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NEW ASPECTS OF CONSERVATION OF HUMAN FETAL PANCREATIC β-CELLS BY THE REGULAR PASSAGE METHOD

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Much progress in recent years has been made in the development of methods of culture in insulin-secreting human pancreatic β-cells with a view to their transplantation into diabetic patients. However, the problem of accumulation and long-term preservation of functionally active pancreatic β-cells has not been solved and remains an urgent task in conservation.

In recent research some Western investigators have shown that long-term culture of isolated islets of Langerhans of experimental animals [12, 13] and of human adults [10] and fetuses [8] is possible, with preservation of insulin-secreting capacity, for between 75 and 97 days, but in only a few publications have cases of long-term preservation of pancreatic β-cells in tissue culture for periods ranging from a few weeks up to 2 months been described [5, 7, 9]. Previously the present writers studied long-term culture of pancreatic β-cells from guinea pigs [2]. The positive results of this work laid the foundations for a similar study of human fetal pancreatic β-cells in culture.

This paper gives data on the possibility of using a technique of cell subculture *in vitro* as an approach to the accumulation and preservation of functionally active human fetal pancreatic β-cells.

EXPERIMENTAL METHOD

A monolayer of primary trypsinized cultures obtained from the pancreas of human fetuses aged from 16 weeks to 9 months of intrauterine development, served as the original material for subculture. Primary trypsinized cultures were obtained by the method described previously [1, 4]. Disaggregation of the monolayer of primary trypsinized and subcultured human fetal pancreatic β-cells, seeding, and culture of the cells were carried out by the method described by the writers previously [2], and devised for guinea pig pancreatic β-cells. A mixture of equal volumes of medium 199, lactalbumin hydrolysate, 20% bovine serum, and 30% of conditioned medium obtained from a monolayer of β-cells was used as the growth medium for the subcultures of human fetal islet cells. The seeding dose and the intervals between passages were determined depending on the age of the experiments. For cytologic study of islet cells a culture of different passages of β-cells was seeded in penicillin flasks with coverslips. Preparations fixed with Bouin's fluid were stained with hematoxylin and eosin and with aldehyde-fuschsine. The insulin content was determined in washings of the cultures of different passages by radioimmunoassay.

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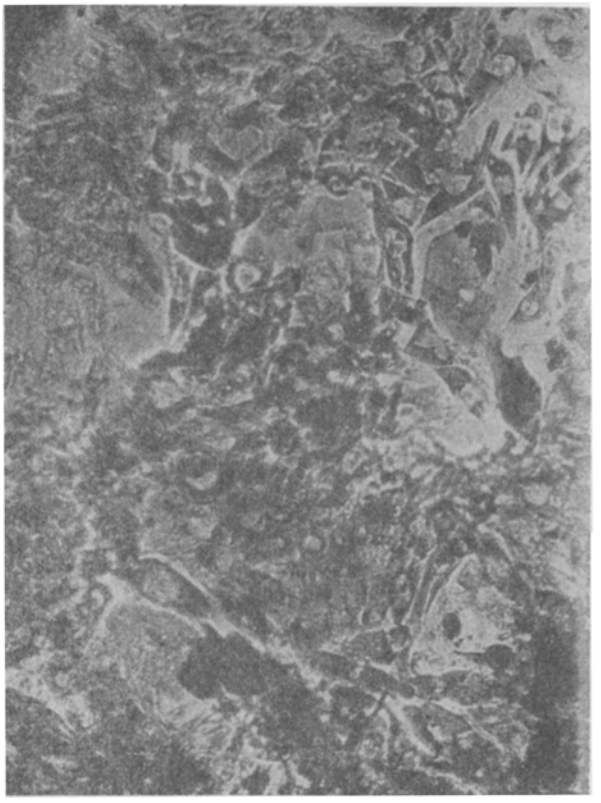

Fig. 1

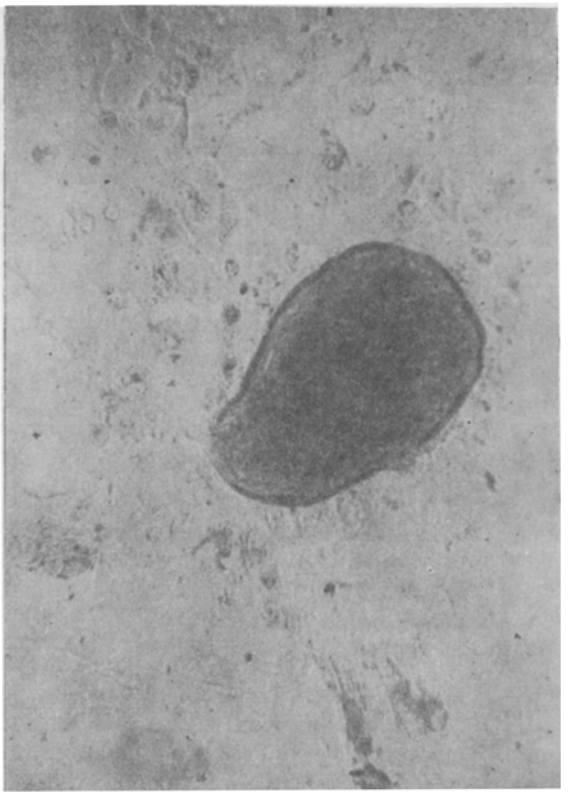

Fig. 2

Fig. 1. Culture of human fetal pancreatic β-cells at the 7th passage. Here and in Fig. 2: vital microscopy, 280 ×.

Fig. 2. Aggregated freely floating microcolony of human fetal pancreatic β-cells against the background of a monolayer focus.

EXPERIMENTAL RESULTS

Vital microscopy of the subcultured human fetal pancreatic β-cells showed that the dynamics of growth and multiplication of the subcultures and of the primary trypsinized cultures was similar in many respects. Growth and formation of the cell monolayer of the β-cell subcultures depended on the seeding dose. With a seeding dose of 8×10^5 cells/ml, 24 h after seeding attachment of individual cells and microcolonies of them was observed. During culture the microcolonies increased in density and acquired the appearance of multilayered foci with time. Elimination of fibroblast-like cells from one passage to the next had the result that by the 3rd and 4th passage the monolayer consisted almost entirely of epithelioid cells. At the 5th or 6th passage aggregation of the nonadherent microcolonies was observed, so that in time large spheres were formed. The formation of these spheres was evidently due to division and growth of endocrine cells.

By regular change of medium the subcultures could be preserved from passage to passage for 30-45 days without any visible destruction of the cells of the monolayer. During longer keeping of the cultures without passage, nonspecific degeneration of the cells and their separation from the glass were observed. The variability of the number, size, and position of the aldehyde-fuchsine-positive granules in cells of cultures at different passages demonstrated the different functional state of the cell monolayer at the time of their cytologic study. Cells of freely floating colonies also were found to be aldehyde-fuchsine-positive. The results of determination of functional integrity of the β-cells of the subcultures at the level of different passages and at the age of 20-25 days by radioimmunoassay showed that functional activity of the islet cells at all passages was maintained at a high level, and varied from 1000 to 1500 μU/ml.

These results do not agree with those obtained by other workers [6, 9] who describe a gradual decrease in insulin secretion by the islet cells during culture, but they agree with data described by those workers [7, 10] who observed preservation of the hormone-secreting capacity of the isolated islets for 270 days.

It must be mentioned in particular that insulin production was considerably higher in cultures consisting of large multilayer colonies with freely floating spheres of β-cells, and amounted to as much as 2000 μU/ml/day. A high insulin level was maintained until the next routine passage, and with several changes of medium.

The phenomenon of long-term preservation of morphologically and functionally active β-cells by regular subculture was repeated many times over. Shortening the intervals between passages enabled a large number of β-cells of a homogeneous population to be accumulated. For instance, at least a fivefold increase in the number of cells could be obtained from one culture in the course of 1 month. Increasing the intervals from 30 to 45 days enabled the culture to be maintained for 1 year. Long-living cultures lost their functional activity because of bacterial contamination.

Cultures which have been maintained by subculture for about 1 year and have gone through nine passages are currently in existence in the laboratory. During long-term observation and study of cultures of human fetal pancreatic islet cells, under certain conditions of culture high potential for growth and reproduction of β-cells is observed.

The study of this problem demonstrated that a large number of homogeneous, hormonally-active islet cells can be accumulated within a comparatively short time, so that it is possible for them to be typed before transplantation. Ability to maintain functionally active β-cells *in vitro* for a long time by increasing the intervals between passages may provide an approach to the conservation of cells for the creation of banks with a view to future transplantation into diabetic patients.

Subculture of human fetal pancreactic β-cells *in vitro* opens up prospects for the study of the secretory activity of islet cells under conditions of humoral disconnection and the creation of an *in vitro* model of hormonal control.

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