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The surface coat of chylomicrons: electron microscopy

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ABSTRACT Electron microscope studies were performed on thoracic duct lymph and on washed chylomicrons from dogs fed corn oil. High-resolution electron micrographs showed the presence of a surface coat that differed from the core material and did not resemble a plasma membrane. This was true for both chylomicrons in whole lymph and those that had been subjected to repeated washing. Apparently, the chylomicrons, while passing from the intracellular to the extracellular space, do not acquire their surface coat from pinched off cellular membrane.

KEY WORDS dog · lymph · chylomicrons · electron microscopy · surface structure

In a previous publication, electron micrographs of ruptured, isolated chylomicrons showed the presence of hollow rings which were thought to represent the surface coat of the chylomicron (1). In the accompanying paper (2) further properties of the surface material of isolated chylomicrons have been presented. Chemically, this material is distinct from that of the chylomicron interior. It consists primarily of phospholipid with some free cholesterol and protein and possibly some triglyceride. The possible sites of origin of the chylomicron surface coat were discussed. One possible manner in which chylomicrons could become covered by a layer of lipid and protein is by a process in which a piece of plasma membrane is pinched off, as it were, during the extrusion of the lipid particles from the intestinal cell into the extracellular spaces. Such a mechanism has been postulated for milk fat droplets (3–6). If this mechanism also operated for chylomicrons one would expect to see "unit membranes" adhering to the oil droplets.

Electron micrographs of chylomicrons have been published previously (7–16). In these studies the fat droplets were sectioned as part of the tissue, which was the prime object of study, or the chylomicrons were centrifuged from blood or lymph, fixed, and then either placed on a grid for viewing by the electron microscope or embedded and sectioned prior to viewing. Although it has been established that there is no endoplasmic reticulum envelope surrounding extracellular chylomicrons (7, 9), none of these studies focused attention on resolving the fine structure of the chylomicron surface.

The present study was aimed at discovering whether the surface of chylomicrons either in lymph or isolated by centrifugation and washing showed any evidence of the trilaminar structure of cytoplasmic membranes.

METHODS

The techniques for collection of lymph from dogs fed an emulsion of corn oil in skim milk as well as the procedures for centrifuging and washing chylomicrons have been described (2). Whole lymph and washed chylomicrons were fixed for 2–3 days in 2% unbuffered osmium tetroxide in distilled water. The prolonged fixation was to ensure complete fixation of the lipid droplets (11). After fixation, some of the preparations were stained in block with a 2% uranyl nitrate solution for 1 hr. All the preparations were dehydrated with ethanol and embedded in Epon 812. Sections (~700 A thick) were examined either with an RCA EMU 3C or with an AEI, EM 6B electron microscope.

RESULTS

Figs. 1 and 2 show the results obtained on washed

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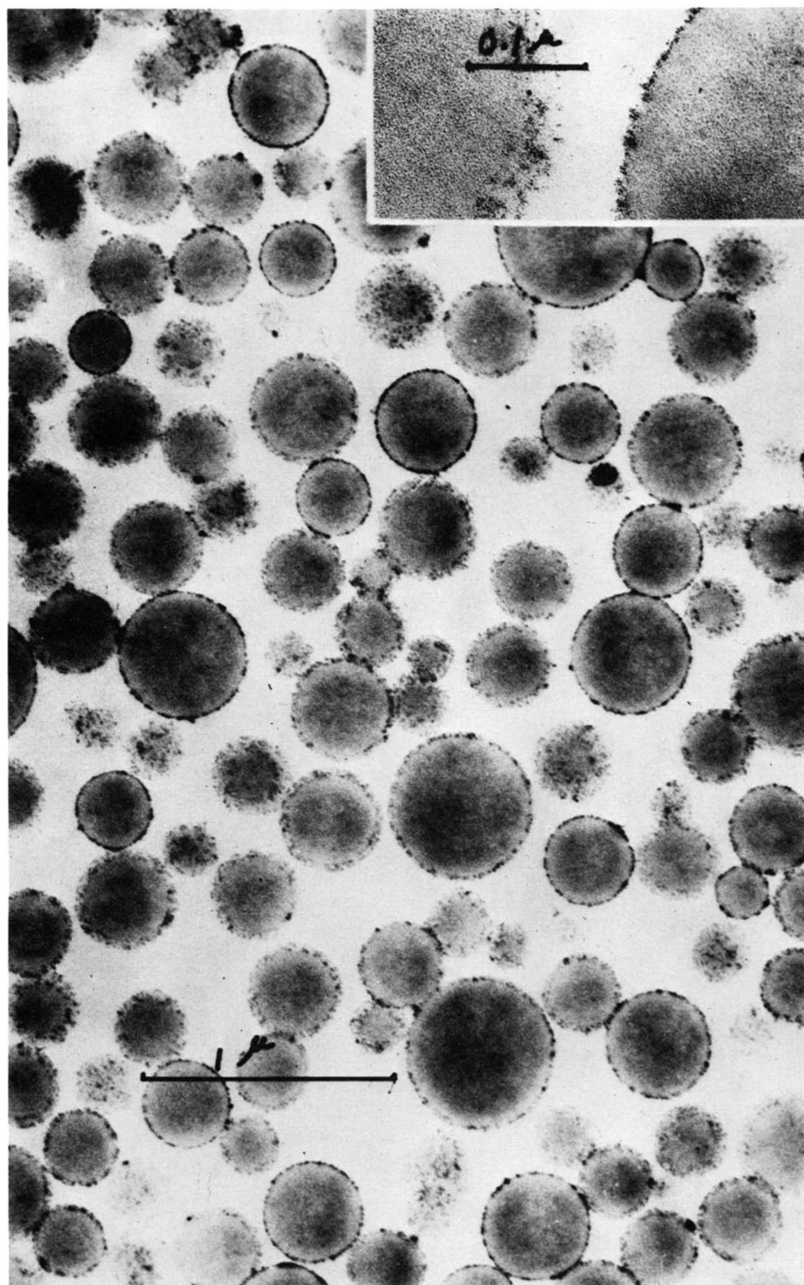

FIG. 1. Washed chylomicrons from dog 1. Dense outer coat bears no resemblance to plasma membranes. Fixation: 2% OsO_4, unbuffered, for 2 days. ×35,000 (insert ×160,000).

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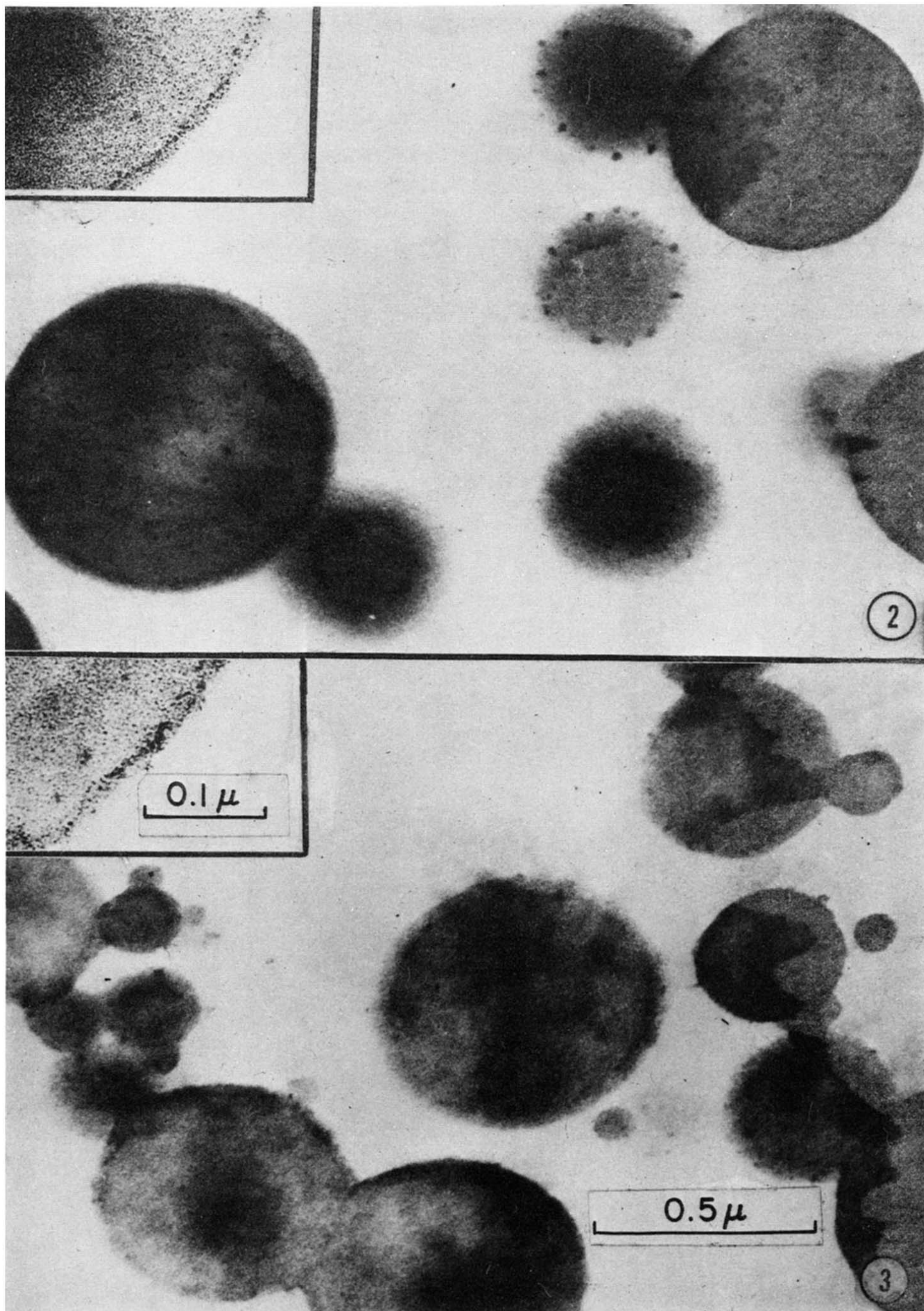

FIG. 2. Washed chylomicrons from dog 2 show a somewhat different organization of outer coat from that in Fig. 1. Fixation: 2% OsO_4, unbuffered, for 2 days followed by 1 hr in 2% uranyl nitrate. ×60,000 (insert ×160,000).

FIG. 3. Chylomicrons in whole lymph from dog 2 fixed and stained as in Fig. 2. ×60,000 (insert ×160,000).

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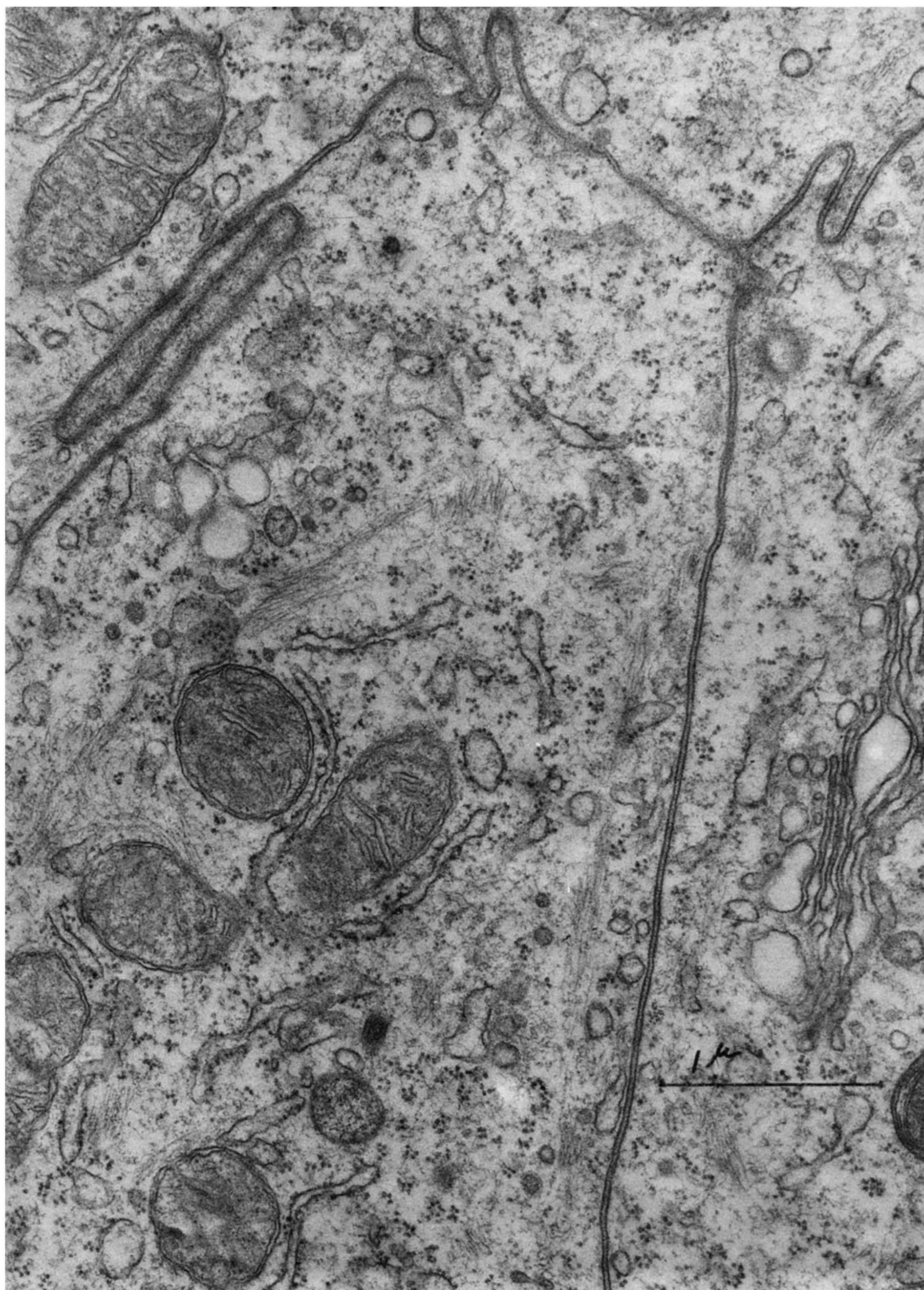

FIG. 4. Section of intestinal epithelium from the mouse. Fixation: 2% OsO_4, unbuffered, for 2.5 days, followed by 1 hr in 2% uranyl nitrate. ×30,000.

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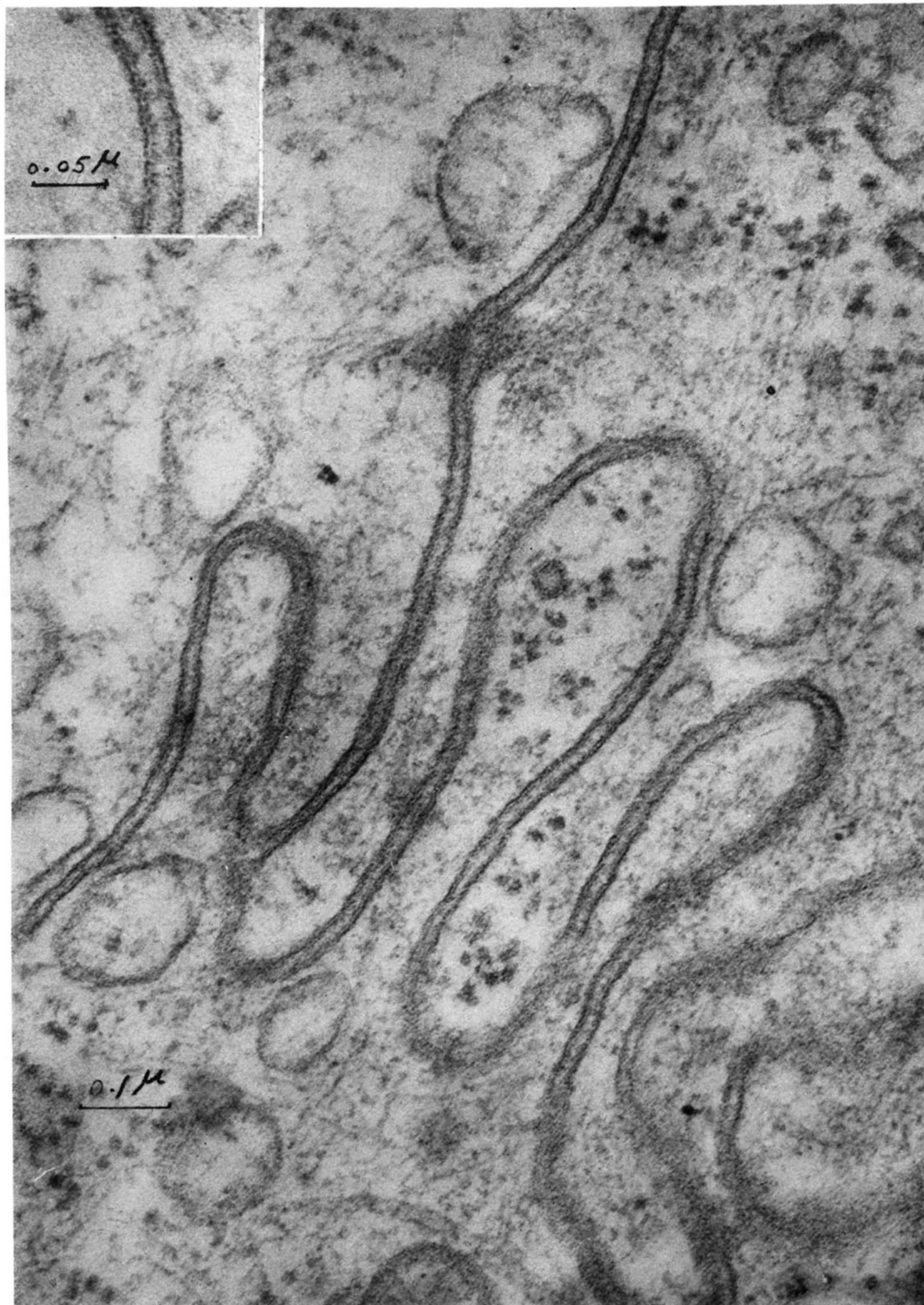

Fig. 5. Same tissue as in Fig. 4, showing adequate preservation of membrane structure. ×120,000 (insert ×200,000).

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chylomicrons from two dogs. Fig. 3 represents whole lymph fixed in OsO_4. The lipid droplets are circular and have no resolvable fine structure. They appear to be surrounded by a coat of irregular shape considerably more osmiophilic than the interior. The coat, which bears no resemblance to plasma membrane, consists either of a dense zone covered by an outer fuzzy layer or either of these components alone. It is conceivable that a plasma membrane might not have been preserved by the unusually harsh fixation employed. A piece of small intestine from a mouse was fixed in 2% aqueous osmium tetroxide for 2.5 days so that we could ascertain whether intestinal plasma membranes are preserved under the conditions of fixation employed for chylomicrons. Adequate preservation of membrane structure was seen especially when, after fixation, the tissue was soaked in a 2% solution of uranyl nitrate for 1 hr (Figs. 4 and 5).

DISCUSSION

Palay and Karlin (7) showed earlier that in the intestine, intracellular lipid droplets are found within vesicular components of the endoplasmic reticulum. Since the endoplasmic reticulum envelope is absent from extracellular chylomicrons, it has been postulated that the fat droplets leave the cell either by a process whereby the membranes of the endoplasmic reticulum surrounding the intracellular lipid droplets fuse with the plasma membrane (7–9) or directly via the lumina of the endoplasmic reticulum (10). Another possibility, compatible with the above observations, is that the formed chylomicrons reenter the cytoplasm from the endoplasmic reticulum before leaving the cell. The mechanism proposed for the extrusion of milk droplets (3–6), whereby a piece of plasma membrane is pinched off by the droplet as it leaves the cell could then also apply to the chylomicrons. If this were so, then some trace of plasma membrane would be expected to adhere closely to the chylomicron core and comprise the chylomicron coat. Such a membrane would be distinct from the loose-fitting endoplasmic reticulum envelope, known to be absent from extracellular chylomicrons (7, 9). No such membrane was seen.

In the accompanying paper (2), three hypotheses were proposed for the possible origin of the chylomicron coat. It was suggested that the coat may arise from (*a*) intracellular components, (*b*) pinched-off plasma membrane or (*c*) extracellular components. The chemical data on the fatty acid composition of chylomicron and lymph phospholipids suggest that the surface coat cannot be derived exclusively from extracellular material. The electron micrographs presented here give no support to the hypothesis that the particle leaves the intestinal cell by pinching off a section of plasma membrane. Therefore, it seems most reasonable that the chylomicron surface coat is derived from intracellular material.

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