CULTURES OF HUMAN FETAL PANCREATIC ISLET CELLS

V. I. Shumakov, V. N. Blyumkin,
R. A. Babikova, V. P. Fedotov,
G. G. Tsertsvadze, N. V. Sadovnikova,
and L. I. Ul'yanova

UDC 612.64.34-085.23

Cultures of islets cells were obtained from the cadaveric pancreas of 16-25-week human fetuses. During culture two waves of mitotic activity were observed. An increase in the insulin concentration in the culture medium took place after a wave of mitotic activity.

KEY WORDS: cell cultures; islet cells; mitotic activity; insulin.

Isolation of hormonally active islets of Langerhans from the human pancreas for allografting into patients with severe forms of diabetes mellitus is an urgent but as yet unsolved problem [9-11]. This is largely because the connective-tissue stroma is very well developed in the cadaveric pancreas of adult human donors, making it difficult to isolate the islets, the weight of which is only 1-2% of the total weight of the gland [5]. The high enzymatic activity of the exocrine portion of the pancreas in the postembryonic period of development is another handicap. The possibility of using the pancreas of the intrauterine human fetus as a source of islet cells producing insulin therefore deserves attention: In this case the islet cells account for up to 30% of the total weight of the gland and enzymic activity of the exocrine portion is absent.

This paper gives details on cultures of islet cells obtained from the pancreas of 16-25-week human fetuses.

EXPERIMENTAL METHODS

The pancreas of 22 cadavers of 16-25-week human fetuses obtained during operations for termination of pregnancy on medical grounds (little caeserean section) was the source of the islet cell cultures. The time between cessation of the fetal circulation and the beginning of culture in vitro was 2.5-3 h. The pancreas was removed under sterile conditions and washed several times in Hanks' solution with antibiotics; after removal of the connective tissue (the capsule and the large bands between the lobules) the gland was divided into fragments measuring about 1 mm. The shredded tissue was treated with 0.1% collalytin solution [1] on a magnetic mixer at 20-22°C for 20-25 min. The resulting suspension was thoroughly washed with Hanks' solution and centrifuged, the supernatant was removed, and the cell residue was resuspended in medium No. 199 with 10% bovine serum. The cell complexes were introduced into 100-ml or 200-ml flasks and into penicillin flasks with coverslips. Culture took place at 37°C. The medium was completely changed 3 days after seeding of the cells. Samples of culture fluid was taken at various times after the change of medium for determination of their insulin concentration by a radioimmunological method (using CEA-IRE-Sorin kits). Cultures grown on coverslips were fixed with Bouin's mixture or 96% ethanol and stained with hematoxylin-eosin and aldehyde-fuchsin.

EXPERIMENTAL RESULTS

Adhesion of complexes of epithelial cells obtained from the cadaveric human fetal pancreas to the glass coverslip as a rule was complete 3-4 days after seeding. During that time the acinar cells of the exocrine portion and also most of the fibroblasts had undergone total destruction and elimination. During the 2-4 days after the change of medium (5-8 days after seeding the cells), a monolayer border was formed on the outside

Department of Artificial Organs and Assisted Circulation, Laboratory of Pathomorphology, Scientific-Research Institute of Transplantation and Artificial Organs, Ministry of Health of the USSR. Laboratory of Biological Standardization of Hormones, Institute of Experimental Endocrinology and Hormone Chemistry, Academy of Medical Sciences of the USSR. Maternity Home No. 26, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 8, pp. 202-204, August, 1979. Original article submitted December 11, 1978.

0007-4888/79/8808-0903$07.50

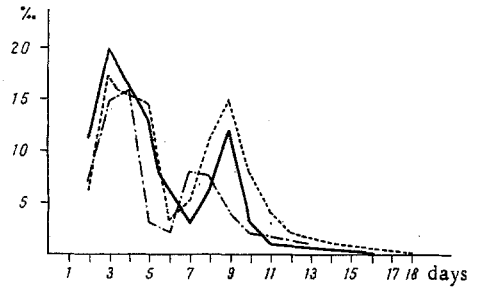

Fig. 1. Mitotic activity of cultures of human fetal pancreatic islet cells. Abscissa, days after seeding cells; ordinate, mitotic index (in ‰). Curves illustrate three different experiments.

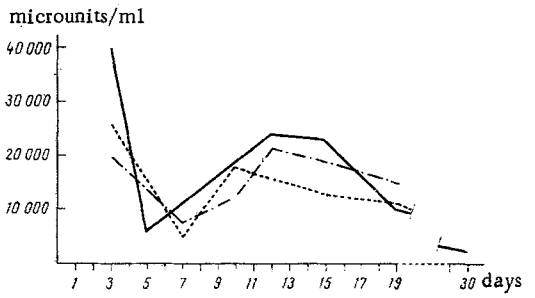

Fig. 2. Insulin concentration in medium at different times of culture of human fetal pancreatic islet cells. Abscissa, days after seeding; ordinate, insulin concentration in culture medium (in microunits/ml). Curves illustrate three different experiments.

from multiple foci of adhesion: This was a zone of growth consisting of small and large (8-30 μ) polygonal epithelial cells and atypical wide laminar "special cells" [6], located along the outer border of the culture. Mitoses were found in the zone of growth, and as a rule during formation of the cultures it was possible to distinguish two waves of mitotic activity (Fig. 1). The first wave of mitoses, as shown by the study of fixed stained preparations, was observed 3-5 days after the beginning of culture. At this time the mitotic index reached 15-20‰. Most of the dividing cells were located in the middle and outer parts of the zone of growth. During the next 2-3 days mitotic activity as a rule diminished (to 2-3‰), but on the 7th-9th day a second wave of mitoses, not as high as the first (mitotic index 8-15‰) was observed. The diameter of the zone of growth around each primary focus of adhesion by this time was 3-4 mm and the fraction of cells with aldehyde-fuchsin-positive granules in their cytoplasm (B cells) was 70-90%. In turn, these could be differentiated into B cells in a stage of accumulation of secretion, and also partially and completely degranulated cells. During aging of the cultures, the primary central focus of adhesion was the first to die: As a result of this, an irregularly circular defect formed in the center of the zone of growth. Later, the destructive processes spread to the monolayer part of the cultures. By the 18th-23rd day, the mitotically dividing cells disappeared, after which massive vacuolation of the cells took place, followed by their detachment from the glass. By frequent partial changes of the growth medium, some cultures could be maintained for as long as 30-40 days.

The insulin concentration in the culture fluid at different times of culture of the human islet cells is shown in Fig. 2. A decrease in the insulin concentration in the medium took place most frequently on the 5th-7th day of culture. In culture No. 1, for instance, the insulin concentration fell from 40,000 to 6000 microunits/ml and in culture No. 3 from 26,000 to 5000 microunits/ml. Next followed a marked rise in the insulin concentration in the medium (most frequently to the 10th-12th day of culture), followed by another fall.

The phenomenon of fluctuations of the insulin concentration in the culture medium requires special discussion. The fall in the insulin concentration could be connected with partial destruction of insulin directly

in the medium [7] and also with utilization of insulin by the cells in culture during growth and differentiation [12]. The decrease in the insulin concentration in the medium was perhaps connected with its utilization by cells preparing for mitosis or by cells just leaving mitosis and entering a new interphase (G_1 period). In turn, the differentiated B cells newly formed as a result of the wave of mitoses could be the source of fresh portions of insulin entering the culture medium [3, 8]. This hypothesis seems soundly based because the second increase in the insulin concentration took place a little later than the second wave of mitosis. Similar results were obtained previously during the study of the dynamics of insulin-forming activity of pancreatic islet cell cultures from bovine fetuses [2]. Finally, when the results are analyzed the possibility of periodic inhibition of function of the B cells in in vitro culture on the attainment of "critical" insulin concentrations in the medium (reciprocal inhibition of secretion) must also be borne in mind [4, 12]. The likelihood of this is all the more difficult to rule out because in the present experiments the cultures were grown under stationary conditions in flasks, and not in continuous-flow systems with constantly renewed medium.

The results indicate that monolayer cultures of human fetal pancreatic islet cells possess considerable insulin-forming activity which persists for a long period of time. Such cultures may perhaps eventually form one basis for the creation of a B cell bank for subsequent grafting into diabetic patients.

LITERATURE CITED

1. R. A. Babikova, V. N. Blyumkin, B. I. Shal'nev, et al., Byull. Éksp. Biol. Med., No. 3, 350 (1977).
2. V. N. Blyumkin, V. P. Fedotov, R. A. Babikova, et al., in: Organ Transplantation in Clinical and Experimental Practice and Artificial Organs [in Russian], Moscow (1978), pp. 98-101.
3. I. S. Komolov and V. P. Fedotov, Probl. Éndokrinol., No. 6, 52 (1974).
4. J. C. Dunbar, W. J. McLaughlin, M. -F. Walsh, et al., Hormone Metab. Res. (Stuttgart), 8, 1 (1976).
5. H. Ferner, in: Gastroenterologie und Stoffwechsel. Aktionen und Interaktionen, Brussels (1974), pp. 75-79.
6. J. Hilwig, S. Schuster, W. Heptner, et al., Z. Zellforsch., 90, 333 (1968).
7. K. D. Kohnert, H. J. Hahn, H. Zuhlke, et al., Biochim. Biophys. Acta, 342, 68 (1974).
8. I. S. Komolov and V. P. Fedotov, Endocr. Exp., 12, 43 (1978).
9. G. Lundgren, A. Andersson, H. Borg, et al., Transpl. Proc., 9, 237 (1977).
10. J. S. Najarian, D. E. R. Sutherland, A. J. Matas, et al., Transpl. Proc., 9, 233 (1977).
11. B. C. Reed, S. H. Kaufmann, J. C. Mackall, et al., Proc. Natl. Acad. Sci. USA, 74, 4876 (1977).