ELECTRON-MICROSCOPIC AUTORADIOGRAPHY OF THE LIVER AFTER ADMINISTRATION OF [^{3}H]CORTICOSTERONE

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Distribution of [^{3}H]corticosterone in the liver cells after adrenalectomy was studied by electron-microscopic autoradiography. The deficiency of endogenous glucocorticoids led to more rapid incorporation of [^{3}H]corticosterone into the liver cells, as shown by the appearance of tracks above the nuclei of the hepatocytes and by an increase in their number above the various cytoplasmic formations.

KEY WORDS: *electron-microscopic autoradiography; liver, corticosterone.*

During the last 10 to 15 years considerable evidence has been obtained to show the effect of hormones on the mechanisms of regulation of intracellular metabolism. On penetrating into the cell steroid hormones form a complex with specific receptor proteins. The resulting complexes can combine with other intracellular macromolecules and be transported into the nucleus, and this is evidently a key factor in the action of these hormones on target organs [3]. An important development in the study of the physiological role of cell receptors for steroid hormones is the analysis of their content and localization in the cytoplasm and nucleus in the presence of different levels of endogenous hormone [1].

The object of the present investigation was to study the distribution of [^{3}H]corticosterone among the subcellular organelles of hepatocytes and the effect of adrenalectomy on this distribution.

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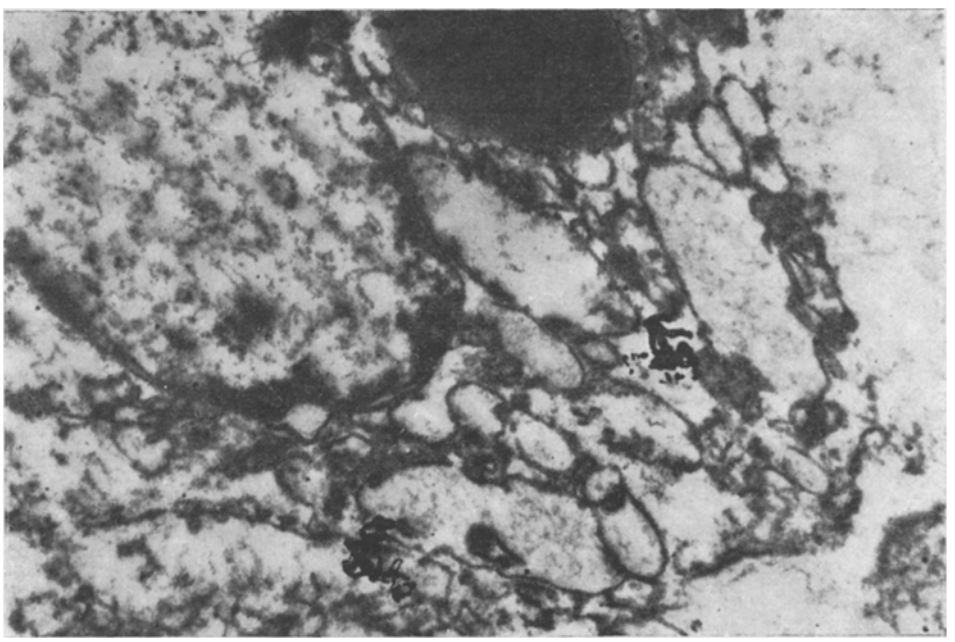

Fig. 1. Autoradiograph showing [^{3}H]corticosterone tracks above membranes of endoplasmic reticulum in liver of intact animals; 50,000×.

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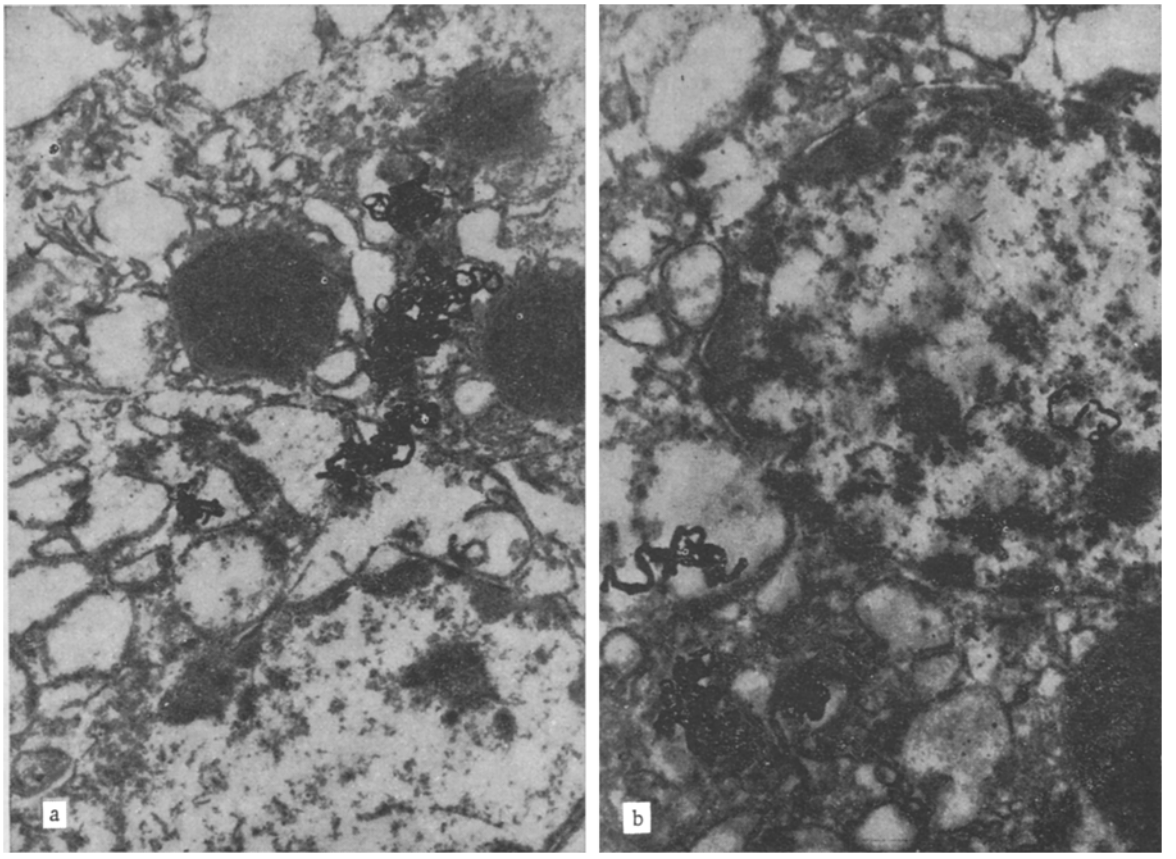

Fig. 2. Autoradiograph showing tracts of [^{3}H]corticosterone above membranes of endoplasmic reticulum (a) and above nucleus (b) in hepatocytes or adrenalectomized animals; 50,000×.

EXPERIMENTAL METHODS

Experiments were carried out on adult male albino rats (weighing 180-200 g), both intact and adrenalectomized (on the 5th day after the operation). Pieces of liver tissue were incubated at 37°C for 40 min in an incubation mixture of the following composition: Hanks' medium, medium 199 (1:1), with the addition of 1 mCi [^{3}H]corticosterone in an excess of unlabeled corticosterone (1:100). After incubation the pieces of tissue were fixed with glutaraldehyde and then postfixed with osmic acid and embedded in a mixture of Epon and Araldite. Sections 800 Å thick were coated with PR-2 emulsion and treated as described previously [2].

EXPERIMENTAL RESULTS

Very few tracks of [^{3}H]corticosterone were found in the cytoplasm of the liver cells of intact animals, and these were mainly above the membranes of the reticulum far from the nucleus (Fig. 1).

Bilateral adrenalectomy caused considerable changes in the ultrastructure of the hepatocytes. The cell cytoplasm was considerably vacuolated on account of dilated cavities of the smooth parts of the endoplasmic reticulum. Few mitochondria were present in the cell, they were greatly compressed, and their matrix considerably condensed. The nucleus was slightly swollen, translucent, and showed characteristic condensation of chromatin near the periphery. The nuclear membrane in places formed considerable swellings on the nuclear surface. The total number of tracks per cell in hepatocytes of the adrenalectomized animals was much larger than in the controls. Tracks were located mainly above the dilated cavities of the endoplasmic reticulum, lipid inclusions, and membranes of the lamellar complex (Fig. 2a). A characteristic feature distinguishing the distribution of [^{3}H]corticosterone tracks in the hepatocytes of the adrenalectomized animals was their discovery above the nuclei (Fig. 2b).

The results of the investigation by electron-microscopic autoradiography thus show that the endogenous glucocorticoid level has a significant effect on the hormone-dependent capac-

ity of the intracellular structures of the hepatocytes. A marked decrease in the hormone content (following bilateral adrenalectomy) led to an increase in the intracellular inclusions of [^{3}H] corticosterone, to a true increase in the number of tracks above the cytoplasmic structures, and to their appearance above the nucleus.

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ELECTRON-MICROSCOPIC AND AUTORADIOGRAPHIC STUDY OF THE PANCREAS AT DIFFERENT STAGES OF POSTMORTEM ISCHEMIA

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Experiments on dogs showed that the maximal allowable period of normothermic postmortem ischemia of the pancreas must not exceed 45 min, for irreversible destructive changes develop later in the ultrastructural formations of cells of the exocrine parenchyma. Cells of the islets of Langerhans are more resistant to ischemia.

KEY WORDS: *pancreas; transplantation; ischemic injury.*

The degree of injury to cells of an organ allografted during the period of normothermic ischemia, lasting from the time of circulatory arrest in the donor to the beginning of perfusion with the preserving solution, has a marked effect on the function of the organ and its survival. The pancreas is an organ which has received least study in this direction, for the high sensitivity of the cells of its acinar system to hypoxia creates difficulties in the way of the appropriate research.

If the duration of ischemia is 60 min, gangrenous-hemorrhagic pancreatitis regularly arises in the graft, whereas after ischemia for 40 min the course of the postoperative period is smoother [1, 2]. Some writers report that after ischemia for 30 min cells of the exocrine parenchyma develop changes consisting of edema, granular and hydropic degeneration, a sharp decrease in the DNA, RNA, and glycogen content, swelling of the mitochondria, disorganization of the cristae, vacuolation of the ergastoplasm, and a decrease in the number of secretory granules [3, 4, 7]. No such information is contained in most papers on this subject, or their authors merely state the duration of the ischemia [5, 6, 8, 9].

EXPERIMENTAL METHODS

Experiments were carried out on 20 mongrel dogs. The animals were anesthetized and killed by injection of air into the heart. The effect of normothermic ischemia on the pancreatic cells was studied between 5 and 60 min after circulatory arrest. Material for investigation was fixed in 2.5% glutaraldehyde, pH 7.4, and then transferred into 1% osmium tetroxide solution, dehydrated in alcohols of increasing strength, and embedded in Araldite.

To obtain autoradiographs, the pancreas was perfused at the same times through one of its main arteries for 1 min with 25 ml of Hanks' solution containing [^{3}H]leucine in a concentration of 10 μCi/ml. After incubation, pieces were removed from different parts of the pancreas and fixed in Bouin's and Carnoy's fluids. Sections 6 μ thick were fixed to a slide, coated with type M emulsion, exposed for 14 days, processed in amidol developer, and stained

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