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In vitro differentiation of mice pancreatic tissue

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Abstract

Pancreatic differentiation was studied using rotary histotypic cultures prepared from mice at different fetal ages. Pancreatic tissues were dissected and incubated for 6 days. The cultures were kept under constant rotation (70 rpm) and media were oxygenated every 24h. Pancreatic cultures prepared from 15 day-old mice showed histotypical differentiation of exocrine and endocrine cell populations, whereas those obtained from 17 and 19 day-old embryos revealed an important progressive reduction of the exocrine cell population, moreover, cultures prepared from 21 day-old embryos showed few and isolated exocrine cells. The results of the present study revealed three important features: a) Embryonic pancreatic tissue cultures develop histotypic exocrine and endocrine cell elements using the above described procedure; b) An interesting and unusual phenomenon was observed as shown by the permanence of the endocrine component with increasing embryo age; c) The intrapancreatic nervous cells demonstrate numerous end terminals containing clear neurotransmitter vesicles and dark neurosecretory ones.

Keywords

Exocrine, Endocrine, Pancreatic Cells, Tissue Culture, Venezuela

1. Introduction

Numerous strategies have been developed to fight Diabetes Mellitus disease (1-11), a human disorder that causes progressive health deterioration, moreover, with incalculable social and economic consequences. Among developed experimental procedures, those based on the implant of all insulin-producing pancreatic islets have been applied successfully (12-22), however, these techniques confront several practical difficulties. Various researchers have proposed to implant cultured insulinergic cells as alternative to the fetal endocrine pancreatic tissues (23-31).

In the past, our research group has implanted histotypic cultures of nervous and suprarenal catecholamine-producing cells (32, 33). These cultures have the advantage of not generating rejection in the host animal because they grow in a simple incubation media free of antibody substances, therefore, the cultured tissues do not "learn" antigenic behavior. Another aspect that is important to emphasize, which represents the main objective of the present work, is the interesting phenomenon of in vitro plasticity inducing transformation of complex multicellular populations. In the case of cultured embryonic adrenal cortex glands, we have observed that after 6 days of rotary incubation and 20 days after implantation, there was an increase in the number of catecholaminergic cells but the growth of other cells is reduced (33). In the present report, we have provided morphological evidence showing that whole pancreatic histotypical cultures, when prepared from mice older than day 19 of embryonic age, developed mainly endocrine tissue with the concomitant disappearance of the exocrine component.

2. Materials and Methods

2.1. Animals

Embryos of the strain Naval Medical Research Institute (NMRI) mice at 15 (E15), 17 (E17), 19 (E19) and 21 (E21) days of development, were used to obtain pancreatic tissue.

The animals were provided by the Central Animal Laboratory of the University of Los Andes, Mérida state, Venezuela. They were treated according to the regulations of the Bioethics Committee of that Laboratory, based on the Standards for the Use of Animals in Research contained in the Code of Bioethics and Safety of National Found for Science, Technology and Innovation (FONACIT, Caracas, Venezuela) and the requirements of the National Institute of Health-USA (NIH) (34,35).

2.2. Ultrastructural Analysis

After 6 days of incubation, the pancreatic cultures were fixed and processed for morphological analysis. Cultured tissues were fixed for 2h at 4ºC in a fixative solution consisting of a mixture of 3% glutaraldehyde plus 3% formaldehyde in 0.1 M cacodylate buffer, pH 6.3 (38), followed by postfixation for 2h in 1% osmium tetroxide prepared in the same buffer. Then washed again in buffer, dehydrated with ethyl alcohol, and infiltrated with epoxy resin.

For light-microscopic observations, 1-µm sections were stained using toluidine blue. Ultrathin sections were stained using a modified uranyl acetate-lead citrate method (39-41) and observed in a Hitachi H-7000 electron microscope.

The data obtained were tabulated in Microsoft Excel program, and proceeded to perform an illustrative table showing schematically the results of all the information collected from pancreatic cell count.

3. Results

3.1. Pancreatic Histotypic Rotary E15-6 Cultures

When pancreatic cultures were prepared using E15 embryos and incubated during 6 days (E15-6), well developed acinar exocrine pancreatic cells were constantly seen (Fig. 1), and cubical or pyramidal cells composing tubular structures were also observed (Figs. 1 and 2). The acinar exocrine complexes showed histotypic characteristics; they are filled with abundant zymogenic granules and in the center of some acini can be clearly seen the initial element or intracanalicular tubules of the exocrine pancreas (Fig. 2), similar to that observed in mature pancreas. The high degree of development of the exocrine pancreas tissue is also revealed by the formation of interacinar tubules that go from acini to the surface of the cultured mass of pancreatic tissue, as shown in Figure 3. Endocrine pancreas was also seen in these cultures (Fig. 3). The endocrine cellular population formed tissular islets as normally seen in situ.

3.2. Pancreatic Histotypic Rotary E17-6 Cultures

The cultures obtained from 17 day-old embryos revealed similar characteristics as those observed in E15-6 cultures. The epithelial cells of the pancreatic tubular excretory (intercalate ductus) system are cubical cells possessing short and scarce microvilli (Fig. 4). Underlying the epithelial cells, elongated connective tissue cells conform the general acinar and tubular arrangement of this complex multicellular tissue. In these cultures, reduced population of exocrine cells began to be observed.

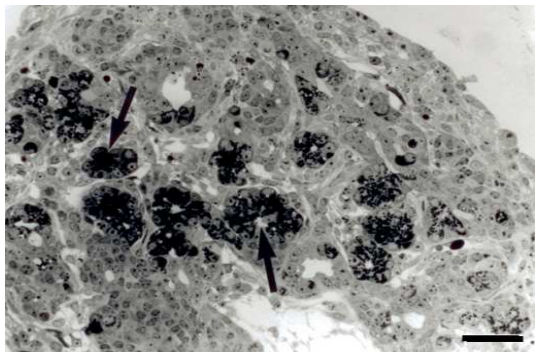

Figure 1. *Light microscopy image of a E15-6 histotypic pancreatic rotary culture. The arrows point to the exocrine pancreatic acini. Notice the abundance of exocrine cells highly loaded with zymogenic material. Bar=10.5 µm.*

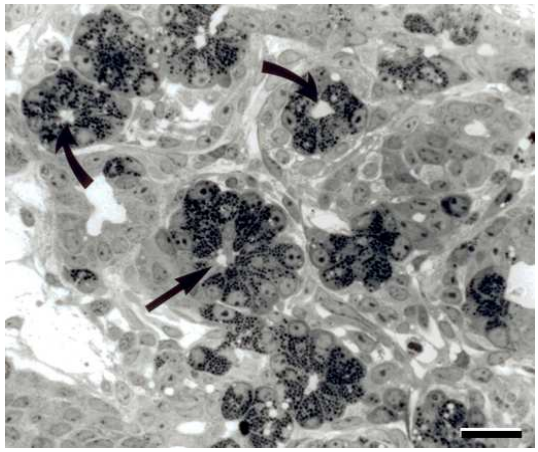

Figure 2. *Higher magnification of a section close to the one shown in figure 1 to illustrate better the cellular organization of the well differentiated exocrine component (arrow) of E15-6 pancreatic rotary cultures. The zymogenic granules are clearly visible as well the central cavity of the acinus (curved arrows). Bar=6.5 µm.*

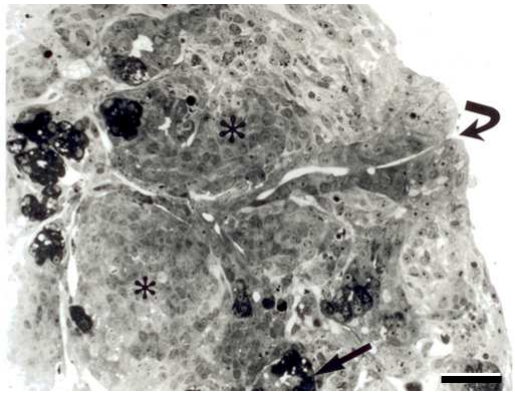

Figure 3. *In this light microscopy image obtained from a E15-6 pancreatic rotary culture, a long tubule is seen having an aperture at the surface of the culture (curved arrow). Well differentiated acinar exocrine cells (right arrow) as well as endocrine cellular component (asterisks) can be observed. Bar=10.5 µm.*

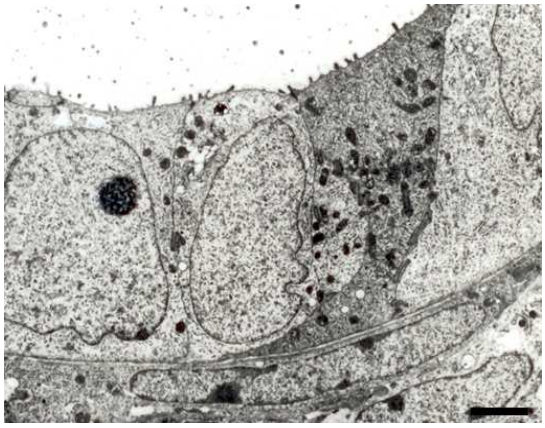

Figure 4. *Electron microscopy photograph obtained from E17-6 pancreatic rotary culture showing the cytotypic cubic cells of the wall of pancreatic excretory tubule. At the surface of these cells, short and scarce microvilli are visible. Nucleus with well developed nucleoli are centrally located within the cytoplasm. Bar=2.7 µm.*

3.3. Pancreatic Histotypic Rotary E19-6 Cultures

Cultures prepared from E19 embryos and incubated during 6 days (E19-6) showed less number of acinar exocrine pancreatic elements, as compared with those from previous ages. Most of the cell population has endocrine characteristics (Fig. 5). An interesting feature of E19-6 cultures is the fact that the acini appear as round and small cellular zymogenic aggregates devoid of secretory tubular system (Fig. 5).

3.4. Pancreatic Histotypic Rotary E21-6 Cultures

When the pancreatic cultures were prepared from embryos at the end of the gestation period and incubated during 6 days (E21-6), it was highly notable the reduced number of exocrine cells. The culture mass was constituted mainly by endocrine cellular elements (Fig. 6); isolated exocrine cells showing scarce zymogenic granules were observed scattered and in very low amount, as compared with the endocrine cells (cf. Fig. 5), which composed also the surface of these cultures.

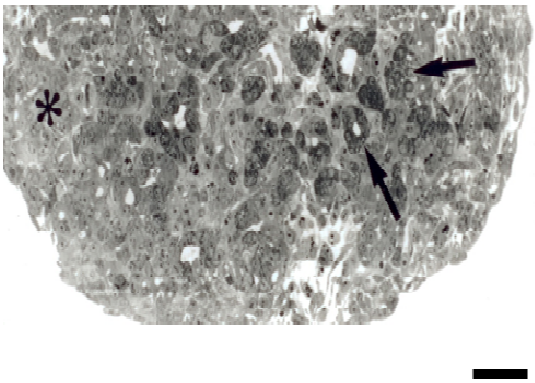

Figure 5. *Histotypic rotary pancreatic culture prepared from a 19 day-old mouse and incubated during 6 days. Notice that the exocrine component of these cultures is substantially reduced, as compared with E15-6 cultures. Scarce exocrine cellular population appears as small and round cellular aggregates (arrows). Pancreatic tubular elements are not present. The main tissular component is constituted by endocrine cells (asterisk). Bar=10.5 µm.*

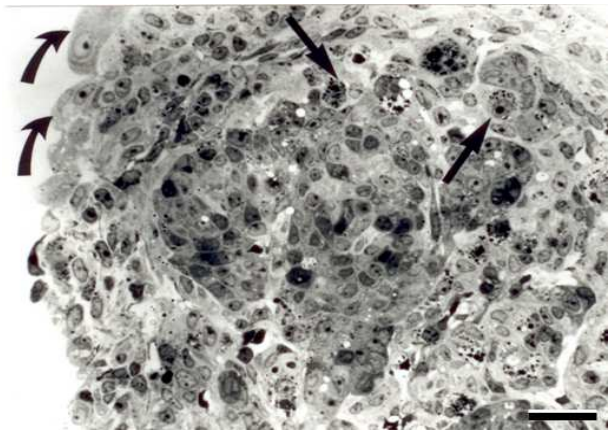

Figure 6. *Higher magnification of a E21-6 pancreatic rotary culture showing solitary exocrine cells, having few zymogenic granules (right arrows). The predominant cellular population in cultures prepared from this age corresponds to endocrine cells (curved arrows) at the surface of the culture. Bar=6.5 µm.*

3.5. Ultrastructural Identification of Exocrine and Endocrine Cytotypic Cells in Pancreatic Cultures

Studies of the ultrastructural characteristics enable the clear distinction between the exocrine and endocrine cells, as well differentiated morphological cellular patterns representative of the main elements of the pancreatic parenchyma. The typical exocrine cells (Fig. 7) showed a highly developed rough endoplasmic reticulum; between this reticulum, numerous mitochondria can be found close to the apical segment of the cytoplasm, and several zymogenic granules could also be clearly seen.

The endocrine islets were constituted by two well identifiable cellular types of the Langerhans population of the mouse (Fig. 8): the alpha cells that have the tendency to be more round and have less dark cytoplasm, and the beta cells that are elongated and with dark cytoplasm.

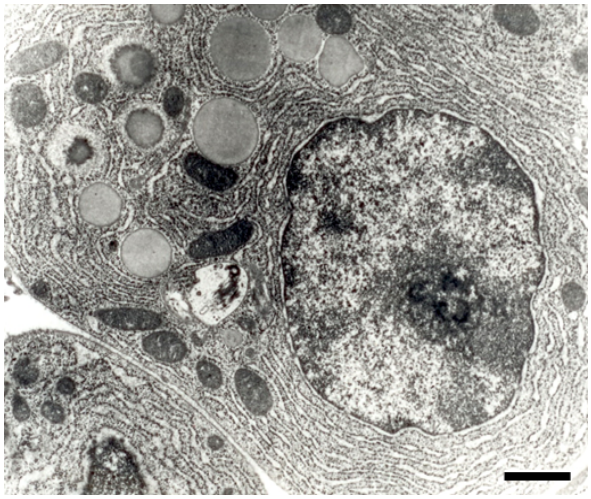

Figure 7. *Ultrastructural image showing typical exocrine zymogenic cells from a E15-6 pancreatic rotary culture. Beside the numerous apical granules of zymogen, a highly developed rough endoplasmic reticulum is clearly observed. The nucleus possesses a well differentiated nucleolus. Numerous mitocondriae are also present. Bar=1.4 µm.*

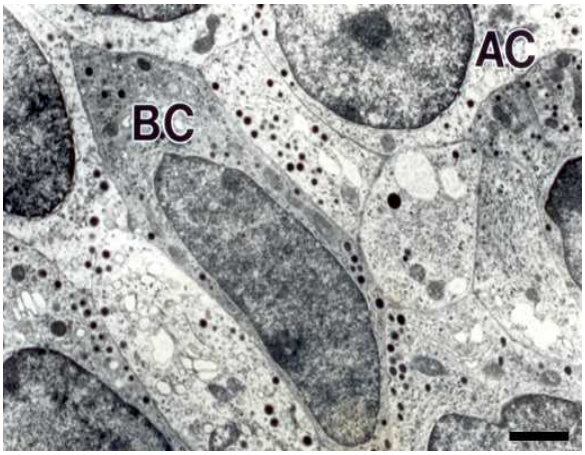

Figure 8. *In this photograph obtained from a E19-6 pancreatic rotary culture, alpha cells (AC) and dark beta cells (BC) are clearly observed. These two cell types are the main constituents of the E19-6 and E21-6 pancreatic rotary cultures. Bar=1.7 µm.*

The average number of acini per 100 µm^2 of surface, in the case of exocrine pancreas, and the amount of alpha and beta cells per 100 µm^2 of surface, in the case of endocrine pancreas, were quantified in pancreatic cultures of different studied ages (Table 1 and Fig. 9). It can be seen that the cultures prepared from 15 day-old mice showed histotypical differentiation of exocrine and endocrine pancreatic cell populations, whereas in E17-6 cultures, there was an increased endocrine cell population as compared with exocrine cells. This fact became more evident in the cultures prepared from 19 and 21 day-old mice, with the concomitant decrease in number of the exocrine cells.

Table 1. *Differentiation of exocrine and endocrine pancreatic cells in rotary histotypic cultures (n=50).*

Culture ages	Average number exocrine cells/100µm^2±SD	Average Number endocrine cells/100µm^2±SD
15-6	9.77±1.36	8.83±0.71
17-6	11.12±0.77	12.07±0.91
19-6	6.90±1.12	15.77±0.93
21-6	4.38±0.65	22.34±0.97

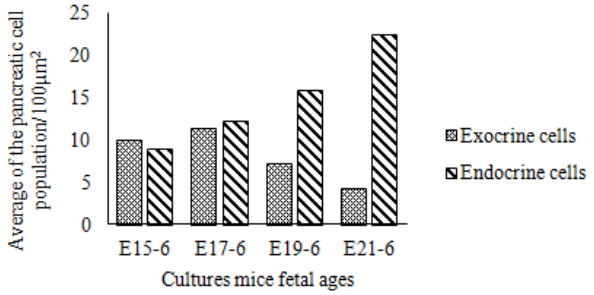

Figure 9. *Exocrine and endocrine cell population in cultured pancreas of different prenatal ages*

4. Discussion

According to the results shown in this research, it is evident that the experimental use of rotary cultures not only allows the production of phenotypic and viable pancreatic cultures, but also, depending on the embryonic age, it is possible to obtain cultures with different pancreatic cellular populations. When cultures are prepared from mice younger than E19, the exocrine pancreas is the dominant cell population, but when cultures are obtained from mice between E19 and E21, the number of the endocrine component progressively increases, which is a very interesting plastic feature expressed in these pancreatic rotary cultures. It is important to remark that this phenomenon was consistently observed independently of the incubation period, which was kept constant for 6 days in all experiments.

Another important aspect that needs emphasis is the fact that the zymogenic cells fully developed their ultrastructural characteristics, similar to the ones they have in normal adult animals (42-46). In relation to the endocrine cells, not only did they express their ultrastructural characteristics, but also they expressed both alpha and beta morphologies without any possible doubt. These two cells have morphologically different cytoplasm and typical granules.

The pancreas plays two main roles: exocrine, through which releases digestive enzymes, and endocrine, that releases hormones like insulin and glucagon. Both functions are exercised under neural modulation control, which integrates the pancreas in regulating functions of various tissues, which are involved in coordinating metabolic processes in the body (47, 48).

It is widely known that pancreatic pathologies are usually very aggressive (49), hence the importance to have therapeutic mechanisms which can improve the prognosis of various diseases. For these mechanisms, transplants using selective cell populations may play an important role, as shown in the present work. Selectivity depends on the moment of development in which is obtained the cultured tissue.

Moreover, in recent years, several research groups (50-53) have focused their interest in using biomodels, as a way to find therapeutic solutions to pancreatic disorders in humans.

5. Conclusions

This work provides the morphological basis for developing strategies for pancreatic tissue transplantation, being able to obtain cultures with the predominance of specific cell types; this technique is promising for the treatment of particular pancreatic pathologies.

Furthermore, the difference in chronological development between pancreatic endocrine and exocrine cells allows the selection of the specific pancreatic tissue culture, including the control of specific hormone production.

Acknowledgments

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