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LIPID DROPLETS OF THE SEBACEOUS GLAND: SOME NEW OBSERVATIONS FROM TANNIC ACID FIXATION

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Abstract. Electron microscopic examination using tannic acid fixation strongly supported the view that lipid droplets in the normal human sebaceous gland originate from smooth-surfaced endoplasmic reticulum. A true limiting membrane was clearly detected around the lipid droplets in the incipient stages but was undetectable in mature droplets.

Key words: Lipid droplet; Limiting membrane; Sebaceous gland; Tannic acid fixation

The morphology of lipid droplets from sebaceous glands has been discussed in a number of reports dealing with droplet ultrastructure (1, 2, 3). However, conflicting results have been presented. The discrepancies presumably stem from the fact that most triglyceride, together with some of the other lipids such as phospholipids, are lost from sebaceous gland cells during dehydration and embedding when conventional techniques are used (4). In the present study, lipid droplets of the normal human sebaceous gland were examined by electron microscopy utilizing tannic acid fixation (5, 6, 7), which is said to enhance membrane contrast more than any other fixation method previously used. The origin of the lipid droplets is discussed in relation to evidence of a true limiting membrane around the lipid droplet.

MATERIALS AND METHODS

Sebaceous glands were obtained from the uninvolved area of surgically excised skin taken from a 29-year-old Japanese male with a solitary pea-sized intradermal nevus on the scalp.

Electron microscopy

The conventional Epon procedure of Luft (8) was used for comparison with tannic acid fixation. Sections were cut in an LKB ultramicrotome and double stained with uranyl acetate and Reynold's lead citrate. The stained sections were observed in a JEM-100B electron microscope with an accelerating voltage of 80 kV.

Tannic acid fixation

Specimens were cut into 0.5 mm^3 tissue blocks and fixed for 2 h in 1% tannic acid in 2.5% glutaraldehyde buffered to pH 7.2 with 0.1 M cacodylate. The pH of the fixative was adjusted to 6.8 with 1.0 N NaOH solution shortly before use. The cubes were rinsed overnight in the same buffer and then osmicated with 1% acid in the same buffer for 2 h. Specimens were rinsed overnight in the same buffer, refixed in the former fixative for 2 h, and then re-osmicated with the latter fixative for 2 h. The subsequent process was the same as for the conventional method.

RESULTS

Conventional method

The sebaceous glands were composed of three kinds of cell: undifferentiated cells, partially differentiated cells, and fully differentiated cells (9).

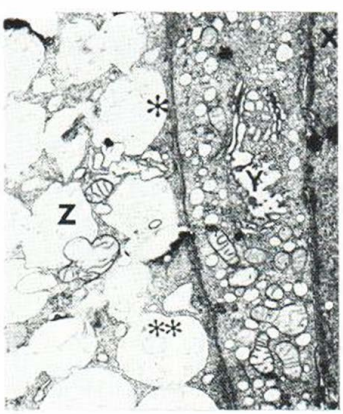

Fig. 1. Electron micrograph of the specimen provided by tannic acid fixation. *X:* Undifferentiated sebaceous cell. Partially differentiated sebaceous cells in early stage (*Y*), and in late stage (*Z*). ×8000.

Fig. 2. Highly magnified view of the area marked by a single asterisk in Fig. 1. *Gol*, Golgi apparatus; *v*, Golgi vesicle; small asterisk, Golgi vacuole; *C*, Golgi cistern; *M*, mitochondria; *Z*, centriole; large asterisk, vacuole; arrow, cell membrane; *L*, lipid droplet; *PDCE*, partially differentiated sebaceous cell in early stage; *PDCL*, partially differentiated sebaceous cell in late stage. ×48 000.

Fig. 3. Highly magnified view of the lipid droplet at the position marked by double asterisk. *sER*, Smooth-surfaced endoplasmic reticulum; *A*, dilated sER; *B*, intermediate stage of lipid droplet between the initial stages and sER; *C*, *D*, initial stages of lipid droplets; arrow, tri-laminar limiting membrane; asterisk, vacuole; *M*, mitochondria; *t*, microtubule. ×48 000.

No new observations were made beyond what has already been reported (1, 2, 3).

Tannic acid fixation method

I. *Low magnification.* The same three cell types were found as in the conventional method. Two kinds of partially differentiated cell (PDC) were distinguishable (Fig. 1), viz. "PDC at an early stage" (PDCE) and "PDC at a late stage" (PDCL). The PDCE (marked *Y* in Fig. 1) was rather dark and flattened, with well-developed Golgi apparatus, mitochondria and abundant vacuoles throughout the cytoplasm. The PDCL (marked *Z* in Fig. 1) contained a number of lipid droplets, which occupied a greater percentage of cell volume than in PDCE.

II. *High magnification.* (A) The PDCE in Fig. 2 showed a well-developed Golgi apparatus (*GOL*) consisting of membrane-limited vesicles (*V*), cisterns (*C*), micro-tubules (*t*), and vacuoles (small asterisk). In addition to a centriole (*Z*) and mitochondria (*M*), the cytoplasm contained a number of widely dispersed membrane-limited vacuoles (large asterisk). The membrane of the latter organelles was 7.5 nm thick, whereas the cell membrane (arrow) was 10 nm thick. Vacuoles (large asterisk) were frequently located in the immediate neighbourhood of the Golgiapparatus and are thus probably derived from it; they may represent a formative stage in the development of the vacuoles scattered throughout the cytoplasm. However, there was no evidence of transitional forms between the vacuoles (large asterisk) in PDCE in Fig. 2 and the lipid droplets in PDCL in Fig. 2.

(B) The PDCL in Fig. 3 contained a number of lipid droplets. The cell matrices were not as dark as in the PDCE. Apart from the lipid droplets (*C* and *D*) and some smooth-surfaced endoplasmic reticulum (*sER*), the most striking feature was the presence of two kinds of membrane-limited structure (*A* and *B*), thought to be transitional forms between the smooth-surfaced endoplasmic reticulum and lipid droplets (*C* and *D*).

Membrane-limited structure *A* in Fig. 3 was thought to be a kind of dilated smooth-surfaced endoplasmic reticulum rather than a form of lysosome, since the matrix of *A* had a lower density than primary lysosomes, and there was no peripheral halo between the limiting membrane and the matrix. In contrast, the matrices of *sER* and *A* were very similar. Structures *A* and *B* were likewise different in an appearance from mitochondria and secondary lysosomes, and thus provided a clear consecutive relation between the lipid droplets (*C* or *D*) and *sER*. Although a true limiting membrane was not observed around the fully matured lipid droplets, some of the early stage lipid droplets (*C* and *D* in Fig. 3) were clearly surrounded by a tri-laminar limiting membrane (arrow in Fig. 3).

COMMENTS

Tannic acid was originally introduced into electron microscopy in 1971 by Mizuhira et al. (5) as a supplementary fixative. A number of investigators reported that it enhanced the contrast of various structures, including membrane systems (9, 10, 11, 12). Kalina et al. (6, 7) have since proved that the ability of tannic acid preceding osmication to reveal highly ordered, preserved lamellar structures is attributable to the interaction of tannic acid with the choline component of phospholipid to form a "complex", which can then be stabilized by treatment with osmic acid.

Despite an extensive literature on the ultrastructure of the surface of lipid droplets in adipose cells, it is still uncertain whether lipid droplets have a limiting membrane. Thus, some workers maintain there is limiting membrane while others discount this (13). Ellis et al. suggested that a thin dense line around the periphery of the lipid droplets found in human sebaceous gland is indicative of a Golgi membrane in the sebaceous cell. Breathnach (14) insists that the lipid droplets of human sebaceous gland are unrelated to any cytoplasmic membrane system.

In the present study, a tri-laminar, true limiting membrane was clearly present around the very initial stages of the lipid droplet, and all the evidence indicated that lipid droplets come from sER. Derivation of the lipid droplet from sER material is supported by the fact that true limiting membrane was clearly present only around lipid droplets, in the very initial stages and never around the mature lipid droplet. If lipid droplets originated from the Golgi apparatus, a true limiting membrane would have to be present throughout their development.

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