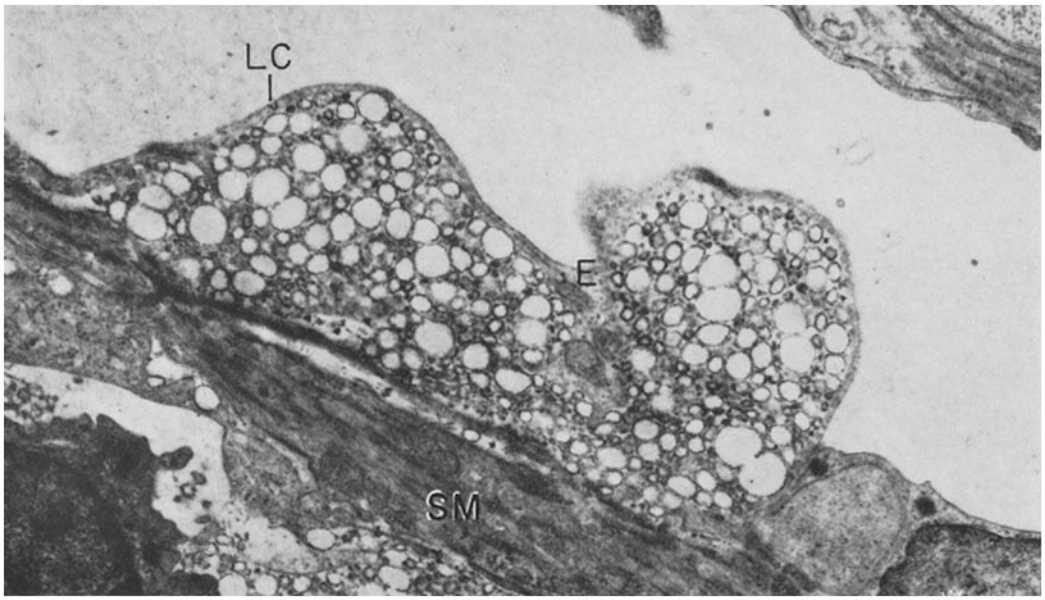


FIGURE 16

The animal has been fed maize oil. The endothelium (*E*) is greatly distorted by a large number of chylomicra which lie between it and the connective tissue. The smooth muscle cell (*SM*), which lies just external to the collection of chylomicra, is obviously much less easily distorted than the endothelium. When the muscle contracted it probably increased the pressure in the tissue around it, thus causing the chylomicra to distort the endothelium.

The tissue was exposed to the dehydrating alcohols for a long time (12 hours). These have removed all the central fat of the chylomicra. There is a large dark caveola in the endothelium (*LC*). Section stained with uranyl acetate. × 9,000.

(Figs. 11, 29 to 32). The situation is similar after one day's starvation (Fig. 28); after 3 days' starvation the lacteals are very narrow and practically empty (Fig. 12). The independent variation in the numbers of the two groups of particles indicates that they are indeed distinct.

c) DIFFERENT FIXING AND EMBEDDING PROCEDURES: When fixed with osmium and embedded in Araldite, the large particles usually have wide dark rims and paler centres (Figs. 10, 17, 19, 20, 23 to 25, 27). If the osmium is applied for 2 days (Fig. 22) the particles are often rather in osmium tetroxide and that the central portions of the large particles are usually still fairly soluble in the dehydrating alcohols. If the tissues are embedded in a water-soluble resin the fat is retained. There are two separate parts to the large particles, probably corresponding to the structure which has been suggested for the chylomicra. It has been thought that these consist of a very thin shell of phospholipid, perhaps with a little protein, which surrounds triglycerides (5, 25, 43). Permanganate fixes phospholipids but little else (26). When it is used, the large particles consist

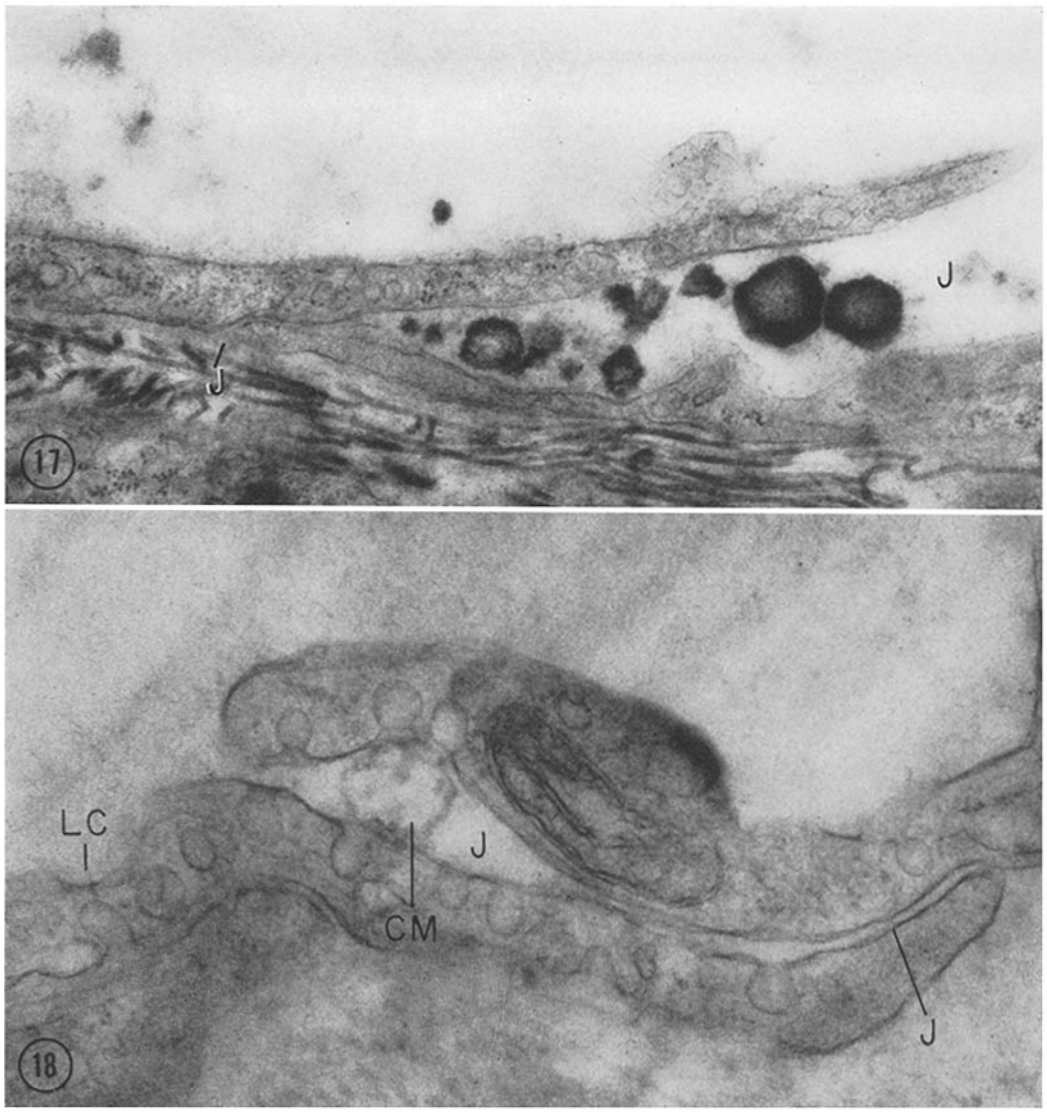


FIGURE 17

The animal has been fed maize oil. There is a partly open endothelial intercellular junction (*JJ*). It contains chylomicra and lipoproteins. Section stained with uranyl acetate. × 30,000.

FIGURE 18

The animal has been fed olive oil and the tissue was embedded in Epon. There is an endothelial intercellular junction (*JJ*) which contains a small section of a chylomicron (*CM*). The central portion of this particle has an electron-opacity similar to that of the empty resin, exept for a few small denser areas. Around the periphery of the particle is a thin dense line. A large caveola (*LC*) is present. Unstained. × 80,000.

of a thin dark rim surrounding an empty centre (Fig. 14, *cf.* Fig. 3). Calcium formol fixation also gives very pale centres, surrounded by thin dark rims. Epon embedding, particularly of large particles derived from olive oil, leaves them with pale centres and thin dark rims (Figs. 13, 18). It has been suggested (Marinozzi, 31) that the osmium attached to the triglyceride portion of

the particle is oxidised by the Epon itself and that this osmium then dissolves in the resin. The osmium attached to the phospholipid rim may be less easy to oxidise and therefore may stay in position. After exposing the fixed material to benzene (Fig. 15), or to excessive alcohol (Fig. 16), all that remains of the particles are the rims. Thus the different solubilities of the rims and the centres support the suggested different chemical compositions.

The characteristics of the small particles indicate that they are lipoproteins. The small particles are retained after permanganate fixation (Fig. 14, *cf.* Fig. 4) and behave like the rims of the large particles when exposed to fat solvents (Fig. 15). They are also retained by calcium formol fixation. Their lipid nature has been confirmed by finding much Sudan IV-positive material in the lacteals of the glucose-fed animals.

Discussion

The group of large particles which are found in the lacteals possess many characteristics which enable them to be identified as chylomicra. They increase markedly in size and number during fat feeding. Their morphology, particularly in Durcupan, is identical with that of isolated chylomicra. Both the isolated chylomicra and those in the tissues seem to possess an outer phospholipid shell which surrounds triglycerides. The rims of the isolated chylomicra usually appear rather granular. This granular appearance may be an artefact caused by the procedures which were necessary to isolate the chylomicra. Thus, Kay and Robinson (25) obtained much larger granules around their chylomicra after fixing them at room temperature and at a pH of 6.1. These conditions are known to produce more artefacts than fixation at 4°C and pH 7.0–7.4 (36). In the tissues embedded in Epon, the rims of the chylomicra are continuous. The major difference between the isolated chylomicra and those in the tissues is that the isolated ones appear empty when they are fixed with osmium and embedded in Araldite. Kay and Robinson also found this, but they prevented it by supporting the chylomicra with agar before dehydrating and embedding. The frequent breaks in the shells of the present chylomicra, which were not supported in this way, indicate that the contents probably leak out and are lost during dehydration and embedding. Embedding in the Durcupan prevents this, demonstrating the value of this substance when dealing with fat-soluble material. Chylomicra in the tissues probably receive adequate support during processing. The chylomicra studied here have all been in lymph. Those in the blood have more protein in their walls (5, 45), and their appearances may be a little different.

The small particles which are seen in the lacteals are very similar in size and form to the isolated lipoproteins. The results of permanganate and calcium formol fixation and the effects of fat solvents also confirm the nature of these particles.

FIGURE 19

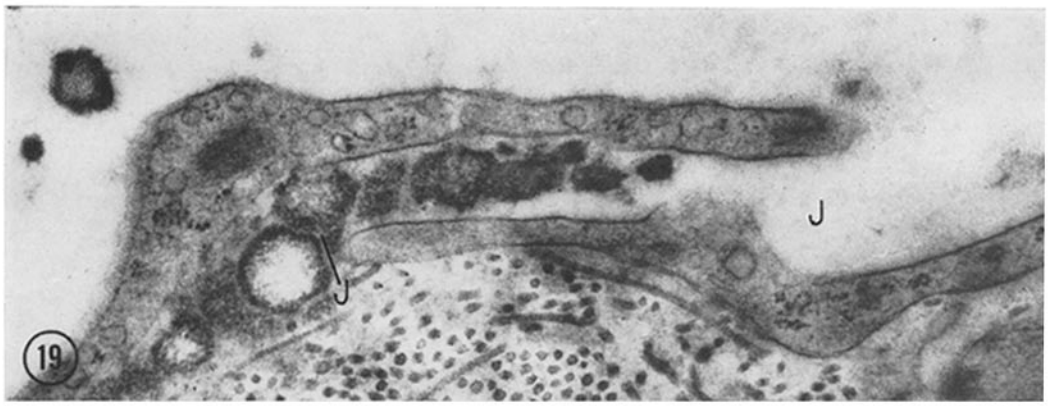
The animal has been fed maize oil. Chylomicra and lipoproteins are passing through an open junction (*JJ*). Section stained with uranyl acetate. × 35,000.

FIGURE 20

The animal has been fed maize oil. Chylomicra and lipoproteins are passing through an open junction (*JJ*). Some collagen fibres extend partly into the junction. Block stained with phosphotungstic acid. × 20,000.

FIGURE 21

The animal has been fed maize oil and the tissue was embedded in Durcupan. The chylomicra (*CM*) are uniformly dark. They are seen passing through the connective tissue and, *via* an open junction (*J*), into the lumen of a lymphatic (*L*). In one of the endothelial cells there is a large caveola (*LC*) containing a chylomicron. Inside the cell are some vesicles (*V*) which contain lipoprotein particles. Some of these particles are also seen in a large caveola (*LC*) on the external surface of the endothelium. A portion of a blood capillary is shown (*BC*). This possesses a slight basement membrane (*BM*), while none is visible deep to the lymphatic endothelium. In the blood capillary endothelium there are a number of fenestrae (arrows). Unstained. × 50,000.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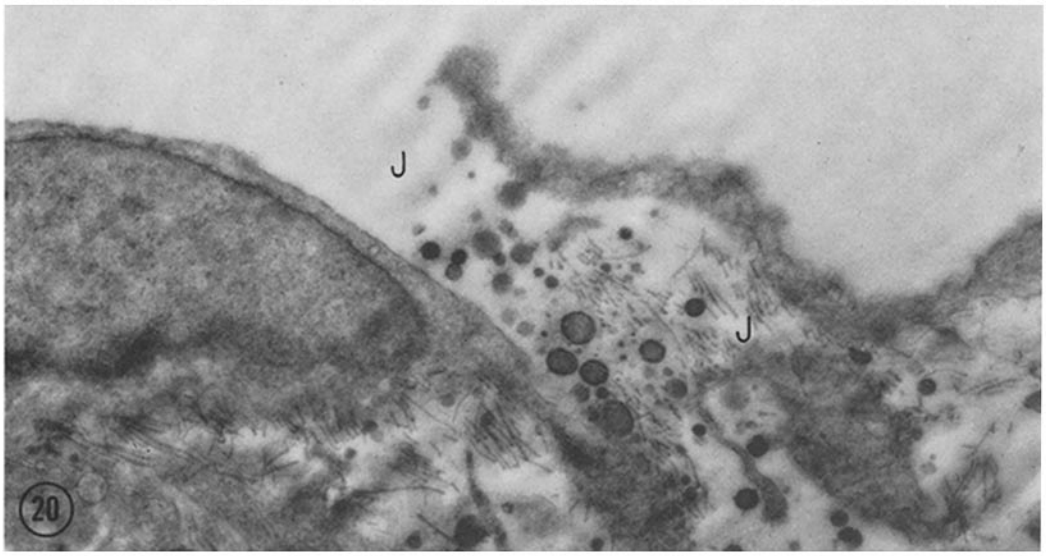
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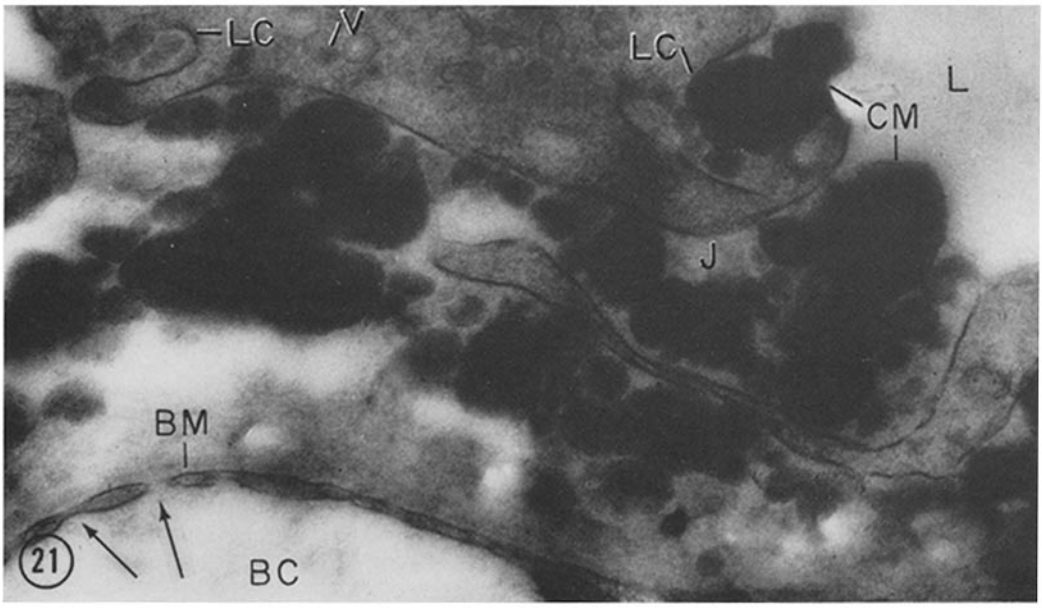
LC
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The appearances of the particles in the lacteals after different diets clearly show the independence of the two groups of fat particles. The fact that some of the larger lipoprotein particles also possess a dark rim after permanganate fixation indicates that their internal organisation may be similar to that of the chylomicra. It is sometimes held that there is no sharp division between these two groups of particles (44). While α and β lipoproteins can be identified by the ultracentrifuge, it was decided not to try to distinguish them at this stage. Their morphology is being studied and will be described later. It is likely that the lipoprotein particles seen in the villus come from the blood rather than from the intestinal epithelial cells. This does not alter the fact that they then pass into the lacteals (51).

Evidence has been presented to show that it is possible to identify chylomicra and lipoproteins in tissue sections. The paths by which these particles reach the lumina of the lacteals will now be described.

B—THE PASSAGE OF CHYLOMICRA AND LIPOPROTEINS INTO THE LACTEALS

Observations

The structure of the lacteals has been described by Palay and Karlin (40). The present observations confirm their findings. The basement membranes are poorly developed (Fig. 21) and the intercellular junctions usually lack adhesion plates

FIGURE 22

The animal has been fed on maize oil and the tissue was fixed in Caulfield's solution for 48 hours. The chylomicra (*CM*) are very black, but are distorted. A chylomicron has partly entered the endothelium via a large caveola (*LC*) in its external surface. Inside the endothelium there is a vesicle (*V*) which contains a lipoprotein particle. Section stained with uranyl acetate. × 45,000.

FIGURE 23

The animal has been fed maize oil. A chylomicron (*CM*) has almost completely entered the endothelium via a large caveola (*LC*). A lipoprotein particle is contained in a vesicle (*V*). Section stained with uranyl acetate. × 45,000.

FIGURE 24

The animal has been fed maize oil. Chylomicra are entering the endothelium via two large caveolae (*LC*). They are also inside the external portion of a junction (*JJ*) which is poorly visible. There is a lipoprotein particle in a vesicle (*V*). At the left of the illustration a chylomicron (*CM*) is contained in the endothelium. *L* indicates the lumen. Section stained with uranyl acetate. × 25,000.

FIGURE 25

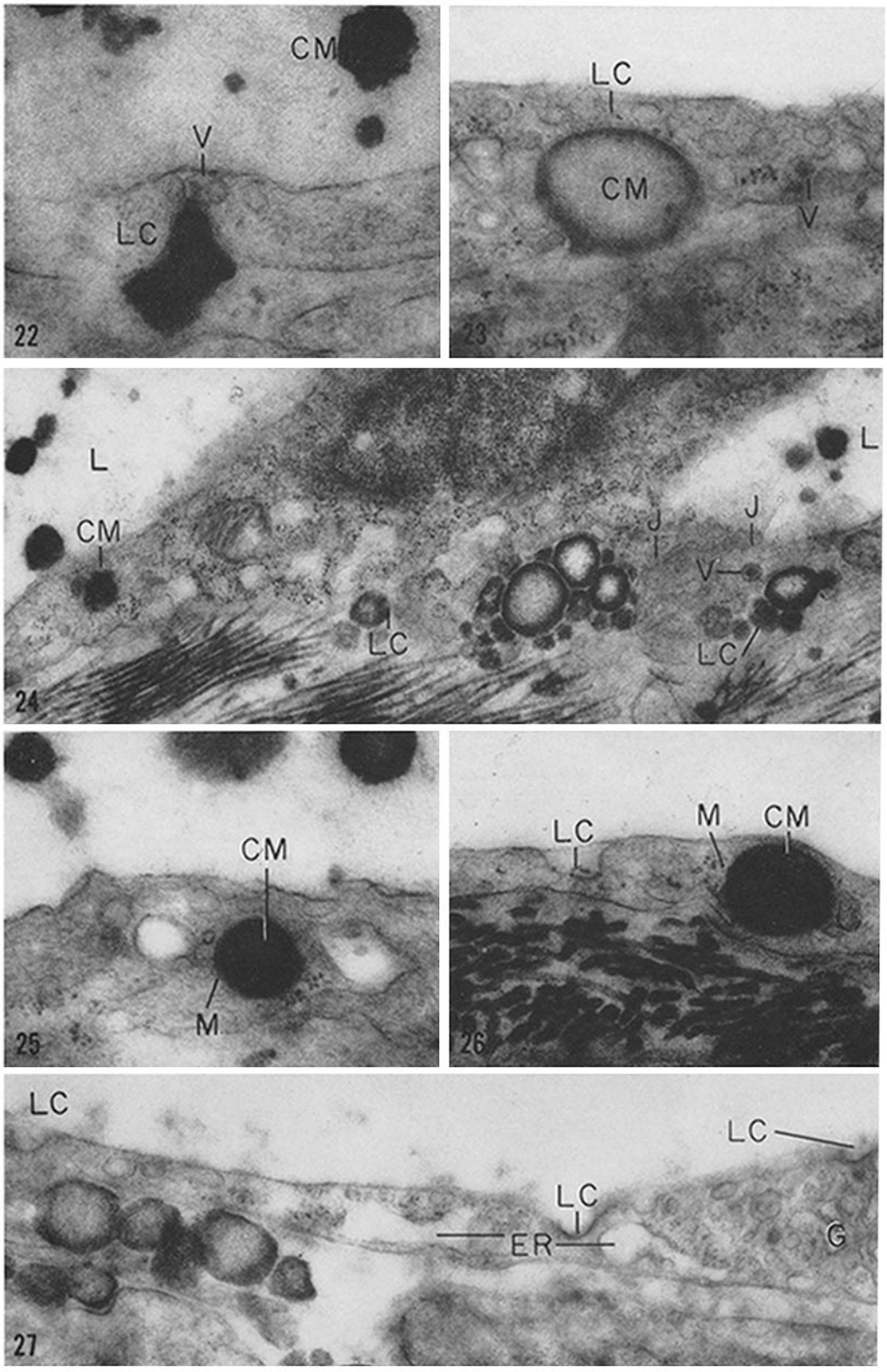
The animal has been fed maize oil. In the endothelium, a membrane (*M*) is visible around a chylomicron (*CM*). There are also two organelles which may be swollen endoplasmic reticulum. One of these contains a lipoprotein particle. Section stained with uranyl acetate. × 45,000.

FIGURE 26

The animal has been fed maize oil. A chylomicron (*CM*) is contained in the endothelium. A membrane (*M*) is partly visible around it. There is a large caveola (*LC*) on the luminal side of the endothelium. Section stained with uranyl acetate. × 40,000.

FIGURE 27

The animal has been fed maize oil. Three large caveolae (*LC*) are visible. Two have dark membranes. Some elements of the endoplasmic reticulum (*ER*) and the Golgi apparatus (*G*) are present. Section stained with uranyl acetate. × 25,000.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(Figs. 9, 11, 17 to 21, 24, 28 to 30; *cf.* Figs. 28, 32). This has been found in other lymphatics (7, 8). The junctions themselves are often partly or completely patent (Figs. 9, 11, 17, 18, 24, 28, 29; Figs. 19 to 21, 30). In this they resemble the junctions of lymphatics near other contracting muscles (*e.g.* diaphragmatic, skeletal, cardiac), but not those in quiescent areas such as the pinna of the ear (7, 8). In contradiction to the report of Ashworth *et al.* (2), but in confirmation of that of Palay and Karlin (40), no "pores" are seen in the endothelium of the lacteals although many fenestrae are seen in the cells lining the nearby blood capillaries (Fig. 21).

The passage of fat through the intestinal epithelial cells has been described by a number of authors (2, 10, 41, 48). The present observations confirm their findings. The chylomicra and lipoproteins then enter the connective tissue of the villus (the "lamina propria") and move towards the lacteals (Figs. 10, 16, 19 to 24, 27, 28). The chylomicra frequently distort the lymphatic endothelium (Figs. 10, 16), but the lipoproteins are too small to do this. The poorly developed or absent basement membranes do not impede the particles.

Chylomicra are often seen in partly or completely open intercellular junctions (Figs. 17, 18, 24; Figs. 19 to 21). Lipoproteins are also often seen in partly or completely patent ones (Figs. 11, 17, 28, 29; Figs. 19 to 21). It is very probable that many of the apparently partly open junctions are in fact completely open but in a plane other than that of the section. This has been confirmed by serial sections of partly open junctions in other sites (7). Some of the partly open junctions were probably in the process of opening completely when they were fixed. Others probably had been fully open and were fixed in the process of closing.

Chylomicra (Figs. 22 to 24) and lipoproteins (Figs. 30 to 32) are often seen entering the endothelial cells on their external surfaces. The lipoproteins usually enter in normal-size caveolae (~500 A), but the chylomicra are far too big to do this. They enter in large caveolae (1,000 A to 1 μ). Chylomicra are seen completely inside the endothelium (Figs. 24 to 26). The fat is so electron-opaque that it is sometimes very difficult to be

FIGURE 28

The animal has been starved for a day. Lipoprotein particles (*LP*) are present in the connective tissue (*CT*), in a partly open junction (*JJJJJ*), and in vesicles (*V*) in the endothelial cells (*E*). There is also a closed junction (arrow), which has an adhesion plate (*AP*). *L*, lumen. Section stained with uranyl acetate. × 35,000.

FIGURE 29

The animal has been fed only glucose for 3 days. A lipoprotein particle (*LP*) is present in a partly open junction (*J*) and in a caveola (*C*). Section stained with uranyl acetate. × 45,000.

FIGURE 30

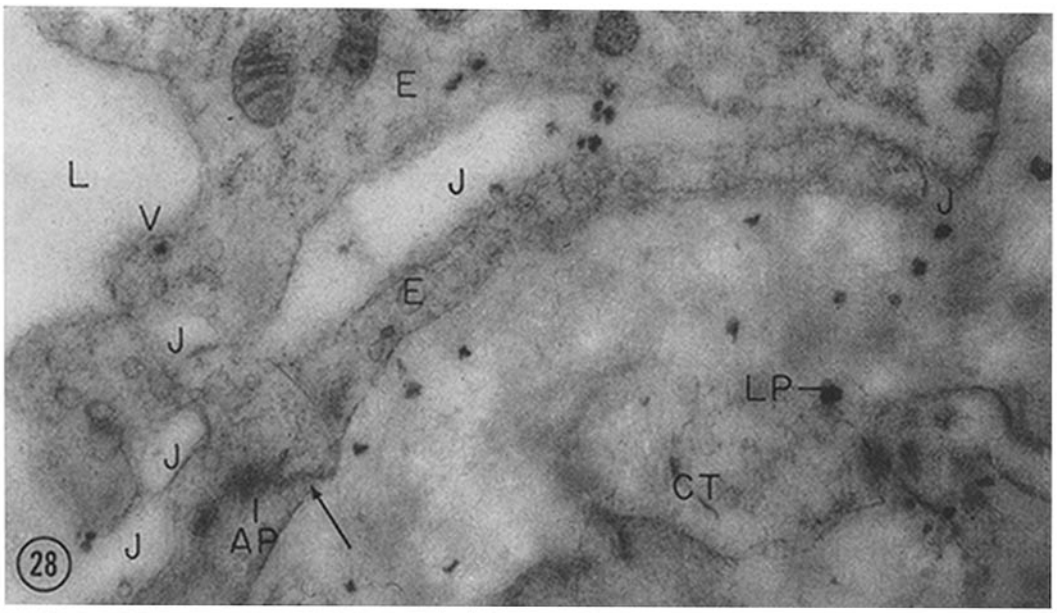
The animal has been fed only glucose for 3 days. Lipoprotein particles are present in caveolae (*C*) on both surfaces of the endothelium, and in vesicles (*V*). There is an open junction (*J*). *L*, lumen. Section stained with uranyl acetate. × 50,000.

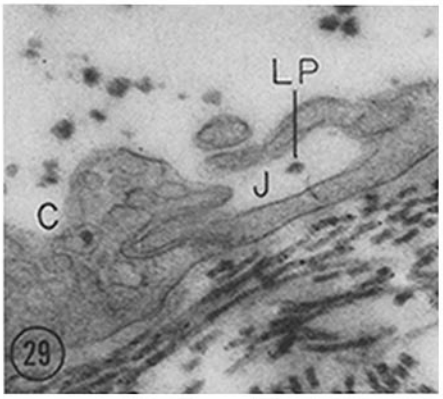
FIGURE 31

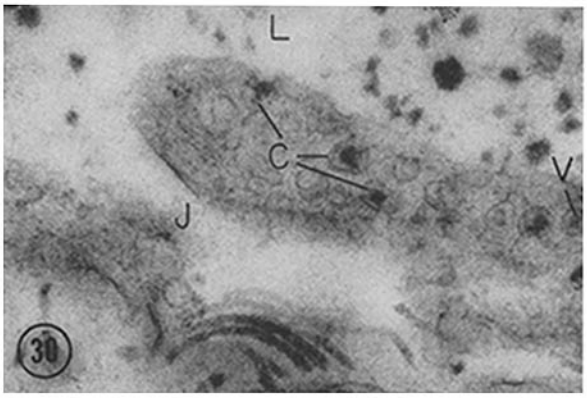
The animal has been fed maize oil. Lipoprotein particles are present in caveolae (*C*) on both surfaces of the endothelium (*E*). *L*, lumen. Section stained with uranyl acetate. × 45,000.

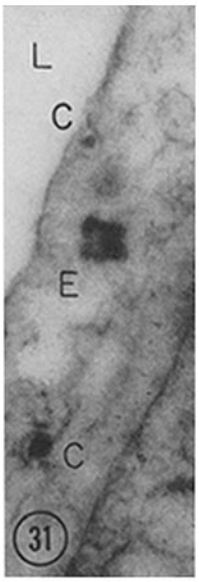
FIGURE 32

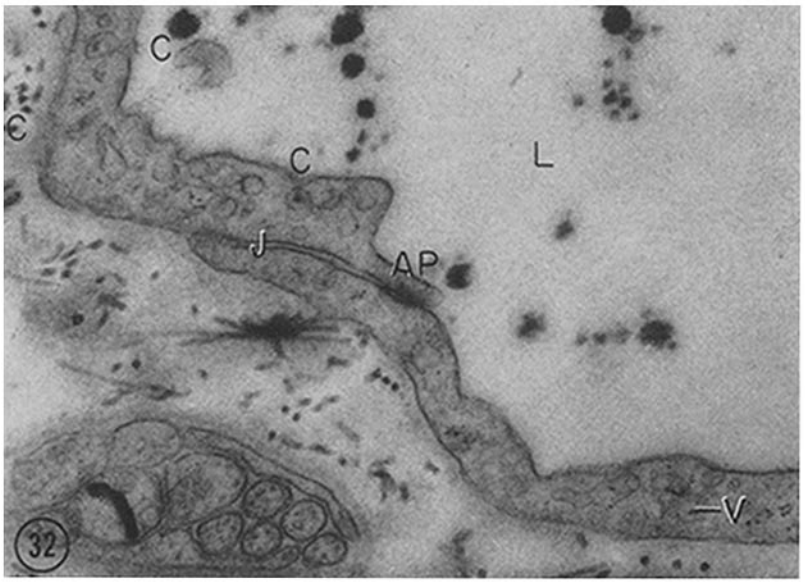
The animal has been fed only glucose for 3 days. Lipoprotein particles are contained in vesicles (*V*) and in caveolae (*C*) on both surfaces of the endothelium. There is a closed junction (*J*) with an adhesion plate (*AP*). *L*, lumen. Section stained with uranyl acetate. × 35,000.


E
L
V
J
J
E
J
LP
J
CT
J
AP
J
28


LP
J
C
29


L
C
V
J
30


L
C
E
C
31


C
C
C
L
J
AP
V
32

sure that there is a membrane around these bodies. Often membranes can be seen partly surrounding them (Figs. 25, 26). There is probably a membrane completely enclosing every particle. The lipoproteins are also often seen in the endothelium in normal-size vesicles (~500 A) (Figs. 21, 22, 24, 28, 30, 32). The membranes can be easily seen around these much smaller particles.

Lipoproteins are often seen in caveolae (~500 A) on the luminal surface of the endothelium (Figs. 29 to 32). It would seem that these are particles which are leaving the cells after having passed through them. However, there are many lipoprotein particles inside the lacteals and it is possible that some of these enter the cells on their luminal aspects. While it is much harder to find chylomicra in caveolae on the luminal side of the endothelium (Fig. 21), there are many large empty caveolae in this situation (Figs. 16, 18, 26, 27). These are present only in the animals which have many chylomicra in their tissues. Since the chylomicra are so large relative to the endothelial cells and to the lipoproteins, it is not difficult to imagine that they might be easily displaced from the caveolae by the flow of lymph or by slight movements during the processing of the tissues. It is still true, however, that there are many chylomicra in the lacteals and that some of these may enter the endothelial cells from the lumina.

One other feature of these large caveolae is notable. Many of those on the luminal surface are much darker than the adjoining plasma membranes of the cells (Figs. 16, 27). French (16) has observed similar dark areas in the plasma membrane at points where chylomicra are in contact with blood capillary endothelium. The large, dark-membraned caveolae are also similar to some which are seen in other situations and which Novikoff (35) considers related to "lysosomes."

In spite of the foregoing descriptions of fat particles entering the lumina of the lacteals, it is most surprising how relatively infrequently one sees particles either in junctions or in the endothelium (Figs. 9 to 11, 16). There are often many particles both inside and outside the vessels, yet the endothelial barrier contains relatively few. This must mean that the particles pass into the lumen extremely rapidly.

Discussion

Passage through Open Junctions

The reasons for rejecting the idea that these open intercellular junctions could be artefacts have been discussed by Palay and Karlin (40) and Casley-Smith and Florey (8). Briefly, these are that the plasma membranes do not appear to be torn, the cells often overlap, and partly open junctions are seen. It is possible that these junctions were mistaken for "pores" by Ashworth *et al.* (2). Open junctions have been found in other lymphatics (7, 8, 17). They are especially frequent when the vessels are near contracting muscle. The junctions are rarely "tied" by adhesion plates and the attenuated basement membranes must give little support. Thus, the junctions in lymphatic endothelium are easily opened, while those of nearby blood vascular endothelium remain closed. The latter are more securely fixed by frequent adhesion plates, more developed basement membranes (3), and the pressure inside the vessels would hold the endothelium against the connective tissue. The junctions in blood vascular endothelium do open, but only after the application of mild pathological stimuli (28, 29). Even here, the only open junctions are those which contain particles. Empty ones are closed by the pressure of the blood.

A number of workers have thought that the villi act as pumps and that their contractions force the contents of the lacteals into the deeper, collecting lymphatics (51). In particular, Verzar and McDougall (49) found that during fat absorption the lacteals were empty in the contracted villi and full in the remainder. Pumps need both inlet and outlet valves. If one of these is lacking, backflow will destroy much of the efficiency of the pump. It has long been known that the deep, collecting lymphatics possess valves which can act as outlet valves for the villus pumps. It would seem that the endothelial intercellular junctions probably act as the inlet valves.

The endothelial cells are very easily distorted. In the present study chylomicra have often been seen deforming them. Endothelial cells, in growing blood capillaries, may herniate through gaps in their basement membranes (11). They are often deformed by other cells in areas of inflammation (42). This pliability of the endothelium means that the free ends of the cells which constitute the open junctions must be easily moved by fluid pressure. In the relaxed villi the fluid and particles flow into the lacteals (49). The pressure difference which causes this flow, and the flow itself, will tend to open the junctions. The cells will be pushed into the lumen away from the connective tissue. The smooth muscle of the villus is situated just

external to the lacteal. When this muscle contracts, the pressure in the vessel will be higher than that in the lamina propria of the villus. If the junctions did not close, much of the contents of the villus would escape back into the connective tissue *via* the gaps between the muscle cells. However, the pressure difference and the potential fluid flow is now in the opposite direction. The pliable endothelial cells will be forced against the less yielding connective tissues and the junctions will be closed (*cf.* blood capillaries). In addition, the contraction of the villi will tend to "telescope" the cells over each other and the overlap at the junctions will be increased. Thus, the junctions can act as inlet valves, opening when the villi relax and closing when they contract.

It seems that other lymphatics also act as pumps. Allen and Vogt (1) suggested that the lacunes on the peritoneal surface of the diaphragm behaved in this way. Material enters these vessels through open junctions which probably open and close with respiration (7, 17). Lymphatics near active skeletal or cardiac muscle probably behave similarly (7, 12).

Majno and Palade (28) saw chylomicra pass through open junctions in traumatized blood vascular endothelium. Palay and Karlin (41) showed that fat particles pass into the lacteals *via* open junctions. The present results have confirmed these findings and extended them by identifying the fat particles as both chylomicra and lipoproteins.

Passage through the Cells

Palade (38) has seen "small fat particles" in vesicles in the blood vascular endothelial cells of starving animals. The small sizes of these particles and the conditions under which they were seen would indicate that they were lipoproteins. In the present study lipoproteins have been seen in caveolae and vesicles which are of normal size (~500 A). Chylomicra occupy much larger organelles. Gordon and King (20) have shown that cellular energy is needed for the entry of large particles (~1 μ) into some tissue culture cells. These authors and others (7, 13, 21) have shown that smaller particles (~200 A) can pass passively into cells and probably into small vesicles without requiring the expenditure of energy. In fact, one test substance used was lipoprotein (13). This would indicate that there is a considerable physiological difference between these two classes of vesicles. In addition, there are dark membranes lining many of the large caveolae. Novikoff (35) has said that similar large dark-membraned caveolae are related to "lysosomes." If so, they are far from being passive structures.

The presence of many particles in the lumina of the lacteals makes it impossible to say with certainty that the particles seen in caveolae on the luminal surface of the cells are emerging after traversing the endothelium. However intraperitoneally injected chylomicra and lipoproteins can leave the lymphatics of the diaphragm by traversing the endothelium (7). (They emerge into the connective tissue around the vessels and are "trapped" there. In this situation they cannot be washed away, nor can they be mistaken for particles entering the cells.) Just because fat particles can pass through the lymphatic endothelium in one set of vessels, it does not necessarily follow that they can do so in the villi. However they probably do so. This path through the lacteal endothelial barrier has not been mentioned by other workers. The passage of material in small pinocytic vesicles (~500 A) across endothelial and other cells has been suggested by many writers (3, 6, 7, 15, 22, 30, 34, 37–39). It has been experimentally verified (7, 15, 22, 30, 39). Clearly, the passage of the lipoprotein in small vesicles would be identical with this process. The passage of the chylomicra in large vesicles might have a different mechanism.

Which Path Is the Most Important?

It is not possible to be certain of the number of particles which pass through the cells relative to those which pass through the open junctions since particles tend to be washed out of caveolae. The lymphatics in the normal ear are about as permeable as the blood capillaries (24, 32, 33, 46). These vessels normally have few open junctions, and it seems that most material passes through the cells rather than through the junctions (7). When the ear lymphatics are subjected to very mild trauma they become much more permeable (24, 32, 33). Under these conditions, there are many more open junctions (7). It seems certain that any great increase above the normal permeability of lymphatic or blood vascular endothelium is caused by the opening of many intercellular junctions. This is also substantiated by the fact that the lacteals (14, 51), the diaphragmatic lacunes (14), and lymphatics near cardiac or active skeletal muscle (12, 51) are all extremely permeable, and these lymphatics possess many

more open junctions than do those in tissues where there is less movement (7). Again, mildly traumatised blood vessels are very permeable and their intercellular junctions are often patent (28, 29). It would seem then that it is the open junctions which give the lacteals their great permeability. Thus, it is likely that much material passes through the patent lacteal junctions rather than through the endothelial cells.

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Addendum in Press:

Jones, Thomas, and Scott (*Exp. and Molec. Path.*, 1962, **1,** 65) have examined osmium-fixed chylomicra in lymph taken from rats fed butter or corn oil. The morphology of the chylomicra from the animals fed corn oil was very similar to that reported here. The chylomicra from the butter-fed animals were often some 5 to 20 times larger, with slightly scalloped borders. In many of their preparations these authors found large numbers of small (~500 A) osmiophilic particles, of whose nature they were in doubt. It is almost certain that these were lipoproteins.