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ESTERASE XXIV: SUBCELLULAR LOCALIZATION OF ORGANOPHOSPHATE-SENSITIVE ESTERASES IN RAT ADRENALS BY AUTORADIOGRAPHY*

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An autoradiographic method was applied in the study of esterase localization in the ultrastructure of rat adrenal glands. Reaction with ^{3}H-diisopropyl fluorophosphate was used to demonstrate esterase activity in various intracellular compartments. The silver grains were observed over lipid granules, mitochondria and smooth-surfaced endoplasmic reticulum, each of which is envolved in steroidogenesis. No silver grains could be detected over the nuclei or over lysosomes. The results are in agreement with those obtained by an ultrahistochemical heavy metal method.

Preceeding investigations of rat adrenals (5) have shown that the reaction products of the non-specific esterase are predominantly observed in the mitochondrial matrix if 8-acetoxiquinoline (Q-O-2) is used as substrate. Contrarily, the main reaction is found in the smooth-surfaced endoplasmic reticulum (SER) and at the periphery of lipid droplets, if 8-acetyl-mercaptoquinoline (Q-S-2) is used.

The intracellular localization of enzymes through their reaction products is always somewhat uncertain under the suspicion that the reaction products are not situated exactly at the enzyme locus itself but that they diffuse to other cell compartments before final precipitation. Both enzyme reactions mentioned above are inhibited by diisopropyl fluorophosphate (DFP). Thus, if the esterase loci found with Q-O-2 and Q-S-2 resp. are not arteficial the silver grains must be localized autoradiographically in mitochondria, SER and lipid droplets, employing the method of OSTROWSKI and coworkers (1), (4) i.e. labeling of the esterases with ^{3}H-DFP.

MATERIALS AND METHODS

Adult male Wistar rats, weighing 320–390 g, were killed by fracturing of the neck. After removing of adrenal glands, small fragments of it were fixed in 1% glutaraldehyde solution for 2 hours, and washed in 10% demithylsulfoxide solution in cacodylate buffer, pH 7.2. Cryostate sections, 30 μm thick, were treated with 10^{-4}M ^{3}H-DFP (0.9 mCi/μM) in cacodylate buffer, pH 7.2 at 20°C for 60 minutes.

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At this point, some sections were treated with unlabelled DFP as the control. The sections were washed in cacodylate buffer pH 7.2 for 3 hours and fixed in 1% osmium tetroxide for 1 hour. After dehydration in ethylalcohol series, each section was embedded in Epon 812. For electron microscopic autoradiography, thin sections were cut and placed on celloidin-coated grids. The wire loap method (2) was used to coat the thin sections with Sakura NR-H2 emulsion. After 2–8 weeks for exposure at 4°C, the sections were developed by Kodak Microdol X and fixed. Staining was done with uranyl acetate and lead nitrate for electron microscopic examination.

RESULTS

Before presenting the results of autoradiographic experiments a brief comment on the morphology of rat adrenal cortical cells will be given. In cortical cells, lipid granules, mitochondria and smooth-surfaced endoplasmic reticulum (SER) are specific organelles for the steroidgenesis, and they are covering a large space of the cytoplasm. The form and the cristae of mitochondria are different in some small degree in different layers of the cortex, for example, a long form with tubular cristae is seen in the zona glomerulosa and a round form with vesicular cristae in the zona fasciculata or reticularis.

The silver grains which suggest the location of ^{3}H-DFP were counted over the cortical cells. They occurred constantly over lipid granules, especially over its

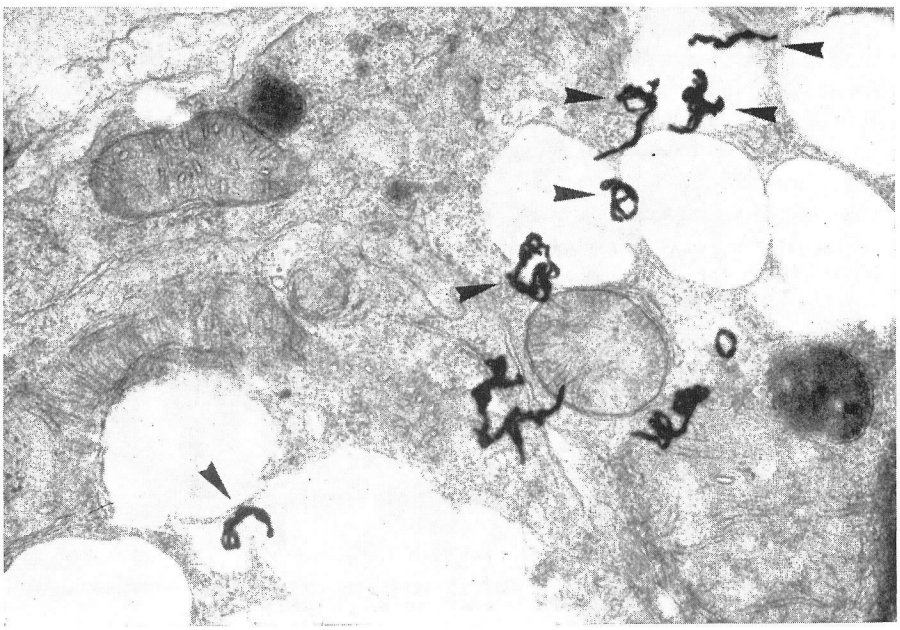

FIG. 1. Electron microscopic autoradiograph of rat adrenal cortical cell in zona glomerulosa, treated with ^{3}H-diisopropyl fluorophosphate (0.9 mCi/μM) for 60 minutes. Filamentous silver grains are located over the lipid granules (►). Developer: Microdol X, Exposure time: 6 weeks, stained with uranyl acetate and lead nitrate. ×27,000

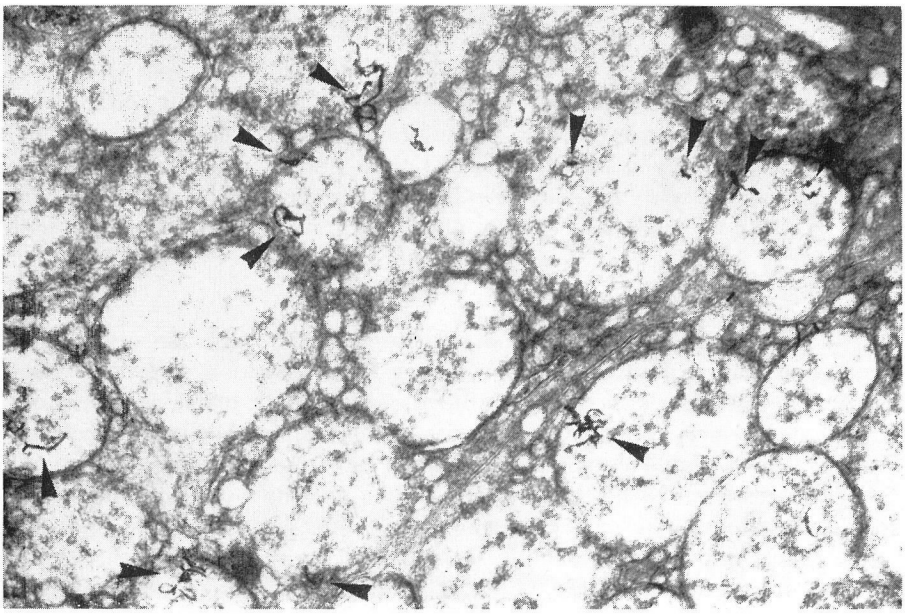

FIG. 2. Silver grains are located over the mitochondria of rat adrenal cortical cell in zona fasciculata (►). ×17,500 Experimental conditions are same as to that of Fig. 1.

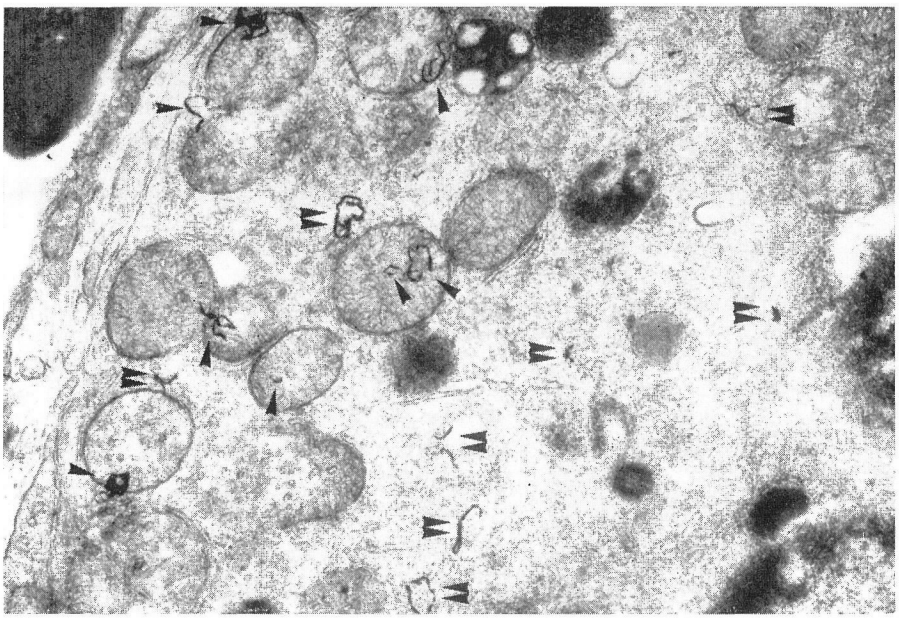

FIG. 3. Silver grains are located over the mitochondria (►) and SER (⪢) of rat adrenal cortical cell in zona reticularis. ×16,500 Experimental conditions are same as to that of Fig. 1.

limiting membrane in each cortical cell (Fig. 1). Secondary they were observed over the mitochondria (Figs. 2 and 3). No grains appeared over the nucleus and lysosomes. Thirdly, grains were observed over the cytoplasm, particularly over the SER area, however it was difficult to associate them exactly with the SER (Fig. 3). A quantitative estimation of the grain number suggests that about fifty percent of radioactivity is attached to the lipid granules, thirty percent to the mitochondria and twenty percent to other cell compartments, to SER amongst others. When unlabelled DFP was applied, no grain was specifically observed.

DISCUSSION

With ^{3}H-DFP the active sites of the esterases are labeled. Since only a small percentage of marker atoms decompose during the observation period the yield of silver grains must be relatively poor. But the distribution of the grains upon the different compartments does well agree with our former cytochemical observations. No label is found over the caryoplasma and over lysosomes. In some mitochondria the silver grains seem to be accumulated while others do not reveal any labeling. This is in accordance with our cytochemical results (5) well which have shown that not by far each mitochondrium contains esterase. Obviously there exist more than one population of mitochondria. Similar observations were recently made with other enzymes (3).

Summarizing our results, the autoradiographic enzyme localization confirms that predominantly the mitochondria, the peripehry of lipid droplets, and the SER including the perinuclear cleft are reckoned with the compartments containing organophosphate-sensitive esterases. The results of the labeling technique are thus in good agreement with our earlier results obtained with the heavy metal method.

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