ELECTRON-MICROSCOPIC AUTORADIOGRAPHY OF THE ADRENAL CORTEX AFTER ADMINISTRATION OF CHOLESTEROL-H^3

D. Kh. Khamidov, Academician K. A. Zufarov,* L. A. Murtazaeva, and I. M. Baibekov

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The distribution of cholesterol-H^3 and its conversion products in the cells of the adrenal cortex was studied by electron-microscopic autoradiography. The highest concentration of tracks was found above the mitochondria and lipid inclusions of the adrenocortical cells.

KEY WORDS: electron-microscopic autoradiography; adrenals; mitochondria; steroid biosynthesis.

Many investigators ascribe a leading role to the mitochondria of the adrenal cortex in steroid production [1, 6]. Electron-microscopic observations have revealed mitochondria with the honeycombed-vesicular structure specific for steroid-producing organs [2, 3, 6, 7]. With an increase in the intensity of adrenocortical function large vacuoles appear inside the mitochondria or intramitochondrial vesicles appear in the cytoplasm [3, 8] (Fig. 1). It is tempting to connect these changes with steroid biosynthesis in the adrenal cortex.

* Academy of Sciences of the Uzbek SSR.

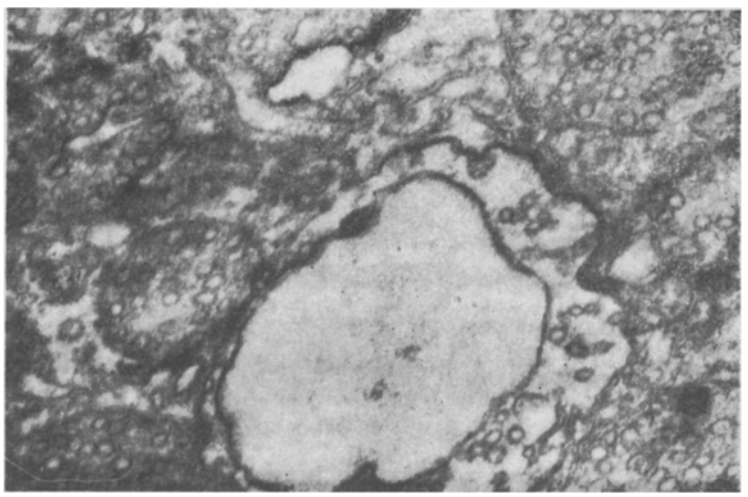

Fig. 1. Various types of intramitochondrial vacuoles in adrenal cortex, 50,000×.

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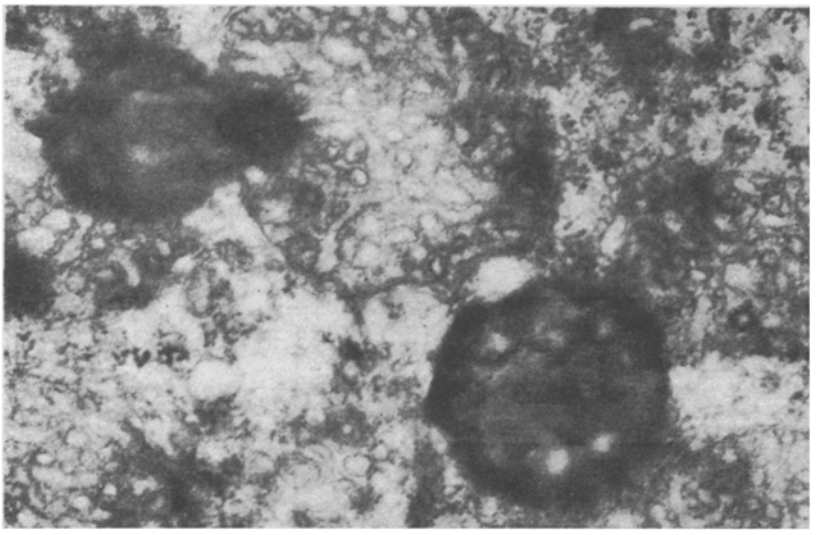

Fig. 2. Tracks of cholesterol-H^3 above lipid inclusions in adrenocortical cell, 50,000×.

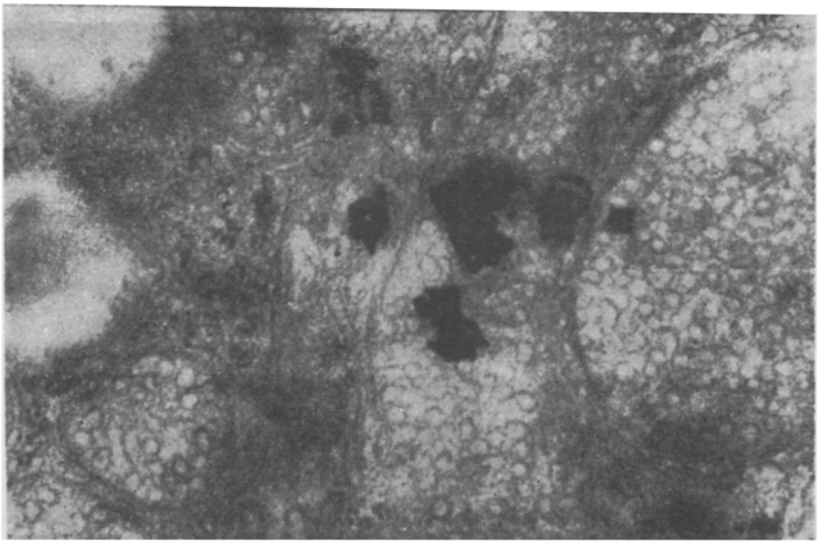

Fig. 3. Tracks of cholesterol-H^3 above mitochondria, 50,000×.

In the present investigation electron-microscopic autoradiography of the adrenal cortex was used after injection of H^3-labeled steroid precursors.

EXPERIMENTAL METHOD

Cholesterol-H^3 (specific activity 750 mCi/g) was injected in a dose of 0.5 μCi/g body weight intraperitoneally into male albino rats weighing 80-100 g. The animals were killed 6, 12, and 48 h later. Pieces of adrenocortical tissue were fixed with glutaraldehyde and then postfixed with osmic acid and embedded in Epon 812. Sections 800 Å in thickness were coated with PR-2 emulsion [1]. The emulsion, melted at 40°C, was diluted with distilled water, and the hardener and plasticizer were added. With the aid of a copper wire loop, Japanese copper grids were coated with the cold emulsion. After exposure for 30-40 days the sections were developed and stained with uranyl acetate and lead citrate and examined in the IEM-7 electron microscope.

EXPERIMENTAL RESULTS

Granules of reduced silver were located mainly above the lipid inclusions in the cell 6 h after administration of cholesterol-H^3. A few of these granules also were observed above the mitochondria, close

to the lipid inclusions (Fig. 2). In the later stages after injection of cholesterol-H^3, tracks were found mainly above the mitochondria (Fig. 3) and far fewer were seen above the lipid inclusions.

The results indicate that cholesterol-H^3 and its conversion products, incorporated into lipid formations of the cell, were subsequently found in the mitochondria, where the cholesterol is converted into pregnenalone [5].

Similar morphological observations have been made on the interstitial tissue of the ovary after incubation with cholesterol-C^{14}. The electron-microscopic structure of the mitochondria of the steroid-producing cells of the ovary and adrenal cortex, moreover, is identical [4].

Whether the observed ultrastructural changes can be ascribed to particular stages of hormone biosynthesis can evidently be decided only after electron-microscopic autoradiography of the adrenal cortex during stimulation of its function.

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