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Ultrastructural Localization of Digitonin-Cholesterol Complex in the Adrenal Cortex

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SUMMARY : The ultrastructural localization of cholesterol was studied by digitonin reaction which has been used to identify the free cholesterol for electron microscopy. Digitonin-cholesterol complex revealed multi-layered cylindrical electron dense lamellae and concentric whorled lamellae. They were found in the cytoplasm closely attached to the plasma membrane in adrenocortical cells, and were also present in extracellular spaces around capillaries in the adrenal cortex. Some of them were present in lipid vacuoles and some of them are attached to the outer membrane of mitochondria.

These findings are consistent with the hypothesis that the free cholesterol is the precursor of steroid hormone in the adrenal cortex. The fact that some mitochondria were surrounded by digitonin-cholesterol complex and these complexes had a close relation with lipid vacuoles, suggested to us that cholesterol might be stored in lipid vacuoles and they might move into mitochondria for the hydroxylation and the side chain cleaving to form pregnenolone in the steroid pathway.

INTRODUCTION

Digitonin reaction has been used for the determination of free 3 β-hydroxysterol in various organs[7] such as liver[3], adrenal glands[1) 3) 5) 7)], aorta[6] and seminiferous tubules[3]. Essentially, the method is based on the formation of a compound of digitonin and free 3 β-hydroxysterols. Digitonin reaction could be used in light microscopy[3], polarization microscopy[1)3)] and histochemical study[1) 3)]. The digitonin reaction was also adapted to the electron microscopic technique[3) 5) 7)]. The digitonin-cholesterol complex formed cylindrical and whorled lamellae that possessed strong osmiophilic properties, permitting the localization and structure of them crystals in ultrathin sections.

The purpose of this study is to identify the localization of the digitonin-cholesterol complex in the cells of adrenal cortex and to discuss the relationship to steroid biosynthesis.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 200-300 gm were used for the experiment. Bilateral adrenal glands were removed after ether anesthesia and adrenals were cut into small pieces and prefixed in 2.7% glutaraldehyde solution for two hours at 4 ℃. This was followed by two rinses (20 minutes each) in phosphate buffer and then by immersion in a saturated solution of digitonin in the same buffer for four hours. After postfixation in 1% osmium tetroxide for a hour, tissues were dehydrated in graded ethanols at 4 ℃ and embedded in Luveak 812. The ultrathin sections were cut on a sorvall MT-2 ultramicrotome and examined with JEM 1200 EX after staining with uranyl

acetate and lead citrate. Small pieces of adrenal glands that were fixed in 2.7% glutaraldehyde without digitonin served as controls.

RESULTS

The digitonin-cholesterol complexes were identified as osmiophilic multilayered cylindrical structures alternating clear spaces in a longitudinal section, measuring 40 mμ in diameter. Cylindrical structures were composed of electron dense membranes and electron lucent cores. The thickness of the individual membrane was about 4 mμ. In cross section, they appeared as concentric whorled lamellae.

Small groups of electron dense digitonin reaction of cylindrical and whorled lamellae were found in the cytoplasm closely attached to plasma membranes in the vicinity of capillaries (Fig. 1). These complexes also appeared focally in the cytoplasm adjacent to intercellular plasma membrane (Fig. 2), but they did not appear so frequently and were not so abundant. These digitonin-cholesterol complexes were scattered along extracellular spaces around capillaries. Digitonin-cholesterol complexes were more abundant in well developed microvilli of plasma membranes facing capillaries (Fig. 3). Cylindrical and whorled lamellae of digitonin-cholesterol complex were observed in lipid vacuoles (Fig. 5) and they were attached to the outer membrane of mitochondria which

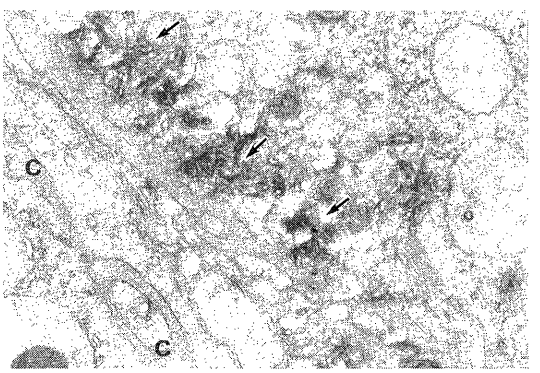

Fig. 1. The digitonin-cholesterol complex (arrows) are found in the cytoplasm closely attached to the plasma membrane near a capillary (C). × 52,000

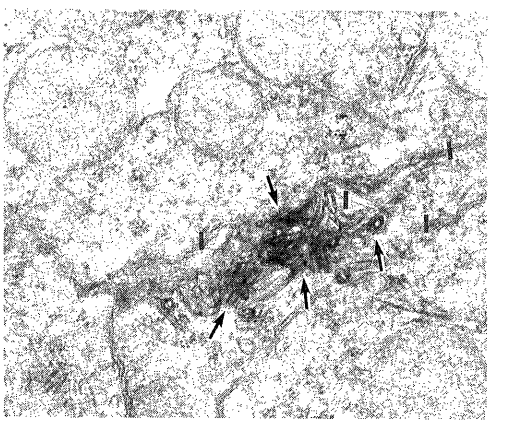

Fig. 2. Arrows indicate the digitonin-cholesterol complex in the cytoplasm adjacent to the intercellular plasma membrane (I). × 52,000

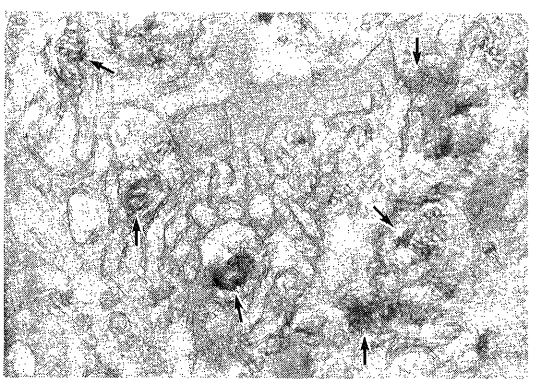

Fig. 3. Abundant digitonin-cholesterol complexes are seen in well developed microvilli of plasma membrane (arrows). × 52,000

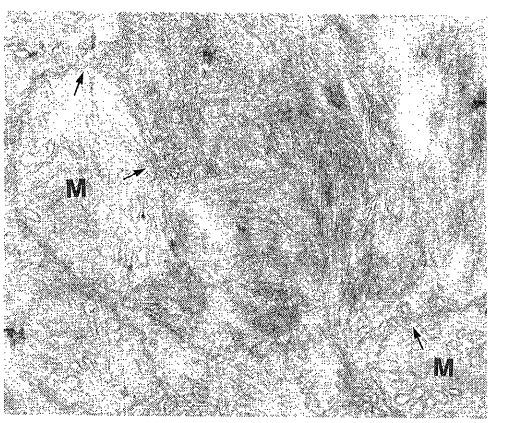

Fig. 4. Mitochondria (M) are surrounded by digitonin-cholesterol complex and some of them are attached to the outer membrane of mitochondria. × 65,000

were surrounded by digitonin-cholesterol complex (Fig. 4). There was no difference in distribution and localization of digitonin-cholesterol complexes between zona glomerulosa and zona fasciculata.

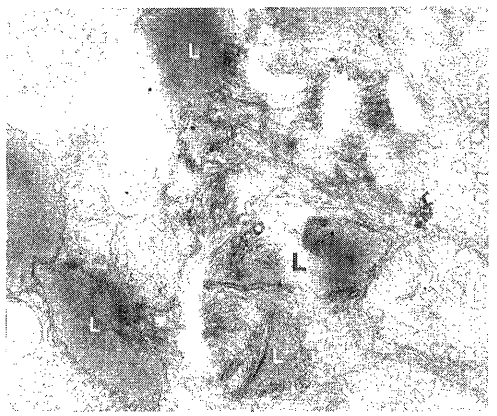

Fig. 5. Digitonin-cholesterol complex are in the lipid vacuoles (L). × 65,000

The reaction products were entirely negative in the cells of the adrenal cortex processed without digitonin in the fixatives.

COMMENT

It has been reported that the digitonin reaction was highly specific for 3 β-hydroxysterols and this reaction has been widely used for separating free cholesterol in various tissues[1) 3) 5) 6) 7)].

Since digitonin does not react with esterified cholesterol, digitonin reaction is a convenient marker to identify non-esterified cholesterol localization in the ultrastructural investigation[3) 5) 6) 7)]. Digitonin-cholesterol complexes appeared as a small group of cylindrical lamellae and concentric whorled lamellae in the cells of various organs[1) 3) 5) 6) 7)]. Autoradiographic study using ^{3}H-cholesterol demonstrated a distribution of free cholesterol in the digitonin-cholesterol complexes[2) 5)].

Though cholesterol is the main precursor of steroid hormones and the raw material for pregnenolone synthesis[4)], the utilization of free or esterified cholesterol in the synthesis of steroid hormone is not known.

In the present study large amounts of digitonin-cholesterol complex were found, mostly in the cytoplasm adjacent to the plasma membrane of adrenal cortical cells, and adjacent to extracellular spaces around the capillaries of the adrenal cortex. Some of them were present in lipid vacuoles and some had a close connection with the outer membrane of mitochondria. These findings suggested to us that free cholesterol might enter into the cytoplasm as a precursor of steroid hormone through the plasma membrane and some of it might store in lipid vacuoles and it might moved into mitochondria for the hydroxylation and side-chain cleaving to form pregnenolone with the functional condition of the adrenal cortex.

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