Application of Microdissecting Method to the Ultrastructural Study on the Pancreatic Islets of Mammals.

Sadayoshi YOKOH, Osamu AOZI and Hideo YOSHIDA.

(Received April 16, 1965.)

It has been well known that the pancreatic islet beta cells take part in insulin formation and release, and that alpha cells are the source of glucagon. But little is known about either the cellular mechanism of their formation and release or the changing modes about the secretion of these hormones under various conditions, in spite of the efforts of many researchers.

Ultrastructural study of the islets should contribute to our knowledge about the mechanism of these problems. But there is a difficulty inherent in studying the mammalian islets of pancreas by means of electron microscopy, namely, it is very troublesome and time consuming to bring the islet cells under the ultramicroscope, because the endocrine parts are scattered as island-like spots throughout the acinar pancreatic gland.

Since Bensley (1911) reported that he had been able to isolate the islets from the pancreas of guinea pig by means of either injection of neutral red or Janus green or without staining, the microdissecting method of islets has occupied attention. Recently Hellerström (1964) reported the microdissecting technic of fresh pure islets for the biological microanalysis.

The availability of the microdissection of the pancreatic islets of mammals for the ultrastructural study is presented in the following report.

I. Materials and Methods.

Animals used for the microdissection were rabbits, mice, rats and guinea pigs. These animals were allowed free access to food and were made to fast for twelve hours before slaughter. They were killed by decapitation and bleeding. The pancreas was removed immediately after sacrifice, and immersed in PALADE’s 1% osmium tetroxide solution buffered to 7.4 pH by veronal acetate solution on the black-painted-upside-down surface of a small dish. The dish was kept at a temperature of 2—4°C. by dry ice during the following procedure. Both freezing and high temperature were carefully avoided. In this solution the pancreas was cut into small pieces with scissors. The dish containing pancreas tissues was illuminated by an oblique ray from a lamp attached to the body of the stereoscopic microscope (Olympus Model SZ) and observed at 20—40 times low magnification. The pancreas tissues in the osmic acid solution were
fixed and became gradually blackish, but as the blacking speed of islets was slower than the acinar tissues, in the course of time islets began to loom and became clearly distinguishable in the blackish background (Fig. 1). Even after twenty or thirty minutes, islets were still recognizable. Six percent glutaraldehyde solution was substituted for the osmium tetroxide as a fixative for microdissection. In this fixative, islets were most distinctly recognized in the mice, but vague in rats. Islets of rabbits were observed to be smaller and more numerous than that of mice under the stereoscopic microscope.

The islets of these mammals generally display a more distinct contrast against the transparent and reddish acinar parenchyma in the fasting period. On the other hand, islets were indistinctly observed when the bleeding at sacrifice was incomplete.

In spite of the stimulation to the conjunctiva and nasopharyngeal mucus membranes during manipulation, osmium tetroxide solution was more convenient than glutaraldehyde solution for microdissection, because of the clear contrast of the islets against the surrounded exocrine tissue.

**Technic.**

As dissection tools, ophthalmologic forceps and 1/2 gauge hypodermic needles attached to glass rods were used, and the edges of the latter were sharpened for cutting the tissue. The microdissection was at first begun with free hand by careful tearing the gland into small pieces with forceps, and then each islet was separated carefully from the acinar tissue by forceps and needles. Since the islets often adhered firmly to the connective tissue of small blood vessels, the preparation was smoothly done with the aid of hypodermic needles. When an islet was picked out directly with the forceps,
Ultrastructural Study on the Pancreatic Islets.

the capsule broke and the content of the islet escaped into the surrounding fixative solution. So it is noteworthy that not only the pure islet but also the detached small part of acinar tissue should be isolated, and the part of the latter should be picked up especially in rabbits because the capsules of the rabbit islets were more fragile than that of other species, as was recognized in the light microscopic observation. Successful dissection required considerable skillfulness, but with some practice the isolation of an islet could be completed within a few minutes.

The isolated islets were placed in another receptacle containing cold osmic acid solution and were fixed for one hour from the beginning of the dissecting procedure. The islets dissected in the glutaraldehyde solution were immersed in cacodylate buffer for twenty-four hours and then refixed in the osmium tetroxide solution. The fixed islets were dehydrated through a series of graded alcohols to absolute alcohol and embedded in epon epoxy resin according to Luft. Sections were cut with a glass knife on a Porter-Blum microtome and were stained with saturated uranyl acetate. They were examined and photographed with Hitachi Electron Microscope HU-11A.

Pancreatic tissue also was placed immediately after sacrifice into Zenker formol solution and paraffin sections were stained by a modification of aldehyde fuchsine or periodic acid-Schiff technic with a trichrome counterstain. In this manner, light and electron microscopic cytological correlation was possible.

II. Observations.

Electron Micrograph of the Microdissected Islets of Normal Rabbits.

Three different types of islet cells were observed in the normal rabbits (Fig. 2). The alpha cells contained extreme electron dense granules and almost equal sized granules which tended to aggregate in cytoplasm as small clusters. This picture of granules coincided with that of light microscopy of the alpha cells.

The beta cells tended to occupy a central part of the islet and were most frequently observed. The numerous secretory granules of various sizes which seemed less dense than the alpha cell granules were distributed throughout the cytoplasm. The third type of cell (delta cell) was rare and usually had no granules or contained only a very small amount of granules.

Alpha cells.

In this type of cell, extreme electron dense granules were enclosed by smooth membranes and separated from the membrane with a narrow halo (100—200 Å). They were approximately 0.11—0.23 μ in diameter, but sometimes smaller and less dense granules enveloped with indistinct membranes were recognizable. These were considered as a phase of granule formation. Nuclei enclosed with double membranes were round or ovoid in shape and less dense in content. There was a certain amount of irregular shaped mitochondria and rough surfaced endoplasmic reticulum in cytoplasm. GOLGI apparatus also was poorly developed in this type of cell. Cytoplasmic membranes complicated each other at approximately 250 Å intercellular space (Fig. 3).

Beta cells.

The numerous dense secretory granules were enclosed in smooth surfaced larger
sacs at 0.05—0.08 μ clear and wide halo. Most of the granules were spherical or ovoid within the limits of 0.15—0.45 μ in diameter and tended to situate near one side of the limiting membranes. The rod shaped granules were scarcely observed in the cytoplasm, and it was also found that spherical granules surrounded by slightly osmiophylic material in large smooth sacs and empty sacs of the same size contained spherical granules.
As an organelle system, well-developed mitochondria and GOLGI apparatus occupied a relatively large area. The latter organelles were recognized as a group of larger vesicles without containing any granules. The endoplasmic reticulum of these cells consisted of a system of rough surfaced fine tubular or vesicular cisterns of various sizes. Nuclei enclosed with pored double membranes were low density but richer in chromatin than that of alpha cells. It was interesting that the slightly osmiophytic
fine fibrillar masses were recognizable surrounding the beta cell nuclei, as the others pointed out (Fig. 4).

The border of the beta cells showed occasional convolutions or invaginations. The intercellular space was narrower than that of alpha cells (Figs. 2 and 4). The electron micrograph at the intercellular space of beta cells was considered to display the release
of granules into the spaces (Fig. 5). The sacs contained granules fused to the cytoplasmic membrane, but the fate of these granules could not be clarified.

**Delta cells.**

It was considered that the specificity of this type of cell consisted of very poorly granulated cytoplasm and well developed mitochondria (Fig. 2). But it could not be clarified whether this cell type was agranular stage of alpha or beta cell or not, as Herman et al. pointed out.

**Capillary and acinar relations of beta cells.**

Through the pancreatic islets, many capillaries penetrated. The capillary wall consisted of occasionally interrupted endothel sheet and connective tissue space between two thin, less dense basement membranes. The connective tissue space was
sometimes observed to be broad, and there, cross sections of fibroblasts were frequently found (Fig. 6).

The beta cells were adjacent to the acinar cells with two modes. The one mode of border was a direct contact of two different types of cells holding an intercellular space between them, as is usually seen in each islet cell border. The other mode was an indirect contact, i.e. beta cell and acinar cell were separated by the connective
Ultrastructural Study on the Pancreatic Islets.

This type of cell contact is usually observed between the capillary and islet cell (Figs. 7 and 8).
III. Discussion.

It might be very important to clarify the mechanism of synthesis, storage and release of insulin and glucagon, not only in order to study the genesis of some diabetes but also to explain the action of anti-diabeticum. Nowadays, the most advanced
Ultrastructural Study on the Pancreatic Islets.

methods for the study of pancreatic islets might be the enzymatic microassay of isolated islets and ultrastructural observation of islet cells. Electron microscopic studies of the islets were performed by many authors, especially the hypothesis for the insulin formation and release presented by Lacy is highly evaluated.

But in spite of these results little is known in this field, because of the difficulty encountered that islets scatter in the exocrine pancreas as above described. One of the authors, Yokoh, had experienced this difficulty in the electron microscopic study of human pancreatic islets. In consideration of this experience, the stereoscopic microdissection method was applied to the electron microscopic procedure in order to overcome this difficulty.

Concerning the microdissection of the pancreatic islets, recently Lacy applied this technic to the frozen dried pancreas of mammals and assayed insulin content and a number of oxidative enzymes. More recently Hellerström (1964) presented the microdissecting method of fresh pancreatic islets in the Krebs-Ringer phosphate buffer or 0.25M sucrose solution. These microdissections of islets were performed for the purpose of biochemical microassay of the islet tissues and did not allow us to get distinct contrast of islets against the exocrine pancreas excepting the mice and guinea pigs.

Bencosme et al. (1958) intended to isolate the pancreatic islets for the first time with the object of ultramicroscopic study. He briefly stated that laparotomy was performed under anesthesia in guinea pig, pancreas was flushed in situ with chilled osmium tetroxide fixative, then individual islets were microdissected with a stereoscopic dissecting microscope, but the islets in cat pancreas could not be visualized.

Our microdissecting method was different from his technic, the pancreatic islets were microdissected from the small pieces of pancreas removed immediately after sacrifice with the stereoscopic microscope, by means of utilizing the difference of blacking speed between acinar part and islet tissue in buffered osmium tetroxide solution. The present method in this paper was applicable to the electron microscopy of the islets of mammals i.e. mouse, rat, guinea pig and rabbit.

IV. Summary

In this paper the authors presented a new microdissecting method for the electron microscopic study of the pancreatic islets of mammals and also insisted that the method was useful to obtain the electron micrograph very easily without any artifacts.
S. YOKOH, O. AOZI and H. YOSHIDA.

衆知のことである。従って先人の多くの努力にも拘らず、ラ氏島の上記機序に関する未開拓の分野は他の組織に比して遙かに多い現状である。著者らはこの点に鑑み、最近注目されている微解剖（microdissection）をラ氏島の電顕的研究に導入し、極めて容易に電顕下にラ氏島細胞をとらえうることを見出した。すなわち細切した脳組織を1％オツミウ酸溶液または6％グルタルアルデヒド溶液中で、実体顕微鏡下にラ氏島の微解剖を行ない、ほぼ純粋なラ氏島組織を得、これについて電顕的操作を加え、ラ氏島を観察した。本論文では本法によって得た正常家児ラ氏島の電顕所見について記述した。

又、オツミウ酸溶液中で微解剖する場合は、脳組織の黒化度が外分泌部と内分泌部では異なるので、他の種々の生理的溶液或はグルタルアルデヒド溶液中に於いて行なうよりも遙かに鮮明なラ氏島を実体顕微鏡下にとらえることができ、従って他溶液中では不鮮明なラット、家児、或は食餌拝取後のラ氏島も明瞭に見える点が甚だ好都合である。

本法は各種哺乳動物ラ氏島の電顕的観察に適用出来る可能性があるものと考えられる。

References.