PRESERVATION OF MORPHOLOGY AND FUNCTION OF GUINEA PIG PANCREATIC ISLET CELLS IN SUBCULTURE

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UDC 612.349.7-085.23

KEY WORDS: passage; subculture; islet cells; insulin.

An extremely interesting aspect of the development of effective methods of treatment of diabetes is culture of the pancreatic islet cells which produce insulin, with a view to their transplantation into diabetics. In recent years several Soviet and other researchers [1-13] have described ways of obtaining and culturing pancreatic cells of experimental animals and of human embryos and cadavers. However, primary trypsinized cultures of islet cells remain viable only for a short time [1, 4, 7]. As a rule aging and death of primary cultures could not be prevented.

The aim of this investigation was to study whether cultures of pancreatic β-cells can be preserved for a long time by subculture and to study their morphology and function at different stages of culture.

EXPERIMENTAL METHOD

Subcultures of β-cells were obtained from primary cultures of guinea pig pancreas. Primary trypsinized monolayer cultures of guinea pig pancreatic islet cells were obtained by the method in [2, 5], using collalytin of Soviet origin. The monolayer thus obtained was removed with 0.02% Versene solution. The cells were sedimented by centrifugation at 1000 rpm, resuspended in medium, and seeded in a dose of 5×10^5 to 6×10^5 cells/ml. To subculture the islet cells a nutrient medium consisting of equal parts of medium 199, 0.25% lactalbumin hydrolysate, and 20% bovine serum was used. Glucose was not added to the nutrient medium. The medium was changed every 2-3 days. Functional activity of the pancreatic β-cell subcultures was determined by the addition of 300 mg% glucose to the medium. The insulin concentration in the culture media was determined by a radioimmunologic method. Cytologic study of pancreatic islet cell subcultures grown on slides was carried out after fixation in Bouin's solution followed by staining with hematoxylin and eosin and aldehyde-fuchsine.

EXPERIMENTAL RESULTS

Vital microscopy of the islet cell cultures of the first passage after 24 h revealed adhesion and spreading of the cells over the surface of the glass. On the 3rd-4th day the pancreatic islet cells were proliferating and joined together by cytoplasmic processes. On the 5th-7th day of culture, intensive growth had led to the formation of a monolayer consisting of epithelial-like polygonal cells and single fibroblast-like cells (Fig. 1). The cells were tightly packed together. On examination of permanent preparations under the light microscope the nuclei of the β-cells usually were round and contained 1-3 nucleoli. The cell cytoplasm was finely granular and eosinophilic; basophilia were observed in the perinuclear zone. By changing the growth medium regularly the islet-cell culture after the first passage could be maintained for 30-40 days. Similar times of survival have been reported by other workers for primary trypsinized cultures [1, 4, 7]. An attempt to maintain β-cells longer *in vitro* without reseeding led to subsequent development of destructive processes in the culture, followed by death.

The 30-day-old subcultures were found to be capable of reseeding, although the new monolayer formed later rather than after reseeding of cultures 4-7 days old. Accordingly, the subsequent passages were begun on the 25th-30th day of growth of the culture.

Department of Morphology, Armenian Branch of the All-Union Scientific Center for Surgery, Erevan. (Presented by Academician of the Academy of Medical Sciences of the USSR R. V. Petrov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 3, pp. 83-85, March, 1983. Original article submitted May 25, 1982.

 0007-4888/83/9503- 0354$07.50

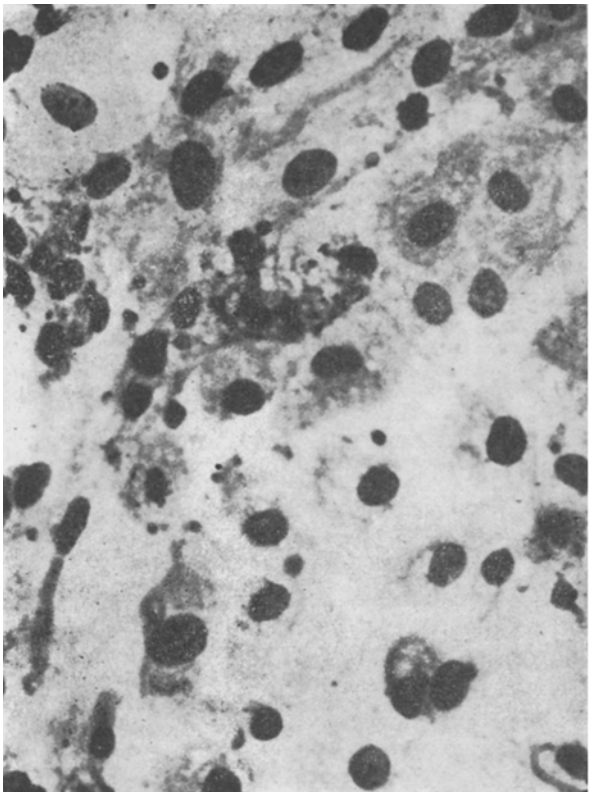

Fig. 1

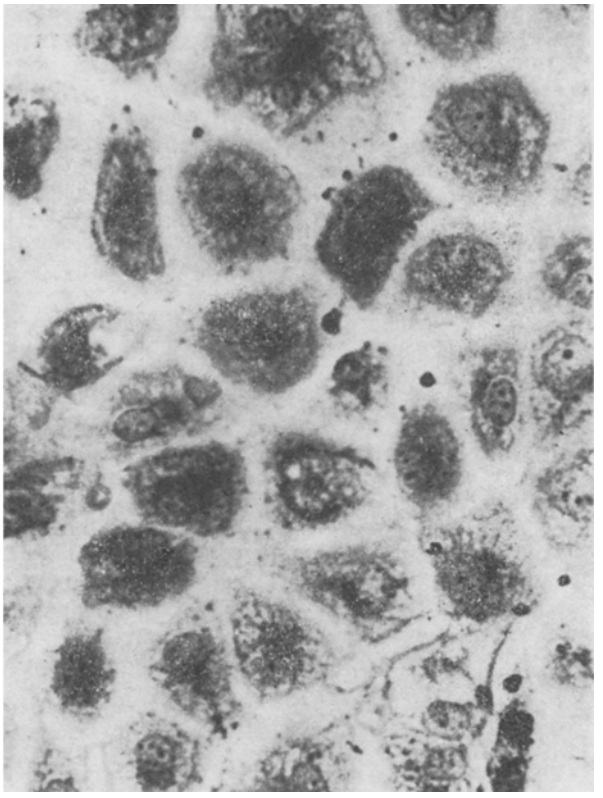

Fig. 2

Fig. 1. Monolayer culture of guinea pig pancreatic islet cells (second passage) growing in medium without glucose. Hematoxylin—eosin, 280 ×.

Fig. 2. Monolayer culture of guinea pig pancreatic β-cells (fifth passage). Aldehyde-fuchsine, 280 ×.

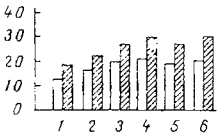

Fig. 3. Insulin level in subculture of β-cells after first to sixth passages. Ordinate, insulin concentration in culture medium (in microunits/ml). Unshaded columns — in medium without glucose, shaded columns — medium after addition of 300 mg% glucose.

The time course of development of the subcultures of guinea pig pancreatic islet cells from one passage to the next was largely similar with that of the primary trypsinized culture. However, there were also some differences. In the course of transplantation the fibroblast-like cells were eliminated and at the fifth passage the monolayer consisted entirely of epithelial-like cells. On staining with aldehyde-fuchsine, positively stained granules could be seen in the cell cytoplasm (Fig. 2). The number of these cells in the subculture amounted to 90%, unlike in the primary culture, in which there were up to 30% of fibroblast-like cells [2]. By regular passage it is thus possible to obtain a culture with a higher content of epithelial-like β-cells. The number, size, and arrangement of the aldehyde-fuchsine-positive

granules in these cells, incidentally, varied evidently because of differences in the physiological state of the cells. After addition of glucose to the medium degranulation and vacuolation of the cells were observed.

The results of determination of insulin production by the subcultured pancreatic islet cells by a radioimmunologic method after successive passages are given in Fig. 3. It will be noted that islet-cell function was preserved after the successive passages, and varied only within narrow limits (18-32 microunits/ml) after glucose loading (300 mg%). This can be explained by the different density of the monolayer in the subcultures.

The guinea pig pancreatic β-cells were maintained in culture for 180 days, having gone through six passages. The phenomenon of such prolonged preservation of insulin-producing ability of the β-cells *in vitro* calls for further special study.

The results of these experiments are noteworthy because they show that a more homogenous population of β-cells can be obtained by regular passages. Preservation of viable, functionally active pancreatic islet cells for 6 months also offers the possibility of accumulating them in sufficient numbers for clinical purposes and for experimental research in transplantology.

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