ULTRASTRUCTURE OF THE PANCREATIC ISLET TISSUE
OF NORMAL AND ALLOXAN-TREATED
COTTUS SCORPIUS

By

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ABSTRACT

The principal islets of Cottus scorpius are very suitable for electron microscopical investigations since they contain only endocrine parenchyma inside the connective tissue capsule. The central region with the β-cells is more osmiophilic than the peripheral region. Thus, it is fairly easy to trim the islets in the desired region and obtain purely endocrine cells with known histological and histochemical features in the electron micrographs.

The peripheral region is mainly composed of two kinds of cells: a granular type, ultrastructurally corresponding to mammalian α-cells, and an agranular one possibly related to the mammalian γ- and/or δ-cells as judged from its fine structure.

The granules of the α-cell like cells are submicroscopical. The agranular cells terminate at capillaries in the same way as the granular cells. Both cell types are essentially unaffected by alloxan administration.

The central region contains granular cells with a fine structure resembling that of mammalian β-cells. These cells are severely damaged by alloxan administration.

Intimately intermingled with these β-cells are granular and agranular cells which are unaffected by alloxan administration. In part, at least they show ultrastructural similarities to the cells of the peripheral region and represent the aldehyde fuchsin and pseudo-isocyanine negative cells of this region.

The pancreatic islet tissue of most strains of teleosts is concentrated into one, or a few, grossly visible structures in the mesentery, the so-called principal islets (»Brockmannsche Körperchen«). In some strains of teleosts, e.g. Cottus scorpius (the daddy or short-horn sculpin) and Lophius piscatorius (angler fish,
goose fish) the principal islets are exclusively composed of endocrine parenchyma. These principal islets offer a favourable object for experimental diabetes research (cf. Falkmer 1961).

In this connection it is important to know the cellular composition of the principal islets of the bony fish to be used. As to teleostean principal islets in general, there is some controversy regarding the cell types which occur in islets with purely endocrine tissue. There is general agreement as to the occurrence of a central region with dark cells and a region with light cells, mainly in the periphery. The disagreement concerns the identification of the various cell types. Most authors maintain that the insulin producing $\beta$-cells are found in the central region (cf. Falkmer 1961) but some claim that the $\beta$-cells are in the light peripheral region (Saviano 1947, cf. Weitzel et al. 1953).

In previous investigations on Cottus scorpius (Falkmer & Olsson 1960, 1961; Falkmer 1961; Falkmer & Hellman 1961), it was found that the $\beta$-cells are present in the central region, intermingled with about equal parts of argyrophilic cells probably corresponding to avian and mammalian $\alpha_1$-cells (Hellerström & Hellman 1960; Hellman & Hellerström 1960, 1961). The peripheral region seems to be composed of a granule-containing cell type corresponding to the $\alpha_2$-cells of birds and mammals (Hellerström & Hellman 1960, 1961) and an agranular type, possibly related to the $\gamma$- or $\delta$-cells of other bony fishes and higher animals.

As several of these findings must be regarded as somewhat tentative, it was considered of interest to try and gain more information about the cellular composition of the teleostean pancreatic islet tissue. We have therefore, now extended our previous investigations on the ultrastructure of the sculpin principal islets (Falkmer & Olsson 1960, 1961) to include a larger animal material and also the use of some other procedures.

**MATERIAL AND METHODS**

Spleen and pyloric pancreatic islets of the scorpaenide teleost Cottus scorpius were used for this study. Small islets were generally fixed in toto while those from large animals were cut in a few pieces or minced. The most successful sections were obtained from tissues fixed in the traditional Palade fluid with an osmotic concentration of 0.44–0.50 M with either sucrose or sodium chloride, which roughly corresponds to a freezing point depression of −0.8° to −0.9°. Very good results were obtained after fixation in 1% osmic acid in 0.45 M collidin (Bennett & Luft 1959). As the central islet region is more osmiophilic than the peripheral one (Falkmer & Olsson 1960, 1961), the methacrylate embedded blocks could be trimmed in the desired islet region with the aid of razor blade hand sections which were examined under the light microscope in a drop of caedax under cover-glass. The blocks were sectioned on a LKB Ultrotome and the sections examined with the aid of an Akashi TRS-50 electron microscope (50 kV) with 50–100 $\mu$ objective aperture diaphragms.

**Alloxan administration.**—Alloxan was administered intramuscularly to 55 animals in doses of 200–350 mg/kg. Animals were sacrificed at intervals of $2\frac{1}{4}$, 3, $3\frac{1}{2}$, 5, 9 and
24 h after injection. Another series of 4 fishes received a second intramuscular alloxan injection of 300 mg/kg within 2 d. Principal islets from the alloxanized fish were treated as mentioned above as well as, for control purposes, paraffin embedded for light microscopy. Blood sugar determinations were performed by the method of Hagedorn-Jensen. The electron microscopical investigations on alloxanized sculpin were mainly performed on animals with hyperglycemia and/or β-cell changes in the paraffin embedded parts of the islets as investigated in the light microscope (cf. Falkmer 1961).

RESULTS

Peripheral Region Cell Types in Normal Islets

P 1. Zymogen cells. – In sections cut out from blocks trimmed at the capsule of the islets, a very characteristic cell type is often found which is identified as zymogen cells. The most peculiar feature of these cells is the abundance of large granules with a diameter of roughly 1.25 µ, apparently zymogen granules. The cytoplasm appears coarsely reticulated due to its richness in vesicles and the elaborate ergastoplasmic structures. These cells are often accompanied by bundles of collagen fibres and in one case a secretory duct was demonstrated among them. Apparently these sections show strands of exocrine cells at the connective tissue capsule.

P 2. Agranular cells. – In peripheral zone sections, about half of the section area, or somewhat more, is occupied by a cell type which very rarely contains granular cytoplasmic constituents (Fig. 1). These cells terminate at capillaries intermingled with their granulated neighbours. Following some of the fixation techniques, the cytoplasm is less dense than that of the other cell types and seems »watery«. The cells contain several elongated mitochondria which are sometimes clumped in juxta-nuclear aggregations. In some cases membrane complexes are seen which apparently belong to an organized ergastoplasm. The nucleus is fairly dense with evenly distributed chromatin and often one distinct nucleolus. The nucleus may be strikingly polymorphous, and is often cut in the form of an arch or horse shoe, the interior of which may contain several mitochondria.

P 3. Granular cells. – These cells are about as common in the peripheral islet zone as the above-mentioned type, and they occur intermingled with them. Although these cells both terminate at capillaries, the two types are easily distinguished by means of the inclusions and – as seen after certain fixatives – by the denser ground cytoplasm of the granular cells. Systems of agranular lamellae are sometimes found in the cytoplasm near the nucleus. apparently as constituents of the Golgi complex. In several cells, a »nebenkern« arrangement of granular membranes in concentric array is found in this cell region. The interior of a »nebenkern« may contain secretory granules. Numerous mitochondria are distributed in the cells. The nuclei are usually ovoid with a caryoplasm of the same appearance as that of the type mentioned.
A typical section from the outer islet periphery showing granular cells (G) and agranular cells (A). Some endothelial cells (E) are seen although the blood vessel has not been cut. 5000 X.

above. Deep cytoplasm pockets are often seen in the nuclei. The most striking feature of these cells is their rich content of small, dense granules with a mean diameter of about 95–135 mμ and enclosed within a thin membrane (Fig. 2).

P 4. Dark granular cells. – Groups of very dark cells were found in the peripheral zone of one of the islets investigated. In fact the density of these cells is so high that it is difficult to demonstrate the different cellular components clearly. It is evident, however, that they may contain lamellar and slightly vesiculated structures and furthermore a large number of granules with a size range which is roughly the same as that of the light granular cells: usually of between 120 and 140 mμ. The dark cells are usually joined together forming branched clusters. These cells also terminate at capillary walls. The nuclei are more like those of the light granular cells than those of the agranular cells.

This cell type is apparently not a regular component of the peripheral islet zone because it is completely absent in most of the islets investigated.
Fig. 2.
Detail from a peripheral zone capillary. (G) = Granular cell. Note the projections into the vascular space (V) which contain granules. 12 500 X.

P. 5. »Type 5 cells«. – In a few cases another rare cell type was also seen in the peripheral region which contains a few round and electron dense bodies with a diameter of about 0.8 – 1.5 μ. These cells may also contain irregular, »lipid« inclusions. In one case a few small granules of the same type as those of the granular cells were found in such a cell. The density and structure of the cytoplasm is similar to the granular cells.

Peripheral Region Capillaries
The blood vessels of the peripheral zone are usually not wider than 2 – 3 μ in diameter – exceptionally up to 5 – 10 μ. The most striking feature of these vessels is the absence of connective tissue fibres surrounding them so that the glandular parenchyma borders directly on the endothelial cells. In some cases a thin homogeneous lamina can be traced which separates the glandular cells from the endothelial cells and which suggests a basement membrane. The endothelial cells have the usual appearance with conspicuously lamellated areas and several cytoplasmic vesicles. In some places the endothelial layer is
extremely thin, but the existence of pores in the endothelial cytoplasm could never be demonstrated. Sometimes granules are found situated in small cytoplasmic protrusions into the vessel cavity (Fig. 2). It has not been possible to determine whether these protrusions are processes of granular cells which in some places actually penetrate the vascular endothelium or whether the granules are situated in the endothelial cells. In a few cases a membrane could be demonstrated surrounding the granules which closely suggest those of the granular cells mentioned earlier.

**Central Region Cell Types in Normal Islets**

In the central region as in the peripheral one, there are granular and agranular cells mixed together around capillaries (Fig. 3).

C 1. *Granular cells Type 1*. – Several of the granular cells are frequently found which contain granules characterized by a marked variation in size (Figs. 3 and 4). They may range in size up to about 0.75 μ. These large granules may show the same internal structure as the smaller ones or appear somewhat less electron dense. These granules are also enclosed by a limiting membrane which, however, is usually overlooked because of the minute space between the membrane and the dense vesicle content. The *Golgi* complex is found in the paranuclear cell region, and in some cases a close relationship between this complex and secretory granules may be observed (Fig. 4). The dense centre of a granule may thus be found in a *Golgi* membrane which forms a pocket around the granule. In other cases, it is wrapped in the membrane which now completely surrounds the dense centre but is still connected to the *Golgi* complex by a thin strand of double membranes. Rows of »organized« ergastoplasm are frequently seen in these cells.

C 2. *Granular cells Type 2*. – Most of these cells are reminiscent of type P 3 cells and contain several uniform granules. Such a typical granule consists of a dense centre, about 100 mμ in diameter, which is separated by an electron-optic empty space 7 – 10 mμ wide from a *ca.* 5 mμ thick membrane. In some cases the dense centre has a completely homogeneous appearance; in other other cases its composition of closely packed subgranules, 15 – 20 mμ in diameter, is clearly demonstrated. More or less disintegrated granules can also be found, and sometimes particles which apparently correspond to these subgranules are found freely in the cytoplasm. The ground cytoplasm is finely granular and lamellated cytoplasmic membranes are sometimes found in the same concentric array as described for the peripheral region cells.

C 3. *Agranular cells*. – This cell type is found among the granular cells and in about the same numbers or possibly somewhat less (Figs. 3 and 5). They are similar to the granular cells in most respects, but lack the rich granulation, and usually have a much less dense cytoplasm and appear vesiculated. Rows
Fig. 3.
Central region section with endothelial (E), agranular (A), and granular cells »Type 1« (G). 7000 X.
Fig. 4.
Paranuclear region of a central zone granular cell showing part of the nucleus (N), a few mitochondria (M), and an abundance of granules of different sizes. Note Golgi complex (G) enclosing buds of secretory granules (arrows upper left). The arrows at lower right point to two ripe granules which are still attached to Golgi membranes. 35 000 X.
of «organized» ergastoplasm are a common feature, and in a few cases the cells have been found to contain a few granules of the same appearance as the small granule type which is found in the granular cells. Mitochondria accumulations are a common feature of this cell type.

Central Region Capillaries

Most capillaries in this region have the same appearance as described for those of the peripheral zone. Not infrequently, however, fairly large capillaries can be seen which are surrounded by a pericapillary space of connective tissue fibres in the same manner as has often been described for various endocrine organs (Fig. 5). Any material which is released from the endocrine parenchyma into the blood has thus to pass two basement membranes, the perivascular space and, finally, the endothelial cytoplasm.

Alloxan Administration

Peripheral Region

As a rule only minor changes occurred – mainly in the form of a slightly vacuolated cytoplasm in some of the granular and agranular cells. No changes were noted in the mitochondria.

Central Region

Five hours after alloxan injection, the islet tissue has undergone a marked change in appearance. Together with several cells of a fairly unchanged C-2-appearance, the sections now contain a number of cells with a reduced density. The cytoplasm of these latter cells seems to be severely destroyed and is coarsely vacuolated. Several granules are found in the meshes of the damaged ground substance. Within one day of treatment, a complete degranulation has occurred in the affected cells with the exception of some single large granules (Fig. 6), while the apparently unaffected granular cells can still be found in about the same number as before. Conspicuous ergastoplasmic structures are now clearly seen in the otherwise almost empty-looking, damaged cells. The micrographs from fish which had received two injections of alloxan during a couple of days were not very informative owing to the severe damage of the islet tissue which made even the cell boundaries difficult to trace. It is evident, however, that even in this material, several granular cells have escaped the general destruction which otherwise characterizes the central region.

DISCUSSION

Before a critical definition can be made of the different cell types which have been listed here, a rather annoying source of error must be discussed. The
Fig. 5.
Central zone section. (E) = Endothelial cell. (G) = Granular cell («C 1»). (M) = Mitochondria accumulations in agranular cells. (P) = Perivascular space. 14 000 X.
Central zone cells alloxanized for 24 h. The affected cells (»C 1«) are now completely degranulated as far as the small granules are concerned. E = Ergastoplasm and N = nucleus of a degranulated cell (D). G = Granule-containing cells (»C 2«).

Cell boundaries indicated by dotted lines. 30 000 X.

Light microscope preparations show that no well defined division in peripheral tissue and central tissue exists. The peripheral tissue penetrates deeply into the central tissue core at irregular intervals, and in some cases the islets even give the impression of being composed of a ground substance of light cells with some centrally interspersed irregular islands of dark tissue. This means that the blocks which were trimmed to obtain the peripheral region tissue may only be assumed with certainty to contain only »light region« cells if they have only been trimmed at the utmost periphery of the islet. The blocks which were trimmed to obtain central region cells, on the other hand, may contain central infoldings of light tissue together with the dark central tissue. It is thus clear that only the peripheral region sections may a priori be considered to contain a pure tissue.
**Peripheral Region**

As regards the first cell type P 1, there is no doubt that it represents acinar cells of the exocrine pancreas. The fine structure of these cells is closely reminiscent of the descriptions given by several authors who have studied the mammalian acinar pancreatic cells (cf. Haguenau 1958; Pipan 1960).

As to the prevalent cell types of the endocrine parenchyma, viz., the agranular »P 2« and the granular »P 3« (cf. »Results«), this new investigation has fully confirmed our previous statement (Falkmer & Olsson 1960, 1961) that they represent two different cell types. Thus, the agranular »P 2« were ultrastructurally rather similar to the δ-cells of the cat (Bencosme & Pease 1958) and the γ-cells of guinea pig (Lacy 1957) whereas the granular »P 3« in many respects suggest the mammalian α-cells (Bencosme & Pease 1958; Stoeckenius & Kracht 1958; Gaede et al. 1959; Nordmann & Wolf 1960). By way of example, the ground cytoplasm of the agranular »P 2« was slightly vacuolated and sometimes contained a few »microbodies« (Bencosme & Pease 1958). The granules of the »P 3« cells showed the high electron optical density and the characteristic uniformity in size which are typical of the α-cells of many species. The granules were as a rule, however, smaller than in mammalian α-cells.

Light microscopical observations on Cottus scorpius (Falkmer 1961) have revealed the occurrence in the peripheral region of non-argyrophilic »granular« cells which were related to mammalian α-cells. In the electron micrographs, however, granular cells were found to constitute about 50 per cent of the cells in this region. This result differs strikingly from the light microscopical observations (Falkmer 1961) in which most of the »light« cells seemed agranular. This difference is, however, easily explained by the fact that the sculpin α-granules are submicroscopical and that the light microscope can resolve as »granules« only the granule accumulations in several of the α-cells.

The rare cell types »P 4« and »P 5« in the peripheral region are difficult to classify. The »P 4« cells, which were found in one islet only, showed all the characteristics of the »P 3« cells except the extreme cytoplasmic density, and may be a natural or artificial state of these cells. The »P 5« cells may in some way be related to or be of the same nature as the »granulated delta cells« of Bencosme & Pease (1958) or δ-cells with large microbodies (Rouiller & Bernhard 1956). The fact that they were seen in a few islets only, makes it difficult to interpret them as regular constituents of the peripheral islet tissue.

The slight vacuolation seen in some of the peripheral cells one day after alloxan administration was not interpreted as an alloxan induced degenerative change, mainly because actual cytolysis never occurred and the mitochondria and the nuclear and plasma membranes were intact in all cases (cf. Williamson & Lacy 1959). Light microscopically, degenerative cytolological changes were never noted in the peripheral region even after repeated alloxan administration (Falkmer 1961).
From these investigations, it seems justified to state that no β-cells are found in the peripheral region of the endocrine parenchyma and that the agranular »P 2« cells possibly correspond to the γ- or δ-cells of higher animals. The granular »P 3« and probably also »P 4« cells are apparently equivalent to mammalian α-cells. The »P 5« cell type remains unidentified.

Central Region

The »C 1« cells of this region are apparently the insulin producing β-cells. They were severely damaged by alloxan treatment and their ultrastructure corresponded well with that of mammalian β-cells (cf. Ferreira 1957; Lacy 1957; Bencosme & Pease 1958; Stoeckenius & Kracht 1958; Gaede et al. 1959; Nordmann & Wolf 1960). Thus, the granules differed considerably in size and were of moderate electron optical density. The cell organelles conformed well with those of the β-cells of higher animals.

The ultrastructure of the »C 2« and »P 3« cells and that of the »C 3« and »P 2« cells is apparently identical. This means that the prevalent cell types of the peripheral region, or cells indistinguishable from these, are also important constituents of the central region sections. Due to the above mentioned difficulties in localizing the central region sections, several of these micrographs may actually show true peripheral region tissue. Nevertheless, the fact that β-cells were constantly and intimately intermingled with the granular »C 2« cells as well as with the agranular »C 3« cells indicates that these types are also constituents of the central region.

Another indication of the occurrence of α-like cells together with the central β-cells is the observation that granular cells of the »C 2« type remained intact and adjacent to the damaged β-cells in the central region after alloxan administration. This is in conformity with the light microscopical observations (Falkmer 1961) that aldehyde fuchsins negative cells of the central region are not damaged by alloxan administration, at least not primarily. These cells, however, differed from the assumed α-cells of the peripheral region in being argyrophilic. Moreover, they contained zinc histochemically and were not vacuolized after formalin and alcohol fixation.

These differences in histochemical reactions but not in ultrastructure between peripheral and central cells may indicate a difference in maturity or other functional state.

It may be concluded that the central region differs ultrastructurally from the peripheral one only in having several β-cells intermingled with the assumed α-cells and the agranular cells.

Capillaries

The relationship found between the parenchymal cells and the capillaries is
essentially in accordance with the observations made in mammals (Lacy & Hartroft 1959). But there are some exceptions to this. The «fibrocytic processes» said to be present between the two basement membranes of mammalian islet tissue are not usually found; and in some micrographs, only one basement membrane can be seen. Here, the endocrine cells consequently border directly on the endothelial cells. This intimate relationship was most conspicuous in the peripheral region of the sculpin principal islets.

Origin of Granules

In the granular cells, both of the peripheral and of the central region, a close relationship was observed between the Golgi complex and the granules. Actually, pictures were seen which suggested that the granules originated from the Golgi membranes (Fig. 5). This is in conformity with prevalent views on the origin of the granules in mammalian islet cells (cf. Ferreira 1957). Pictures suggesting the formation of granules from the endoplasmic reticulum as suggested by Chin et al. (1959) were not seen although occasionally an intimate relationship was noticed between the granules of the α-cell like cells and the «nebenkern» arrangement of the ergastoplasmic membranes.

Consequently, our previous suggestions (Falkmer & Olsson 1960, 1961) that the ultrastructure of the cells of the pancreatic islet tissue of the marine teleost Cottus scorpius conforms essentially with that of mammals has been confirmed in these more comprehensive investigations.

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REFERENCES


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