Specific Lamellar Structures of Agranular Endoplasmic Reticulum in the Senile Mouse Adrenal Cortex*

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Summary. In the adrenal cortex of old mice, lamellar bodies of various shapes frequently appear in the zona reticularis cells. These structures consist of electron dense plates, about 20nm in width, closely piled at regular distances of about 25nm. Each plate displays two sheets of unit membranes enclosing a dense material and with several clear spaces between them. These paired membranes usually terminate in round saccules at both ends, but occasionally continue to the tubules of surrounding agranular endoplasmic reticulum. Saccules in several rows, in combination with a part of the lamellae, may be arranged circularly in the cytoplasm. From these observations, it is proposed that the lamellar body is composed of a cup-shaped mass of flattened cisternae of the agranular endoplasmic reticulum and each of the cisternae is attenuated and closed except at their marginal sacculations. The concentric or horseshoe-shaped profile of the lamellar body observed in this study is accounted for by different section planes through the cup-shaped structure.

Specific lamellar structures of agranular endoplasmic reticulum occasionally appear in adrenal cortical cells of mice (Sato, 1967; Shelton and Jones, 1971; Moore and Callas, 1975; Fujita, 1977), but their detailed ultrastructure has not yet been elucidated. During the course of an electron microscopic study of senile mouse adrenal glands, highly organized lamellar structures were frequently encountered in parenchymal cells of the inner zone of the cortex. This paper describes the three-dimensional organization of these structures.

MATERIAL AND METHODS

Six healthy senile male mice aged 23 months and a control group of six healthy mature male mice aged 6 months were used for this study. All mice were DDK strain, and had been maintained in our laboratory. The animals were first anesthetized by ether, and then the adrenal glands were removed after intracardial perfusion with 2% glutaraldehyde in 0.1 M phosphate buffer. The perfused glands were cut into small

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pieces and fixed in 2% glutaraldehyde for 3 hrs. The tissues were washed overnight in a buffer solution containing sucrose, and then postfixed with 2% osmium tetroxide in Millonig buffer for 1.5 hrs. All tissues, after dehydration in a graded series of acetone, were embedded in Epon 812. Ultrathin sections were cut with a Porter Blum ultramicrotome, doubly stained with uranyl acetate and lead citrate, and examined with a JEM T8 electron microscope.

RESULTS

Specific lamellar structures of a linear, curved, horseshoe or concentric shape were frequently noted in parenchymal cells of the inner zone, especially in the zona reticularis adjacent to the medulla in the senile mouse adrenal cortex. The cells containing such lamellar structures possessed well-developed, intimately connected tubules of agranular endoplasmic reticulum (SFR) and ovoid and occasionally elongated mitochondria with tubular cristae (Fig. 1). The fine structures of these cells corresponded to those reported previously (ZELANDER, 1957, 1959; MÖLBERT and ARNESEN, 1960; SATO, 1967; SHARDEIN et al., 1967; IDELMAN, 1970), except for the increase of lipofuscin and ceroid pigments and the appearance of occasional bizarre mitochondria (unpublished data).

These lamellar structures were variable in their profile appearance (Fig. 1–4) but had the following common features: 1) they consist of electron dense plates, about 20 nm in width and closely stacked upon each other at a regular distance of about 25 nm; 2) the origin of the plates composing the lamella is never from the same level

![Fig. 1.](image)

Fig. 1. Cells of zona reticularis in a senile mouse. Lamellar structures of various shapes are seen in the cytoplasm. Other cell organelles such as mitochondria, free ribosomes, Golgi apparatus and endoplasmic reticulum show no difference from those of the control animal, except for increased lipofuscin or lysosomal granules. × 8,000
but disaligned by a constant distance between adjacent plates; 3) each end of the plate usually terminates in a round saccule of about 150 nm in diameter; 4) some of the plates in the same lamella are continuous to the surrounding SER, though in rare cases continuous to rough ER (Fig. 8), instead of ending in saccules; and 5) each plate,

Fig. 2-5. Different profiles of concentric and horseshoe-shaped lamellar bodies. The differences apparently depend upon the direction of sectioning through a stack of cup-shaped cisternae of agranular endoplasmic reticulum accompanied by marginal saccules (see the Fig. 6). The profiles 2-4 seem to be produced when the cup is cut transversely (Fig. 2), obliquely (Fig. 3), and longitudinally (Fig. 4) to long axis, respectively. The pattern in Figure 5 is thought to occur when the cup is cut circularly along its rim with numerous saccules. Arrows show that some of the electron-dense plates composing the lamellar structure continue to the endoplasmic reticulum (Fig. 2). ×20,000
under high magnification, comprises two sheets of unit membranes enclosing a dense osmiophilic material, within which is contained several clear spaces of up to 50 nm in diameter at irregular intervals (Fig. 7). From these findings, it can be supposed that each of these plates may be a flattened SER cisternae, the inner cavity of which is narrowed except in the area of the dilated peripheral saccules.

The horseshoe-shaped and concentric profiles were the most highly organized

Fig. 6. Three-dimensional diagram of the lamellar structure in the reticularis cells of the senile mouse adrenal cortex. The body consists of a stack of cup-shaped cisternae of agranular endoplasmic reticulum, which are closed except at their margins with dilated saccules. Four different profiles produced by different section planes are illustrated.
among the lamellar structures observed here (Fig. 2-4). It seems reasonable to suppose that the two types of profiles may have been formed by a dense, cup-shaped body of SER cisternae. The horseshoe-shape seems to occur when the cup is cut parallel to its long axis, while the concentric one must appear when it is cut transversely. The thickest concentric lamellae encountered in this study consisted of 30-layers but several of the outermost cisternae did not form a complete circle (Fig. 2, 4). In addition, some of the concentric or horseshoe-shaped profiles contained mitochondria and other organelles in their center (Fig. 5). Furthermore, in rare instances, an annular array of several rows of round saccules of 120nm in average diameter were seen in the cytoplasm, in combination with a part of the lamellar structure (Fig. 5). These may be cases in which the layered cup-shaped cisternae of SER had been cut circularly along their rims, and this suggests that the rim is studied with a row of numerous saccules.

On the basis of these observations, a three-dimensional diagram of the concentric and horseshoe-shaped bodies is given in Figure 6

**DISCUSSION**

Lamellar structures similar to those reported here were first demonstrated by SATO (1967) and SHELTON and JONES (1971) in the fasciculate cells of the normal mouse adrenal cortex and briefly described as “stacks of agranular endoplasmic reticulum” and “lamellar collections of SER” respectively. Subsequently, one of the figures of the reticularis cell of mouse adrenal cortex by MOORE and CALLAS (1975) shows a straight lamellar body but without any comment on it. Recently FUJITA (1977) described the same sort of body in an old mouse aged 22 months. As the lamellar structures

*Fig. 7. High magnification of a straight lamellar structure. It consists of parallel electron-dense plates, each comprising two unit membranes containing a dense osmiophilic material and several clear spaces between them. Both ends of each plate usually connect to round saccules, but occasionally to tubules of the surrounding endoplasmic reticulum (arrow). One plate is shown connected to a large vacuole, but this is an exceptional incidence. ×52,000*
hitherto demonstrated were smaller in size or simpler in structure as compared with those observed in the present study, none of the authors seem to have special attention to them.

Somewhat similar lamellar structures have been described in epithelial cells of the mouse ascending colon (Michaels, 1975): they resemble our lamellar bodies in that they consist of a stack of lamellae thought to be a modification of endoplasmic reticulum and sometimes are concentric in structure, but differ in that they contain numerous vesicles in their intercisternal spaces.

Occurrence of electron-dense materials within the cisternae of SER in steroid-synthesizing cells has been reported in the adrenal gland (Dietert and Scallen, 1969) and testis (Dietert and Scallen, 1969; Russo, 1971) of rats and mice following administration of triparanol, inhibitor of cholesterol synthesis, and interpreted as an accumulation of cholesterol precursor (Russo, 1971). Thus, it is conceivable that the electron-dense material retained in the lamellar, flattened cisternae observed in the present work might also be a substance produced during cholesterol synthesis under some inhibitory condition of SER-associated enzymatic activity, and that the lamellar structures might have been caused by accumulation of such substance into the flattened cisternae probably formed by a process of apposition and fusion of tubules of the surrounding SER.

Fig. 8. A straight lamellar structure continuous with smooth endoplasmic reticulum at one end and with rough endoplasmic reticulum at the other end. A connection with rough ER is rarely found. ×30,000
The lamellar structures were also found in the control young mice, but they were rather rare and mostly small in size and simple in structure. Accordingly, it is possible that these structures increase in number, and become larger in size and more complex in form with age. Similar structures of simpler type are found also in Leydig cells of old mice (unpublished data). Therefore, it may be assumed that such lamellar structures indicate one type of age-associated degenerative changes of SER in the steroid-producing cell of mice. Occurrence of the lamellar bodies noteworthy is specific for this animal species. No other species but the mouse has been reported to have similar structures in steroid-producing cells.

REFERENCES


