Fine structure of frozen-etched lipid granules in the fat body of an insect

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Abstract Lipid storage in fat-body cells of adult female black flies was examined using freeze-etching electron microscopy. Frozen-etched lipid granules exhibited a laminated structure. The molecular arrangement of the lipid granule may depend on the physiological condition of the insect and may be involved in the control of lipid metabolism in the fat-body cell.

Supplementary key words electron microscopy · concentric lipid lamellae · phospholipid · simuliid fly

THE INSECT FAT BODY is considered to be similar in function to mammalian liver and adipose tissue with respect to lipid metabolism (1). This is supported by many biochemical studies (see reviews, Refs. 2 and 3). Much lipid is stored in fat-body cells by various insects in the form of triglycerides and other lipids, such as phospholipids. These lipids serve as an energy reserve and as building blocks for cellular structures during metamorphosis (4).

The freeze-etching technique with electron microscopy was developed by Moor and Mühlethaler (5), who applied it to the study of phospholipids in yeast cells. The technique permits an ultrastructural study of lipids *in situ* without involving chemical reactions, such as those in thin-section electron microscopy. In this paper the fine structure of lipid granules in the fat-body of a black fly, *Simulium vittatum* Zett., is examined using freeze-etching electron microscopy.

MATERIALS AND METHODS

Larvae and pupae of *S. vittatum* were collected from the Grand River at Highway 6, Caledonia, Ontario. The rearing method of Yang and Davies (6) was used to obtain adult flies. Fat bodies of newly emerged female black flies were dissected out in Schneider's *Drosophila* medium (Grand Island Biological Co.) mixed with glycerol in the proportion of 4:1 (v/v). Fat bodies were held in this medium for 2 hr. The pretreated fat bodies were placed in a gold specimen holder; they were briefly frozen in Freon 12 and then stored in liquid nitrogen. Freeze-fracturing was conducted in a Balzers freeze-etching apparatus (Balzers AG, Liechtenstein) according to the technique of Moor and Mühlethaler (5). Etching was performed for 1 min. Platinum–carbon replication was produced immediately after etching with a shadow angle of 45°. The platinum–carbon replica was released by dipping the sample holder in 30% KOH for 2 hr, leaving the replica just above the fluid surface. The digestion with KOH was required for fat-body tissue because, being rich in protein and lipids, it stuck firmly to the replica and was cleanly released only by this special treatment. The freed replica was mounted on a 300-mesh copper grid and examined in a Zeiss EM 9 electron microscope.

RESULTS

A large lipid granule observed in the fat-body cell measured 6.2 μ in length and 4.1 μ in width (Fig. 1). The fractured face of this granule shows a structure of smooth-surfaced, concentric lamellae, each of which was approximately 40–60 A (400–600 nm) thick. The space between two lamellae cannot be measured from the micrograph, but it appears that the lamellae are in close contact with each other. A regular succession of more than 20 lipid lamellae is observed from the outside toward the center. Separate globules appear to be wrapped within the outside lamellae. From the outside their profiles can be seen as a group of elevations. One of these separate globules in the center of the micrograph shows also a series of lamellae with the same thickness as the main lamellae of the lipid granule.

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FIG. 1. A frozen-etched lipid granule in the fat-body cell of a newly emerged female black fly showing the convex surfaces of concentric lamellae. Arrow in circle indicates shadowing direction. × 36,000.

A cross-fractured lipid granule also revealed that separated globules are wrapped within the outer lamellae (Fig 2). Each of these globules exhibits a concentric pattern of smooth-surfaced lamellae showing either convex or concave faces. Between these globules appear some amorphous areas which could be uncrystallized lipid, such as triglyceride, differing from the lamellar configuration of the polar lipid molecules.

Usually two lipid granules (sometimes one) were observed centrally in each fat-body cell, surrounded by much smaller, but variously sized, protein granules (Fig. 2).

DISCUSSION

In newly emerged adult black flies, fat bodies containing large amounts of protein and lipid were studied histochemically by Chen (7), but she gave no indication of the chemical nature of this lipid. The fine structure of the frozen-etched lipid granules, observed in the present study, was similar to that of phospholipids seen with the freeze-etching technique either *in situ* (5) or in diluted dispersions of phospholipid in water (8, 9). Phospholipid is known to exist in insects in large amounts (4, 10). However, in the black fly fat body, the chemical composition of the lipids remains to be investigated. Since *S. vittatum* females are autogenous, requiring no blood meal after emergence for ovarian development (7), it is reasonable to assume that the larva stores phospholipid in the fat body along with other lipids and proteins.

The present observations suggest that "phospholipid" in the cytoplasm of frozen-etched fat-body cells behaves just as in aqueous solution. The fractured surface of etched lipid lamellae in these cells is smooth. Fluck,

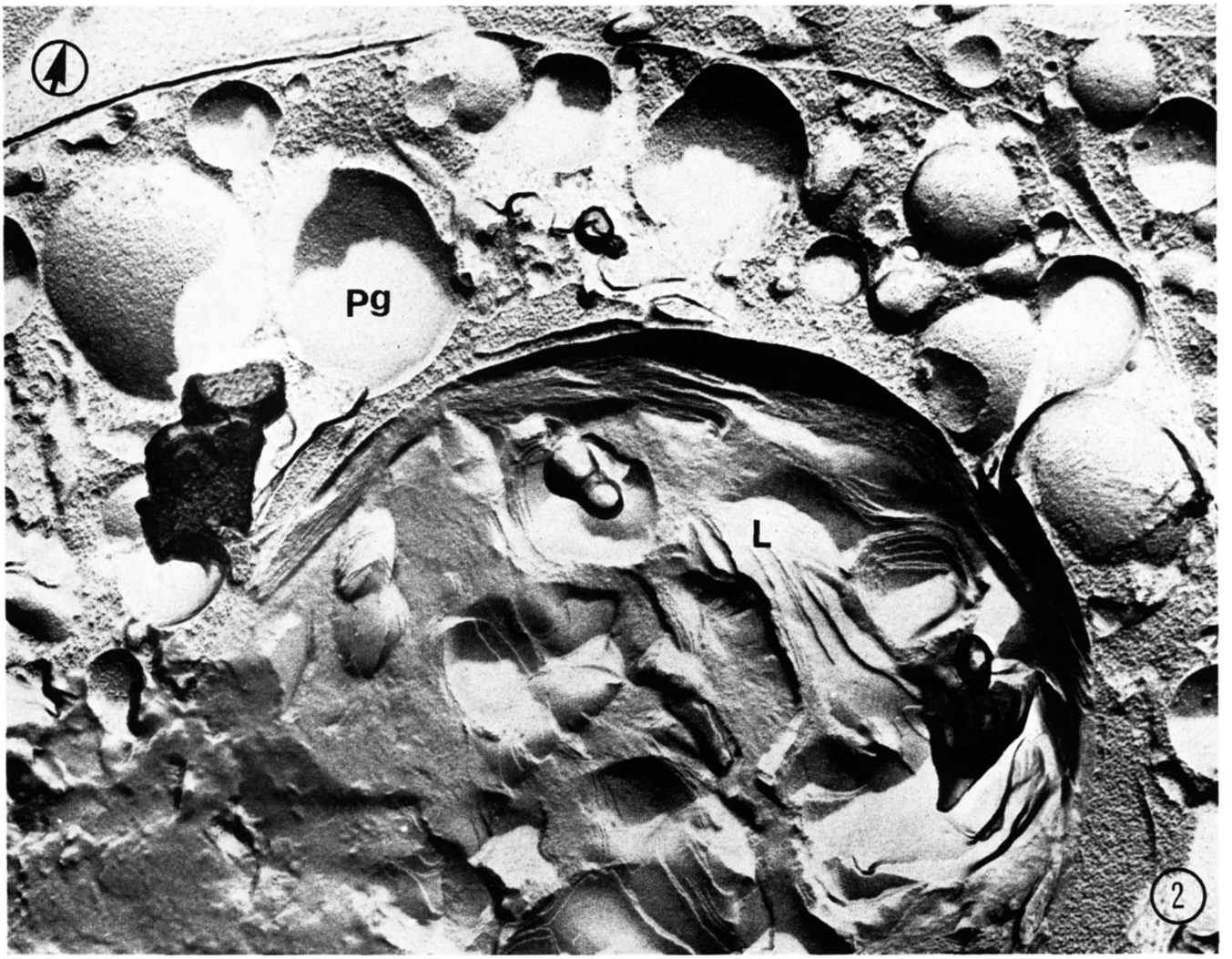

FIG. 2. A frozen-etched fat-body cell of a newly emerged female black fly. A lipid granule (*L*) in cross-fractured face reveals a series of small granules, each wrapped within laminated "shells" showing both convex and concave faces. Numerous protein granules (*pg*) are also characteristic. × 36,000.

Henson, and Chapman (8), who studied frozen-etched lipid suspensions, argued that fractures passed between lipid–water interfaces and that etching had removed etchable material (presumably water) from the lipid surface. However, Deamer and Branton (11) presented experimental evidence proving that frozen lipid bilayers are split along their hydrophobic central plane. Thus the lipid–water interface remained unobserved in our preparations.

The lamellar packing of "phospholipids" in such granules in the black fly fat body may be of physiological importance. Bangham (12) pointed out that the molecular arrangement of the phospholipid presents certain problems concerning enzyme degradation, since no reaction site is available for enzyme hydrolysis unless the surface charge is modified. This modification depends on the ion concentration of insoluble anion or cation, the overall pH of the aqueous solution, and the concentration of diglyceride. From these observations, it is reasonable to assume that the lamellar structure of the lipid granule has physiological significance, since in insects most of the lipid stored in the fat body is available for various physiological processes (2, 4). The specific structure in the lipid granule may function in controlling lipid metabolism in the fat-body cell and depends on the physiological condition of the insect.

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