ISLET CELL CULTURES OBTAINED FROM BOVINE FETAL PANCREAS

L. A. Kirsanova, V. G. Ablamunits, N. N. Skaletskii, and V. V. Severin

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The problem of isolation and culture of the islets of Langerhans in the pancreas has recently assumed a largely applied character, due to the use of islet-cell (IC) cultures for transplantation into patients with insulin-dependent diabetes. The encouraging results of such transplantations, consisting basically of stabilization of the course of labile forms of diabetes, in the treatment of diabetic angiopathies and neuropathies [5, 7], and also the achievement in some cases of temporary insulin-independence of the recipients [9, 12] have acted as the stimulus to continued intensive scientific research in this direction. One of the most important aspects of this problem is the choice of the source of IC cultures. The most meaningful results have been obtained by clinical transplantation of allogeneic islets or IC cultures [4, 5, 7, 9, 12], although the limited amount of donated material available, as well as legal and ethical problems connected with the use of human fetuses, have made the study of alternative sources of IC highly topical — namely the pancreas of animals. Most research has been devoted to the pancreas of rodents, dogs, and hogs, whereas bovine pancreas (BP) has received little study as a source of IC [1, 2, 8, 10, 11].

We described previously some features of the histological structure of the bovine fetal pancreas [3] and, in particular, the structure and process of formation of so-called acino-insular complexes. According to our observations, the largest number of acino-insular complexes is observed in the pancreas from fetuses with a crown-rump length of 27-35 cm. The aim of this investigation was to study the possibility of obtaining hormonally active cultures of IC from the pancreas of such fetuses.

METHODS

Altogether 15 pancreases from bovine fetuses with a crown-rump length of 27-45 cm were used. The duration of heat-induced ischemia was 1-3 h. The subtotally removed pancreas was placed in a Petri dish, and 5-6 ml of a solution of collagenase (1 mg/ml) was injected into the parenchyma. The pancreas was cut into 4-6 parts which were transferred into a flask and incubated for 40 min at 37°C. Digestion was stopped by the addition of cold (+4°C) Hanks' solution. The contents of the flask were vigorously shaken and filtered through a metal sieve with mesh of 0.6 mm. The tissue suspension thus obtained was washed off twice with Hanks' solution and once with MEM medium during centrifugation (100 g, 10-15 sec). Microfragments were resuspended in 40 ml of MEM, containing a double set of amino acids and vitamins, 10% fetal calf serum HEPES (20 mM), L-glutamine (2 mM), and gentamicin sulfate (80 mg/liter). Into each well of a 24-well planchet (Costar) was added 2.0 μliters of the suspension. Culture was carried out at 37°C in a humid atmosphere containing 5% CO_2. Microfragments for histologic investigation (immediately after dissociation of the pancreatic tissue and after 24 h in culture) were fixed with Bouin's fluid and embedded in paraffin wax. Sections 5-7 μ thick were stained with hematoxylin and eosin and with Gomori's aldehyde-fuchsine. Material for electron-microscopic investigation was fixed with 1% OsO_4 solution, dehydrated in alcohols of increasing concentration, and acetone, and embedded in Epon. Ultrathin sections were studied in the JEM 100B electron microscope.

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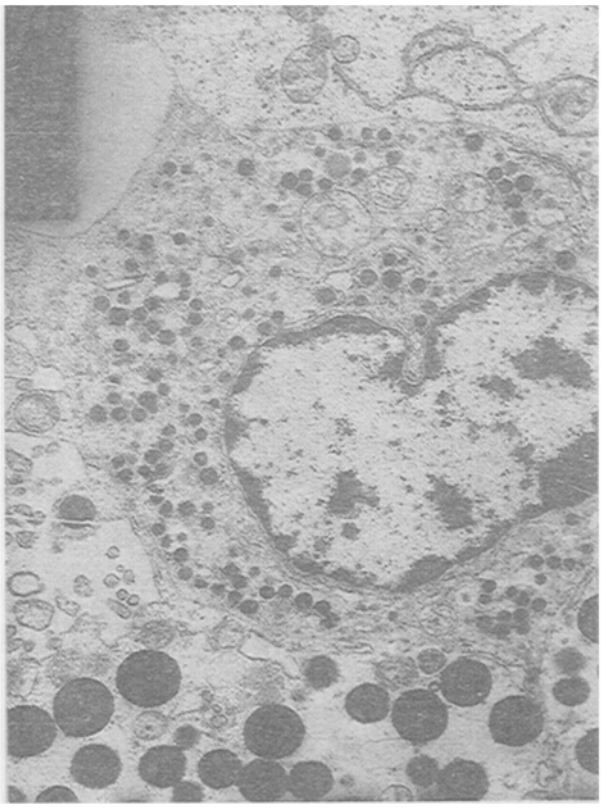

Fig. 1

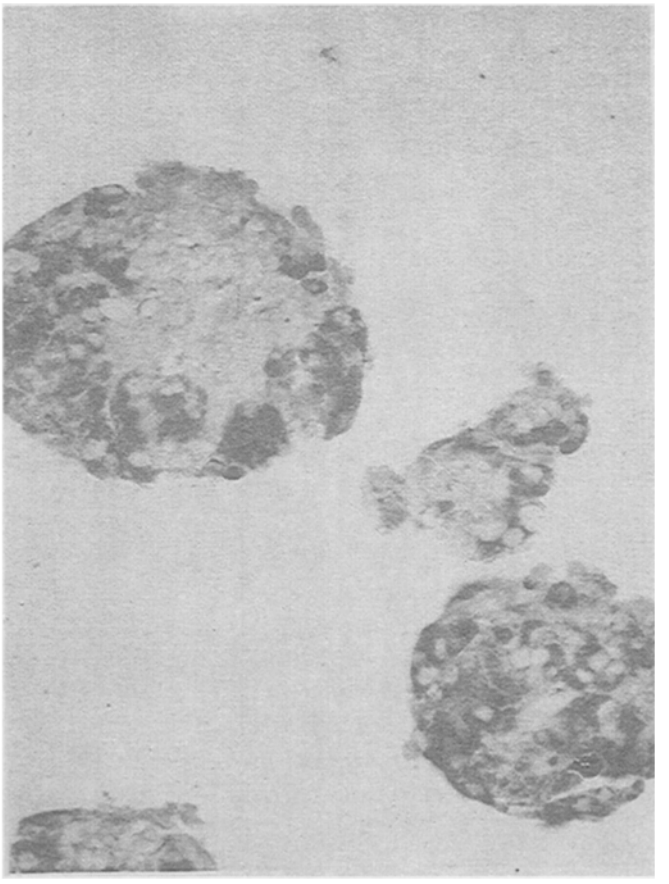

Fig. 2

Fig. 1. β-Cell of pancreas of a bovine fetus with crown-rump length of 32 cm. Secretory granules at different stages of maturation. 11,000×.

Fig. 2. Numerous β-cells in pseudoislets. Aldehyde-fuchsine. 400×.

Insulin secretion was determined by continuous flow method. Pancreatic microfragments, collected from two wells, were placed on a nitrocellulose filter (pore diameter 2.5 μ) in a capsule, through which was passed, at a rate of 1 ml/min, the MEM medium containing, consecutively: 1) 5 mM glucose, 2) 16.5 mM glucose, 3) 16.5 mM glucose and 5 mM theophylline, 4) 16.5 mM glucose, 5 mM theophylline, and 0.4 μM glucagon. Samples, each consisting of 0.2 ml, were taken every 3 min and frozen for subsequent determination of insulin by radioimmunoassay.

RESULTS

Histologic investigation of the bovine fetal pancreas showed good preservation of the original material. On staining with aldehyde fuchsine well-granulated β-cells in discrete islets and β-cells with various degrees of granulation, components of acinoinsular complexes were seen. Electron-microscopic investigation showed (Fig. 1) that the nuclei of the β-cells were oval in shape, sometimes with an irregular lobular outline; they contained 2-4 nucleoli and condensed chromatin, connected with the inner nuclear membrane. The cytoplasm of the β-cells was of average electron density. Numerous free ribosomes and polysomes connected with tubules of the endoplasmic reticulum were seen in it. The mitochondria as a rule were circular in shape. Elongated mitochondria were occasionally observed. Typical secretory granules with an electron-dense core and a peripheral electron-translucent zone were seen in the cytoplasm of the β-cells. The granules were uniformly distributed throughout the cell cytoplasm.

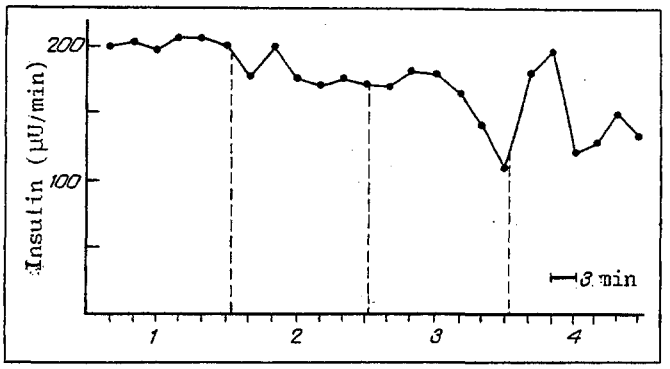

Fig. 3. Insulin secretion by pseudoislets in MEM medium containing 5.5 mM glucose (1), 16.5 mM glucose (2), 16.5 mM glucose and 5 mM theophylline (3), and 16.5 mM glucose, 5 mM theophylline, and 4 mM glucagon (4).

As a result of digestion of the pancreatic tissue by collagenase followed by manipulations, the pancreas was dissociated into tissue microfragments consisting of typical acinar and endocrine cells. Nuclei with pycnotic changes were often observed in the acinar cells located at the periphery of the microfragments.

During culture for 24 h morphogenetic processes connected with adaptation of the cells to the conditions of culture took place in the microfragments. Acinar cells underwent destruction, and endocrine cells preserved their structure. According to the data of light microscopy, virtually no typical acini were found in the microfragments. However, electron-microscopic investigation revealed not only destroyed acinar cells, but also a small number of preserved cells with zymogen granules in their cytoplasm.

After 24 h in culture the microfragments had the appearance of compact oval formations measuring 0.1-0.5 mm. The main mass of these formations, which may be called pseudoislets, consisting of polygonal cells with pale homogeneous cytoplasm and round nuclei. A few cells with pycnotic nuclei and vacuolated cytoplasm were found at the periphery and in the thickness of the pseudoislets: these were dystrophic cells of the exocrine part of the pancreas. On staining with aldehyde-fuchsine, numerous β-cells with the characteristic lilac granules in the cytoplasm, were seen (Fig. 2). An electron-microscopic investigation showed that the ultrastructure of these β-cells was virtually indistinguishable from that in the original material.

Investigation of functional activity of the pseudoislets by the continuous flow method showed that insulin secretion in MEM containing 5.5 mM glucose amounted to 200 ± 9 μU/min (Fig. 3). We found no evidence that the pseudoislets could increase their insulin secretion in response to an increase (up to 16.5 mM) in the glucose concentration, and addition of theophylline actually reduced insulin secretion. Only the subsequent addition of glucagon caused a temporary increase in secretory activity of the β-cells. These preliminary results are definitely similar to those obtained by other workers who studied functional activity of IC of the human and porcine fetal pancreas [6, 13, 14].

This approach, which we used for dissociation of the bovine fetal pancreas, thus enabled a culture of IC with a high content of hormonally active β-cells to be obtained. The characteristics of function of IC during longer-term culture will be the subject of future research.

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