CELL CULTURES FROM THE BOVINE FETAL PANCREAS OBTAINED BY THE USE OF COLLALYTIN

R. A. Babikova, V. N. Blyumkin,
B. I. Shal'nev, and L. B. Polonskaya

UDC 612.64'34-085.23

The morphological characteristics of primary monolayer cultures obtained from the bovine fetal pancreas are described. Dispersion of the pancreatic tissue was achieved by combined treatment with solutions of trypsin and collalytin, a preparation with collagenase activity. The cultures thus obtained included many epithelial cells corresponding in their morphological and functional characteristics to β cells of the islets of Langerhans: Aldehyde-fuchsinophilic granules were found in their cytoplasm; degranulation of these cells took place if the glucose concentration in the nutrient medium was increased.

KEY WORDS: *cell cultures; pancreas; β cells.*

The isolation of the islets of Langerhans and the production of hormonally active monolayer cell cultures from the pancreas of animals and man are important in connection with experimental transplantation and endocrinology [1-9]. Dispersion of the tissues of the pancreas and culture of its cells, however, are beset by a number of technical difficulties. In recent years various methods of combined treatment of the pancreas with solutions of trypsin and collagenase have been used for this purpose [5-8]. Collagenase is used to obtain isolated islets of Langerhans [6, 8] and also to obtain monolayer cultures containing a high proportion of β cells [3, 4, 7].

This paper describes primary monolayer cell cultures obtained from the bovine fetal pancreas with the aid of the Soviet preparation collalytin, which has collagenase activity.*

EXPERIMENTAL METHOD

The pancreas of bovine fetuses aged 4-6 months was minced in a mixture of Hanks's solution and 0.25% trypsin solution. The minced tissue was then treated with equal volumes of a 0.3% solution of collalytin and a 0.25% solution of trypsin. For 15-20 min the tissue suspension was dispersed on a magnetic mixer at room temperature (20-22°C) and then centrifuged at 800 rpm for 10 min and resuspended in medium No. 199 with 10% bovine serum. The suspension was seeded into a Carrel dish and, after the cells had settled for 20 min, the supernatant and cells still in suspension were carefully removed. By means of this method monolayer cultures containing a considerable number of islet cells can be obtained [5]. Some of the cell cultures were grown after a change of medium in an increased concentration of glucose (300 mg %) to stimulate the secretory function of the β cells. Cultures continuing to grow in medium No. 199 with the ordinary glucose concentration acted as the control. For the cytological investigation the cultures were grown on coverslips. Preparations fixed in Bouin's mixture or in 96% ethanol were stained with hematoxylin–eosin and aldehyde–Fuchsin

*Collalytin was obtained from the Laboratory of Technology of Organ Preparations, All-Union Scientific-Research Institute of the Meat Industry.

Laboratory of Pathomorphology, Institute of Transplantation of Organs and Tissues, Ministry of Health of the USSR. Laboratory of Technology of Organ Preparations, All-Union Scientific-Research Institute of the Meat Industry, Ministry of the Meat and Dairy Industry of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 3, pp. 350-353, March, 1977. Original article submitted September 3, 1976.

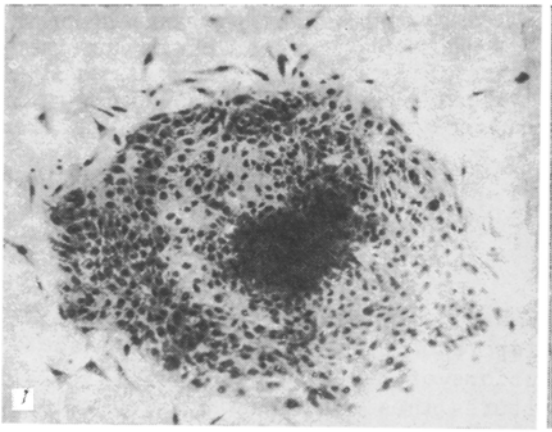

Fig. 1

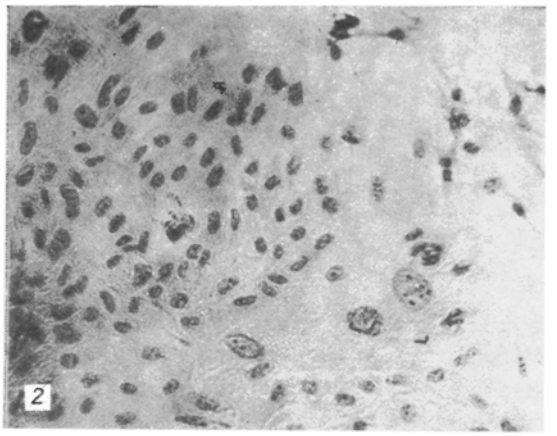

Fig. 2

Fig. 1. Islet of pancreatic epithelial cells (48 h in culture); focus of primary adhesion in center; a few fibroblasts can be seen outside the epithelial islet. Hematoxylin–eosin, 80×.

Fig. 2. Monolayer culture of bovine fetal pancreatic cells (6 days after seeding): completion of formation of epithelial layer; a few fibroblasts can be seen in the top right-hand corner. Hematoxylin-eosin, 200×.

[5] to reveal specific granules in the β cells.

EXPERIMENTAL RESULTS

Treating the fragments of pancreatic tissue with collalytin led to rapid separation of the cell complexes in accordance with the arrangement of the interlobular and intralobular connective-tissue layers. In particular, the islets of Langerhans were isolated in this way and they were clearly detectable under the low power of the microscope. Combined treatment with collalytin and trypsin later caused loosening of the intercellular bonds and isolation of single cells. Cells and cell groups settling in the course of 20 min on the bottom of the Carrel's dish (after removal of the supernatant and the cells remaining in suspension) adhered to the glass. Many single cells and groups of cells adherent to the glass could be seen 24 h after seeding in the native cultures and fixed, stained preparations. Groups of exocrine cells in a state of autolysis, together with accumulations of debris where such cells had died, also could be seen at this period. Observations showed that 1-2 days after inoculation of the cell suspension nearly all the acinar cells were eliminated, and the cultures subsequently grew by proliferation of undifferentiated and islet cells.

Proliferation of the epithelial islets began 48 h after seeding. Their outlines were clearly defined (Fig. 1). Single fibroblasts could be seen between the epithelial islets. By the 3rd-4th day epithelial cells dividing by mitosis were visible in the area of the islets: The mitotic activity at these times was 10-30 ‰. Intensive proliferation of the epithelial islets continued for 5-10 days (2-7 days after the change of medium) and in some cases ended with the formation of a monolayer of epithelial cells (Fig. 2). Starting from the 10th-12th day destructive changes were seen in the epithelial cells at the site of the primary foci of adhesion, culminating in their separation from the glass. Irregularly circular "windows" were formed in these situations in the cultures. Depending on the number of fibroblasts between the epithelial islets, less compact areas of the culture of syncytial character or more compact areas, wedged between the epithelial complexes and moving them apart, could be formed between the epithelial islets. However, in most cases even after 3-4 weeks in culture no proliferation of the fibroblasts took place actually in the epithelial masses, and a clear boundary remained between the epithelial and connective-tissue parts of the culture. This was not observed in monolayer cultures from the pancreas obtained by trypsinization in the usual way without the use of collalytin.

The commonest cells in the epithelial areas of the monolayer cultures isolated from bovine fetal pancreas were polygonal cells of the zone of growth and also large pale cells with a wide homogeneous cytoplasm. In addition, at the edge of the epithelial masses there were cells of fusiform or triangular shape, in some cases separating from the main layer. These cells corresponded to the "special cells" [5] or fibroblast-like β cells [1] described in the

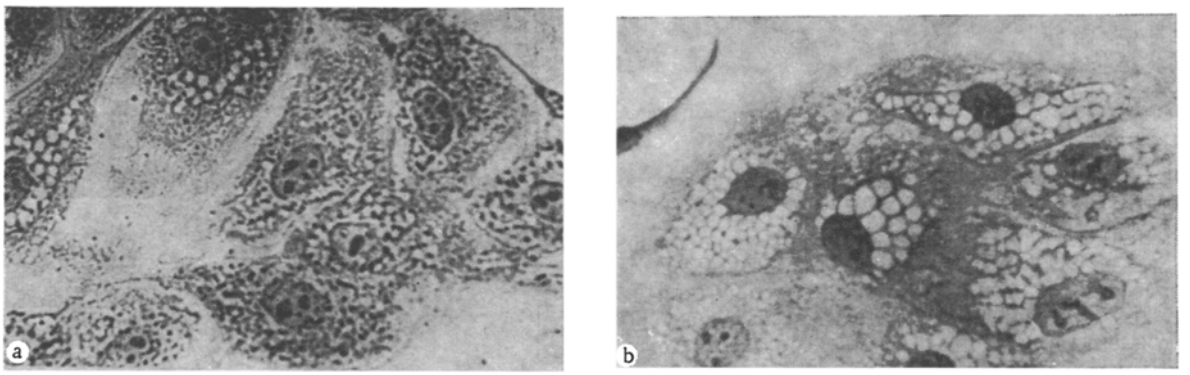

Fig. 3. Monolayer culture of bovine fetal pancreatic cells (6 days after seeding): a) aldehyde-fuchsinophilic granules in cytoplasm of epithelial cells; b) disappearance of specific granules, vacuolation of cytoplasm of epithelial cells after culture for 24 h in solution containing increased concentration of glucose. Aldehyde–fuchsin, 600×.

literature. Their nuclei were spherical or ovoid and contained 2-5 nucleoli and finely dispersed chromatin.

In cultures obtained from bovine fetal pancreas and stained with aldehyde–fuchsin specific granules were found in many of the polygonal and "special" cells (up to 60-70%). Granules were most numerous in the perinuclear zone of the cytoplasm. Cultivation in medium with an increased concentration of glucose led to definite degranulation of these cells: Their cytoplasm became honeycombed (Fig. 3).

On the basis of the cytological features, the presence of specific granules, and the phenomenon of degranulation observed in an increased concentration of glucose in the growth medium, the cells described above thus correspond to β cells of the islets of Langerhans.

LITERATURE CITED

1. I. S. Turchin, "The study of the structure and function of the cells of endocrine glands by the tissue culture method," Author's Abstract of Doctoral Dissertation, Kiev (1975).
2. W. F. Ballinger and P. E. Lacy, Surgery, 72, 175 (1972).
3. W. L. Chick, V. Lauris, J. H. Flewelling, et al., Endocrinology, 92, 212 (1973).
4. W. L. Chick, A. A. Like, and V. Lauris, Science, 187, 847 (1975).
5. I. Hilwig, S. Schuster, W. Heptner, et al., Z. Zellforsch., 90, 333 (1968).
6. P. E. Lacy and M. Kostianowsky, Diabetes, 16, 35 (1967).
7. A. E. Lambert, B. Blondel, J. Kanazawa, et al., Endocrinology, 90, 239 (1972).
8. S. Moskalewski, Gen. Comp. Endocrinol., 5, 342 (1965).
9. C. Weber, R. Weil, R. McIntosh, et al., Surgery, 77, 208 (1975).