Department of Histology and Embryology (Prof. P. Em. PETKOV), School of Medicine, and
Regeneration Laboratory (Prof. G. GALABOV) of the Bulgarian Academy of Sciences,
Sofia, Bulgaria

Electron Microscopic Investigations on the Langerhans Islets of the Golden Hamster

P. PETKOV, R. GALABOVA and St. MANOLOV

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The electron microscopic structure of the endocrine pancreas of some mammals, mainly laboratory animals, such as rat, rabbit, guinea pig, dog, mouse, cat, etc., has been the object of a series of investigations (LACY, 1957; LACY, CARDEZA and WILSON, 1959; CARAMIA, 1963; THÉRET and TAMBOISE, 1963; BJÖRKMAN, HELLERSTRÖM and HELLMAN, 1963; HERMAN, SATO and FITZGERALD, 1964; MUNGER, CARAMIA and LACY, 1965; LOGOTHETOPOULUS, 1966; LAZARUS, SHAPIRO and VOLK, 1968; KOBAYASHI and FUJITA, 1969). BOQUIST (1967, 1968) studied the histochemistry and the ultrastructure of the Langerhans' islets in the Chinese hamster.

The light microscopy and the histochemistry of the Langerhans islets of the golden hamster (*Mesocricetus auratus* Waterhouse, 1839) were studied by a few authors (MÜLLER and GÜTHERT, 1960; PETKOV et al., 1968) without concerning, however, their ultrastructure.

The purpose of this work was to study the ultrastructure of the endocrine pancreas of the golden hamster and the localization of heavy metals in it.

Materials and Methods

The pancreases of fifteen hamsters were investigated. The organs were removed from the animals under ether anesthesia and fixed in glutaraldehyde and osmium tetroxide and immersed in durcupan. Sections were stained with uranyl acetate.

Zinc was detected after the method of MÜLLER and GEYER (1968) as modified by us. A small tube was inserted in the abdominal cavity under urethane narcosis connected with the Kipp generator from which H_2S was obtained. The Langerhans islets were put in glutaraldehyde and dissected by means of stereomicroscope after the method of HELLERSTRÖM (1964). Several minutes postfixation with osmium tetroxide followed. The physical manifestation was displayed on the grid carriers. The contrasting of the preparations was achieved by means of uranyl acetate and Reynolds solution.

The sections were examined in an electron microscope Hitachi HU-11A.

Results

The localization of the different cell types in the Langerhans islets of the golden hamster is quite typical: the A cells are situated in the periphery of islets, the B cells occupy their central part, while the D cells (A_1 according to Swedish authors) are inserted between the A and B cells (PETKOV et al., 1968) (Fig. 1).

The identification of the different cells is possible not only because of this typical

Fig. 1 A A cells, B B cells, D D cells, DB dense body. $\times 44,000$

arrangement of the cells, but also because of the character of their specific granules.

A cells

The nuclear form of the A cells is generally round; nuclei with a folded nuclear membrane are found also. The mitochondria are oval or elongated and very sparse. The endoplasmic reticulum is very weakly expressed. The Golgi complex is most often localized near the nucleus. The specific alpha granules have a round shape with dimensions $130\text{--}360\text{m}\mu$. They possess a high electron density and are closely wrapped in a membrane (Fig. 2). In rare cases, when the membrane is separated from the granular core, the space between them is occupied by a fine electron dense material.

The specific alpha granules are often located in one of the cell poles.

We have not observed any alpha granules released from the cell by emiocytosis, nor alpha hollow granules with low electron density in the cells.

Very seldom were observed dense bodies.

B cells

The B cell nuclei are round and contain a considerable amount of chromatin. Their ergastoplasm is abundant, having many ribosomes. Free ribosomes are also found. B cells with different density of the ergastoplasm are observed (Fig. 3). Mitochondria are more numerous than in the A and D cells. The Golgi complex is very well developed (Fig. 4) and is situated near the nucleus. The specific beta granules are round shaped. Their electron density is smaller than that of the alpha granules. The granules are wrapped in a smooth sac. Between the sac and the

Fig. 2 The specific granules of the A cells are usually with high electron density and are closely wrapped in a membraneous sac. $\times 13,500$

Fig. 3. The endoplasmic reticulum of the B cells is developed in different degrees. "Empty" sacs are observed (arrows). $\times 14,400$

Fig. 4. Formation of beta granules in the Golgi complex. $\times 54,000$

Fig. 5. The shape and the size of the beta granule core varies to a great extent. $\times 91,600$

Fig. 6. Rod-like shaped and fusing beta granules. $\times 69,500$

Fig. 7 A cilium on the surface of the B cells with characteristic structure. $\times 70,000$

intermediate part of the granules one can clearly distinguish a broad bright halo. The form of the granule core varies very much: spherical, elongated, star-shaped or rod-like (Fig. 5, 6). Sometimes two granule cores were observed in the same sac. "Empty" granule sacs were also observed (Fig. 3). The size of beta granules varies

from 130–600 m μ .

Sometimes lysosomes are to be found in the granulated reticulum. Dense bodies of various size and electron density are often observed. Sometimes they are vacuolized. Very seldom fine fibrilles are found around the nucleus.

Rarely cilia can be observed in the B cells (Fig. 7). A release of the secretory beta granules was recognized neither in the intercellular space nor in the pericapillary spaces.

D (A₁) cells

As it was pointed out the D cells are situated between the A and B cells. They show some similarities to A cells in so far as the nucleus, the ergastoplasm, the Golgi complex and mitochondria are concerned.

The specific delta granules are smaller in size than the alpha and beta granules: with round and more often elongated form (Fig. 1). Their diameter varies from 80–150 m μ . Very often elongated delta granules are observed, their length reaching 200–570 m μ . The electron density of the delta granules is intermediary regarding the A and B granules but different degrees of electron density were observed. "Empty" granule sacs were not found. The membrane of the D granules is densely attached to their core.

Fig. 8. Beta granules showing a positive zinc reaction. The reaction is negative in the alpha granules. $\times 77,800$

Electron microscopic demonstration of zinc*

Zinc is to be found neither in A nor in D cells (Fig. 8, 9). The reaction is

* The problem of the specificity of the reaction is discussed in another work in press.

Fig 9 Negative zinc reaction in the granules of the D cell. Adjoining B cell whose granules display rich zinc contents. $\times 51,000$

Fig 10. All beta granules showing presence of zinc. Neither the membrane of the beta granules, nor their halo contain the metal $\times 70,000$

positive for all B cells. Fine silver granules by which zinc is visualized, are found in all beta granules (Fig 10). They are localized only in their electron dense part and are displayed neither in the halo nor in their membrane. The reaction is negative in the ergastoplasm, in the mitochondria and in dense bodies.

Discussion

The electron microscopic investigation confirms the histological findings, namely: the A cells are situated in the periphery of the islets, the B cells in their central part and the D cells between both cells (PETKOV et al., 1968). The electron microscopic identification of the A, B and D cells is carried out according to the characters of their specific granules.

The structure of the alpha granules in the golden hamster is similar to the alpha granules described in other mammals (LACY, 1957; BENCOSME and PEASE, 1958; CARAMIA et al., 1965; YOKOH et al., 1965; GALLI and GUIDOTTI-LOLLI, 1966; FUJITA and MATSUNO, 1967; FALLER, 1969).

Most probably the synthesis of the content of alpha granules occurs in the ergastoplasm while their package is made in the Golgi complex as it was supposed by MUNGER (1962), KOBAYASHI (1966), LAZARUS et al. (1968).

Contrary to some authors we have not observed any release of alpha granules from the cell membrane in the pancreas of the normal golden hamster, nor their presence in the intercellular or pericapillary space. It seems reasonable to presume that under normal conditions the secretion of glucagon from the contents of the alpha granules is performed without any morphological change of the cell membrane.

The comparatively more sparse endoplasmic reticulum with a few ribosome and the small amount of mitochondria seem to indicate, that the A cells should have a comparatively low metabolism.

The presence of rather more numerous and larger mitochondria in the B cells, the richness of the ergastoplasmic reticulum and the well developed Golgi complex imply that the B cells are in higher metabolic activities in comparison with the A cells. These findings could be related to the fact that the B cells are richer in enzymes in comparison with the A cells (WEGMANN and PETKOV, 1965, 1966; WEGMANN et al., 1967; PETKOV et al., 1968). The polymorphism of the beta granules in all probability is an expression of various phases in the process of the synthesis and secretion of insulin. The presence of "empty" granule sacs seems to suggest that in this case the B cells are quite active. The formation of the beta granules, as many authors presume (LACY, CARDEZA and WILSON, 1959; WILLIAMSON, 1960; LAZARUS, VOLK and BARDEN, 1966), is accomplished in the ergastoplasm, while the "package" takes place in the Golgi complex. So far as the possibility of the B cells secretion is concerned under normal conditions by emiocytosis, as some authors admit to be: LACY and HARTROFT (1959), BJÖRKMAN et al. (1963), LAZAROW, BARDEN and LINDAL (1964), WILLIAMS and ENSINCK (1966), BURTON and VENSEL (1966), GOMEZ-ACEBO et al. (1968), we have not observed any indisputable picture of such a manner of granule release. We accept as more probable that the secretion of the granule's contents is effectuated in a soluble way—diacrinic type secretion (LEVER and FINDLAY, 1964; FUJITA and MATSUNO, 1967).

The D cells closely resemble the A cells, as the structure of their organoids is

concerned. Their specific granules have much less electron opacity and display a difference relating to their size and form.

Zinc, detected after the method of MÜLLER and GEYER (1968) is localized only in the specific granules of the B cells, i. e. in the area where actually the insulin is stored. Contrary to the investigations of BOQUIST (1967) and PIHL (1968) who detected simultaneously heavy metals in the A, B and D cells of the pancreatic islets of the Chinese hamster, we have observed zinc only in the B cells.

Zinc is found neither in the site of insulin synthesis nor of its secretion, i. e. neither in the endoplasmic reticulum and the Golgi complex, nor in the cell membrane. This fact might be explained with the comparatively weak concentration of zinc in the phases of its synthesis and secretion. It is histochemically visible only in the granules when its concentration is increased.

Summary

Three cell types are identified under electron microscopic examination of the endocrine pancreas of the golden hamster: A cells show dense granules closely ensheathed by a membraneous sac; B cells, with granule core of various form: round, star-shaped and rod-like and with dense membrane which forms a halo. The granules of the D cells are spherical or elongated with lower electron density than that of the alpha granules.

Zinc is found only in the specific beta granules of the B cells and not in other cell organelles.

Résumé

Dans le pancréas du hamster doré avec le microscope électronique trois types de cellules sont identifiées: des cellules A à granulations denses étroitement entourées d'une membrane; des cellules B à granulations de différentes formes: sphérique, allongée, en bagette, stelliforme et avec une membrane qui forme une halo. Les granulations des cellules D sont sphériques ou allongées et leur densité électronique n'est pas si marquée que dans les cellules A.

On trouve le Zn uniquement dans les granulations B et non pas dans les autres organelles cellulaires.

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Prof. P. PETKOV
Department of Histology and Embryology
School of Medicine
Sofia 31, Bulgaria