Light and Electron Microscopic Examination of Exocrine Pancreas Using Zinc Iodide-Osmium Tetroxide Technique

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Summary: Zinc iodide-osmium tetroxide (ZIO) fixation/staining technique is a metallophilic technique which has been used for the examination of various tissues and cell types. We examined the ZIO (+) cell types in rat exocrine pancreas to obtain further evidence for the significance of the reaction. Among mostly non-reactive pancreatic acinar cells there were ZIO (+) acinar cells of varying staining intensity. Zymogenic granules and centroacinar cells were completely non-reactive. Our electron microscopic findings support the view that the reactivity of the technique used is cell specific but not cell type or organelle specific.

ZIO (Zinc iodide-osmium tetroxide) fixation/staining technique is a metallophilic technique which has been first introduced by Champy in 1913 as a mixture of osmium tetroxide and sodium iodide for staining autonomic nerves. In 1959–62 Maillet modified the technique using divalent cations, including zinc instead of sodium¹⁾. Several modifications of the technique has been used by many researchers for the examination of many tissues and cell types including Langerhans cells of the skin^{2,3)}, dendritic cells of the lymphoid organs^{4,5)}, blood and bone marrow cells⁶⁾, and synaptic vesicles in the nervous tissue^{7,8)}. An application of the technique was reported and reviewed by Dağdeviren *et al.* in 1994⁹⁾, indicating the presence of ZIO reactive cells in a variety of tissues at light and electron microscopic level. We examined the ZIO (+) cell types in rat exocrine pancreas to obtain further evidence for the significance of the reaction.

Material and Methods

To obtain tissue samples of exocrine pancreas five Wistar rats weighing 150–200 g were used.

Animals were killed by decapitation under ether anesthesia and pancreas of the animals were quickly removed. Freshly obtained tissues were immersed in the ZIO solution used by Niebauer *et al.*³⁾ and kept in the dark for 24 hours in this solution at room temperature. The solution is prepared by mixing 2 g metallic iodine and 6 g metallic zinc powder with 8 ml distilled water. This is added to 80 ml distilled water slowly as it is an exothermic reaction. The solution is filtered after 5 minutes to remove excess zinc and is added to an unbuffered 2% solution of osmium tetroxide at a ratio of 4 parts to 1. It is prepared fresh and kept in dark before use. Fixed and stained tissue samples were processed for routine EM analysis. Tissue samples were dehydrated by graded alcohol, infiltrated and embedded in TAAB resin kit. Semithin sections were cut, stained with Methylene blue-Azur II, examined and photographed using an Olympus C35 light microscope. Ultrathin sections were cut with glass knives and collected on coated copper grids and stained with uranyl acetate and lead citrate. The sections were examined and photographed using a Zeiss EM 9S electron microscope.

Results

In the exocrine pancreas samples among mostly non-reactive pancreatic acinar cells there were ZIO (+) cells of varying staining intensity (Figure 1a and Figure 1b). Centroacinar cells were distinctively unreactive with ZIO and clearly distinguished by their staining pattern with methylene blue- Azur II and their specific location.

Zymogenic granules within the apical cytoplasm of both ZIO (+) and ZIO (-) acinar cells were distinctly non-reactive and intensely stained with methylene blue. As at the light microscopic level the ZIO reaction was confined to individual acinar cells located among non-reactive cells at ultrastructural level. All cytoplasmic elements in ZIO reactive cells were intensely stained with ZIO reflecting a cell specific pattern rather than an organelle specific pattern as seen in figure 2. Dilated cisternae of granular endoplasmic reticulum were strongly reactive in the ZIO (+) acinar cell while the same organelle is non-reactive in the ZIO (-) acinar cell.

Discussion

ZIO technique was widely used in neuro-anatomical studies to demonstrate nerve fibers and synaptic vesicles^{7,8}). Though this technique clearly distinguishes certain organelles or cell types its mechanism still remains to be clarified. Application of this technique on a variety of organs revealed that ZIO reactivity is present in various cell types including Langerhans cells, dendritic cells of lymphoid organs, skeletal muscle and glandular tissue⁹). We examined rat pancreas to determine the ZIO reactive cells in light and electron microscopic levels in detail. As at the light microscopic level the ZIO reaction was confined to individual acinar cells located among nonreactive cells at ultrastructural level. There were distinctly ZIO (+) cells in both exocrine and endocrine pancreas. The staining intensity of individual cell was variable. None of the centroacinar cells were found to be reactive. Our findings reveal that the reaction is cell type specific

in one aspect being only present in subgroup of acinar cells and being totally absent in centroacinar cells. Meanwhile not all the members of a cell group are reactive. Careful examinations on adjacent cells revealed that if the reaction is present it is observed in the entire cytoplasmic elements of the reactive cell while the non-reactive neighboring cell is non-reactive. As a conclusion it can be suggested that the reaction is certainly cell specific but not cell type or organelle specific as adjacent cells of same type and their organelles are reactive in one of the cell but nonreactive in the adjacent one. Our electron microscopic findings support the view that this fixation/staining reaction depends on the functional state of individual cells probably related to the cytoplasmic pH of reactive cells. As centroacinar cells are totally nonreactive in all samples, the specificity of reaction is thought to be restricted to actively protein synthesizing cell types rather than those cells regulating ion-transport.

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Explanation of Figures

Plate I

Fig. 1.a: Semithin section of rat pancreas. Most of the acinar cells were non-reactive. ZIO (+) cells are clearly distinguished some showing a stronger reactivity (arrows). There are also moderately and weakly reactive cells (*). Centroacinar cells were non-reactive (arrowheads). Zymogenic granules both in ZIO (+) and ZIO (-) cells are distinguished. ZIO/ methylene blue-Azur II stained, original magnification $\times 40$.

Fig. 1.b: Another section from rat pancreas through several acini containing fewer Zymogenic granules. As in figure 1a, the ZIO reactivity pattern was variable in different acinar cells; some were weakly, some were moderately and some others were strongly reactive. ZIO/ methylene blue-Azur II stained, original magnification $\times 40$.

Plate II

Fig. 2. High power electron micrograph of adjacent acinar cells demonstrating the fine structural features of these cells. The reaction is confined to one of the acinar cells delineated by its plasma membrane. Granular endoplasmic reticulum of the ZIO (+) cell was also reactive while the granular endoplasmic reticulum of the adjacent cell was not. Lead citrate/uranyl acetate stained, original magnification $\times 14200$.