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ELECTRON MICROSCOPY OF CHYLOMICRONS

by

T. L. Hayes, F. T. Lindgren, and J. C. Schooley

INTRODUCTION

Several of the lipid-containing macromolecules are large enough for visualization with the electron microscope. The serum lipoproteins have been studied in this laboratory for some time, and this note reports some recent work done on the large lipoprotein complex, the chylomicrons.

As originally used, the term chylomicron referred to particles in the size range of 0.5 μ to 1.5 μ in diameter that were observed in the dark-field microscope. In more recent work, the chylomicron class has been extended to include all the light-scattering lipid particles that can be separated from blood or lymph by a few minutes of high-speed ultracentrifugation.

Chemically, the chylomicrons consist primarily of triglycerides, but also contain phospholipid, cholesterol, and protein. Several models have been proposed for their structure, and it was hoped that electron microscopy would provide more direct information concerning the nature of these particles.

METHODS

Chylomicrons of approximately Sf2,000 to 10⁵ were isolated from human serum by using a swinging-bucket ultracentrifugal technique. The top 1 ml was pipetted from the tube, and an aliquot mixed with 10 parts by volume of a 1 per cent solution of OsO₄ buffered to pH 7.5. After 24 hours at room temperature, the chylomicrons were either diluted in distilled water for direct viewing in the electron microscope or were centrifuged to the bottom of the tube, washed, dehydrated, and embedded in methacrylate for sectioning.

A synthetic vegetable oil emulsion (Lipomul-Oral, Upjohn) was used as a source of non-chylomicron triglyceride particles.

Chylomicrons were also studied in lymph obtained by cannulation of the thoracic duct of rats. The lymph was mixed with 10 volumes of the OsO₄ solution, and after 1/2 hour at 0°C, the chylomicrons and cells were centrifuged to the bottom of the tube, washed, dehydrated, and embedded.

Finally, chylomicrons were viewed in lymph in situ in the thoracic duct of the rat. Two ties were placed around the duct in such a manner that the duct remained filled with lymph. The piece of duct was then removed and placed immediately into the OsO₄ solution for 1 hour at 0°C. The ties were left in place throughout the washing, dehydration, and embedding.

Sectioning was done with a Porter-Blum ultramicrotome with a diamond or glass knife, and all material was examined with an RCA EMU-2E electron microscope.

RESULTS AND DISCUSSION

The chylomicrons isolated from human serum by our techniques were found to be spherical particles ranging from 700 to 5000 Å in diameter and occurring singly or in clusters of two or more. The work was supported in part by research grant H-1882(C6) from the National Institutes, U.S.P.H.S., Bethesda, Maryland.
Figure 1. Isolated human chylomicrons; OsO_4 treatment. Magnification: x 55,000.

Figure 2. Lipid particle from vegetable oil emulsion; OsO_4 treatment. Chromium shadow. Magnification: x 82,500.
Figure 3. Isolated human chylomicrons; OsO₄
treatment. Sectioned. Magnification: x 55,000

Figure 4. Isolated human chylomicrons; OsO₄
treatment. Sectioned. Magnification: x 50,000
Figure 5. Rat lymph; OsO₄ treatment. Magnification: x 17,500. C, chylomicrons; L, lymphocyte; M, mitochondria; N, nucleus.

Figure 6. Rat thoracic duct; OsO₄ treatment. Magnification: x 17,500. C, chylomicrons; L, lymphocyte; E, endothelial cell; Col, collagen.
in groups (Figure 1). Following the OsO₄ treatment, the lipid particles seemed quite stable to air drying, retaining their spherical shape (Figure 2). The chylomicrons appeared smooth, and there was no indication of surface irregularity that might be associated with a protein or lipoprotein layer.

The sectioned chylomicrons, however, showed two aspects of fine structure. First, collections of dense particles about 50 Å in diameter were found, usually near the surface of the chylomicron but occasionally as clumps of particles ranging a considerable distance towards the interior (Figures 3 and 4). These dense particles may be material adsorbed to the chylomicron during the preparation procedures, or they may represent structural subunits. Second, there appears to be a fine structure in the interior of the chylomicrons. This consists of particles about 30 Å in diameter and some 100 Å long (Figures 3 and 4). These dimensions are similar to those of the high-density serum lipoprotein that has been postulated to be part of the chylomicron complex. Their presence in the interior of the chylomicron, however, suggests that the protein moiety may be dispersed throughout the particle. In contrast, no internal fine structure was seen in the sectioned lipid particles from the vegetable oil emulsion.

The rat chylomicrons were readily identifiable in the lymph obtained by cannulation (Figure 5) and in the thoracic duct (Figure 6). There was essentially no difference in the appearance of the particles obtained and prepared by these two different techniques. This suggests that centrifugal isolation following OsO₄ treatment does not alter their gross structure.

In all cases, the treatment with OsO₄ preserved the structure of the chylomicrons through subsequent dehydration, embedding, and drying procedures, and it would seem that this technique offers considerable promise as a tool for the study of the structure and metabolism of these large lipoproteins.

LITERATURE CITED