# EFFECT OF SERUM AND GLUCOSE ON PROLIFERATION OF DIFFERENT TYPES OF HUMAN EMBRYONIC PANCREATIC CELLS IN PRIMARY CULTURE

**A. V. Timofeev, B. B. Egorov, V. I. Chizhevskaya, P. V. Pozdnev, and A. R. Kazachkov**

UDC 612.349.014:612.646].086.833

**KEY WORDS:** pancreatic cell culture; proliferation; growth factors; glucose; autoradiography

The endocrine cell population of the mammalian pancreas is formed during embryonic development by proliferation and differentiation of precursor cells located in the epithelium of the pancreatic ducts [3]. In adult animals the endocrine part of the pancreas in renewed by multiplication of certain cells of the ducts and undifferentiated islet cells [4]. The principles of development and renewal of the endocrine pancreas have been successfully studied in vitro, when the islet cells of rodents usually served as the test object [2]. Cultures of the embryonic human pancreas have rarely been used in such experiments, due to the difficulty of obtaining the original material and its biological nonhomogeneity. Yet the study of morphogenetic processes in cultures of human embryonic pancreas is of considerable interest, because such cultures are used for transplantation into diabetic patients [1]. The aim of this investigation was to assess the proliferative potential of different types of cells in cultures of the human fetal pancreas and to study the susceptibility of different types of cells to proliferative stimuli.

## EXPERIMENTAL METHOD

The pancreas from eight human fetuses at 17-18 weeks (group 1) and of 14 fetuses at 23-24 weeks (group 2) were used to prepare histotypic cultures. Pieces of pancreas measuring 0.2-0.8 mm were cultured in dishes made of nonadhesive plastic in an atmosphere with 5% $CO_2$ in medium RPMI-1640 (glucose concentration 5 mM) with 10% fetal calf serum (FCS), 5 mM essential amino acids, and 5 mM vitamins. Spherical floating fragments 0.1-0.8 mm in diameter were formed from the pieces of pancreas 4-5 days after their inoculation (Fig. 1). The parenchyma of the fragments consisted mainly of epithelial cells (94-98% of the total number of cells). Ductlike structures (DLS) were preserved in both groups of fragments. Fibroblasts (2-6% of the total number of cells) were found inside the fragments and on their surface. To study the effect of FCS and glucose on proliferation of the cells the fragments were transferred to 6-well planchets made of nonadhesive plastic and were cultured for 2, 5, or 10 days in medium with 5 or 10% FCS or in medium with 10% FCS and 17 mM glucose. Fragments of each pancreas were distributed so that one well containing from five to nine fragments corresponded to each version of the experiment. In all cases 20 kBq/ml of $^3$H-thymidine (520 GBq/mmole) was added to the medium. The medium in the wells was changed once every 2 days. The insulin concentration in the conditioned medium was determined by radioimmunoassay. The fragments were fixed at the above-mentioned times in Bouin's fluid and embedded in paraffin wax. Sections (5 $\mu$) were covered with type M emulsion, exposed for 14 days, and stained with hematoxylin. At least 3000 cells were counted in each preparation, consisting of sections of fragments from the same well. The total index of labeled cells of all types ($ILC_t$), the index of labeled DLS cells ($ILC_{dis}$), the index of labeled epithelial cells not present in the composition of the DLS ($ILC_e$), and the index of labeled fibroblasts ($ILC_f$) were calculated. All indices were calculated as the ratio of the number of labeled cells of each type to the total number of cells, and expressed as a percentage. The percentage of labeled cells in DLS ($PLC_{dis}$) also was determined. The results were analyzed by Student's test.

Laboratory of Cell Transplants, Research Center for Medical Biotechnology, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR I. I. Votzin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 8, pp. 206-208, August, 1990. Original article submitted December 29, 1989.

 0007-4888/90/0008-1124$12.50 

TABLE 1. Changes in Proliferative Activity of Different Types of Cells in Fragments of Human Fetal Pancreas Depending on Composition of Culture Medium and Age of Fetuses

| Group of pancreas (age and number of fetuses) | Composition of medium | Duration of culture, days | $ILC_t \pm S_x$ | $ILC_e \pm S_x$ | $ILC_{dis} \pm S_x$ | $ILC_f \pm S_x$ |
|---|---|---|---|---|---|---|
| 1 (17—18 weeks, n = 8) | 5 % FCS | 2 | 6,5±0,6 | 4,95±0,45 | 1,5±0,35 | 0,05±0,01 |
| | | 5 | 20,4±2,5 | 16,04±1,2 | 3,75±0,8 | 0,6±0,05 |
| | | 10 | 37,2±8,6 | 26,8±7,3 | 9,0±1,6 | 1,4±0,12 |
| | 10 % FCS | 2 | 8,2±2,1 | 4,65±1,9 | 3,45±0,28 | 0,1±0,03 |
| | | 5 | 41,4±6,3 | 33,3±6,2 | 6,0±1,6 | 2,1±0,31 |
| | | 10 | 65,5±10,2 | 44,95±8,3 | 14,25±2,6 | 6,3±1,2 |
| | 10% FCS + 17 mM glucose | 2 | 13,08±0,8 | 9,85±1,1 | 3,0±0,35 | 0,15±0,06 |
| | | 5 | 67,7±11,7 | 58,7±8,6 | 6,3±1,03 | 2,7±0,8 |
| | | 10 | 95,4±12,3 | 75,6±10,4 | 14,4±1,2 | 5,4±1,2 |
| 2 (23—24 weeks, n = 14) | 5 % FCS | 2 | 3,5±0,4 | 2.86±0,12 | 0,56±0,14 | 0,08±0,01 |
| | | 5 | 9,8±1,3 | 7,46±0,88 | 1,44±0,62 | 0,9±0,12 |
| | | 10 | 20,2±4,8 | 16,6±4,3 | 2,0±0,41 | 1,6±0,4 |
| | 10 % FCS | 2 | 8,1±0,8 | 5,61±0,6 | 1,44±0,1 | 1,05±0,1 |
| | | 5 | 21,3±3,4 | 17,8±2,8 | 2,0±0,3 | 1,5±0,33 |
| | | 10 | 40,8±6,3 | 32,6±5,7 | 4,8±0,81 | 3,4±0,81 |
| | 10% FCS + 17 mM glucose | 2 | 15,6±1,2 | 12.44±1,1 | 1,76±0,36 | 1,4±0,4 |
| | | 5 | 42,1±6,8 | 35,7±5,9 | 3,6±0,82 | 2,8±0,15 |
| | | 10 | 60,7±8,5 | 50,2±7,7 | 6,0±0,85 | 4,5±1,05 |

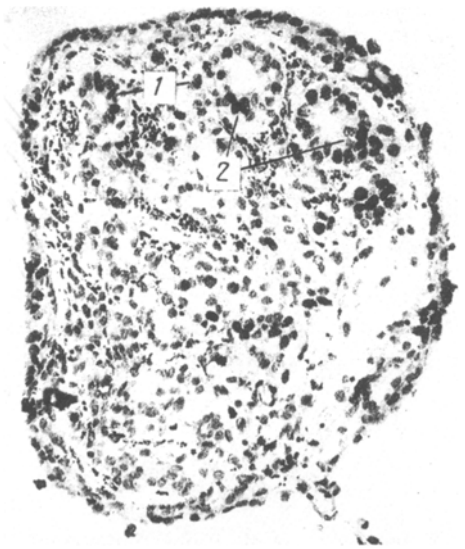


Fig. 1. Section through floating fragment of pancreas of 18-week human fetus on 5th day after transfer into medium containing 10% FCS, 17 mM glucose, and $^3$H-thymidine. Autoradiograph (200×, stained with hematoxylin). 1) DLS, 2) $^3$H-thymidine-labeled cells in composition of DLS.

## EXPERIMENTAL RESULTS

Throughout the period of the experiment all cultures remained viable (they secreted at the rate of 7 nmoles insulin pez well per day). The rate of proliferation of all types of cells depended on the age of the fetuses and the composition of the medium (Table 1). Under all conditions of culture, $ILC_t$ in fragments of group 1 rose more rapidly than in the fragments of group 2. In medium with 10% FCS, $ILC_t$ in fragments of both groups increased much more rapidly than in medium with 5% FCS. During culture in medium with 10% FCS and 17 mM glucose, $ILC_t$ in fragments of both groups on the 5th and 10th days significantly exceeded $ILC_t$ in fragments of both groups on the 5th and 10th days glucose. Changes in $ILC_e$ were due primarily to changes in $ILC_t$, i.e., to proliferation of epithelial cells not present in the DLS. Analysis of changes in $ILC_{dis}$ and $PLC_{dis}$ (Fig. 2) showed that the proliferative activity of the DLS cells depended on the age of the fetuses and on the concentration of FCS, but

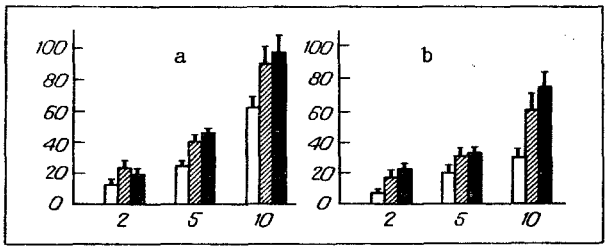


Fig. 2. Changes in $ILC_{dis}$ in fragments of human fetal pancreas at different stages of intrauterine development and during culture under different conditions. Abscissa, duration of experiment (in days); ordinate, $ILC_{dis}$ (in %). a) Fragments of group 1, b) of group 2. Unshaded columns — medium with 5% FCS, obliquely shaded — medium with 10% FCS, black columns — mediums with 10% FCS and 17 mM glucose.

did not depend on the glucose concentration in the medium. The proliferative activity of fibroblasts in both groups of fragments depended only on the FCS concentration in the medium.

It can be concluded from the results that: 1) fragments of pancreas contain two populations of epithelial cells which are capable of proliferating: a small population of DLS cells and a larger population of other epithelial cells; the proliferative potential of the two populations in vitro depends on the age of the fetuses; 2) proliferation of DLS cells is controlled by FCS, but is not controlled by glucose; 3) the proliferative activity of the other epithelial cells depends both on the FCS concentration and on the glucose concentration in the medium. The proliferative response to these factors is more marked in fragments of group 1.

It can be tentatively suggested that DLS in vitro contain multipotent stem cells, during proliferation of which epithelial cells of DLS and the direct precursors of endocrine cells are formed. These multipotent cells proliferate in response to the action of growth factors of FCS, but do not respond to glucose, the specific stimulator of proliferation of the pancreatic endocrine cells. Conversely, precursors of endocrine cells and mature endocrine cells react both to growth factors of FCS and to glucose. Variations in the pool of proliferating epithelial cells in pancreatic fragments from fetuses of different ages in vitro may be due to a decrease in the number of multipotent cells and precursors of endocrine cells in the course of pancreatic embryogenesis in vivo. These suggestions can be tested in experiments with the parallel use of immunocytochemical and autoradiographic methods.

## LITERATURE CITED

1. V. I. Shumakov, V. N. Blyumkin, S. N. Ignatenko, et al., Probl. Endokrinol., **31**, 18 (1985).
2. B. Amory, J.-L. Mourmeaux, and C. Remacle, In Vitro: Cell Develop. Biol., **24**, 91 (1988).
3. G. Teitelman, J. Lee, and D.J. Reis, Develop. Biol., **120**, 425 (1987).
4. S. Tsubouchi, E. Kano, and H. Suzuki, Anat. Rec., **218**, 111 (1987).