A, B, D CELLS AND A FOURTH CELL TYPE IN LONG-TERM CULTURES OF FETAL RAT PANCREAS

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SUMMARY

Whole pancreases from fetal rats of 13 days and 18 days gestation were explanted onto rayon grids and grown in organ culture. Cultures were fixed in Bouin's fluid, sectioned and stained with the fluorescent antibody techniques for glucagon and insulin, aldehyde fuchsin for B cells, pseudoisocyanin for D cells and a silver technique for the fourth cell type.

The 13-day explants were fixed after 10 days in culture. A, B and D and the fourth cell type were seen, indicating that precursors of all four endocrine cell types must be present in the fetal pancreas shortly after the formation of the pancreatic bud (11 days). Further, the presence of these four cell types in the walls of tubules in these cultures indicates the tubules as the site of origin of all the endocrine tissue.

The 18-day explants were collected every other day of culture from 2 to 30 days in a long-term experiment. A number of large islets with well granulated B cells was still present after 30 days of culture. The relative abundance of cell types at different stages was estimated as follows: 18-day fetal controls, A > B = 4 > D; after 2 to 10 days in culture, B > A > 4 > D; after 18 to 30 days in culture, B > D > A > 4.

Key words: pancreatic islets; islet cell types; organ culture.

INTRODUCTION

There is increasing interest in organ culture of the fetal pancreas as a potential method by which islets of Langerhans intended for transplantation to diabetics could be stored. Nearly all of the work that has been done with cultured fetal islets has concentrated on the B cell because of its importance as the source of insulin, and also because techniques for demonstrating the other cell types were lacking. However, A cells in cultured islets were observed by Orci (1) and by Pictet and Rutter (2) who also saw a fourth cell type. Both of these studies utilized electron microscopy, and it has been our experience that the non-B cell types are often extremely difficult to distinguish by EM in the fetal pancreas.

Recently, we reported histochemical techniques for demonstration of fetal A, B, D cells and a fourth cell type (3). Using these techniques we followed the development of these four cell types from the earliest possible staining in the fetus through gestation and the neonatal period to adulthood (unpublished data). The present work was undertaken to see if it were possible to use these techniques to demonstrate all four islet cell types in culture. Additionally, most of the work done with pancreas in organ culture has involved time periods only up to 12 days (4, 5), 14 days (6), or 15 days in the case of isolated islets (7). In this study we sought to follow the progress of all four islet cell types for culture periods up to 30 days.

MATERIALS AND METHODS

Organ culture. Rats of the Sprague-Dawley strain were used. Pregnancies were timed from the moment of a witnessed mating. Whole pancreases (dorsal and ventral primordia), or sometimes only the dorsal portion, were collected from fetuses of 13 days and 18 days gestation. These explants were placed on rayon grids and cultured using the watch-glass method and a liquid medium (4). No antibiotics were used.

Explants from 13-day fetuses were cultured for 10 days while 18-day explants in culture were collected every other day, starting at 2 days and ending after 30 days of culture. Cultures from eight
separate runs were pooled for this study. Whole litters were collected from twelve pregnant females and explants were taken from 58 fetuses. In addition, control pancreases were collected from four adult and 30 fetal and neonatal rats, representing each in vivo age to correspond with each culture. For instance, if an 18-day explant were cultured for 4 days, control pancreas was collected at 22 days (18 + 4).

**Staining.** All material, including cultures and noncultured control pancreas, was prepared with Bouin’s fixative. Sections from adult controls were also included with each of the stains used. Tissue was embedded in Paraplast and serially sectioned at 4 or 5 μ. Relative numbers of each cell type were estimated at each stage in culture but no attempt was made to actually quantitate them.

Alpha cells were stained with the indirect immunofluorescent technique, using an antiguacagogen serum prepared in sheep (kindly supplied by Dr. Pedro Cuatrecasas of the National Institutes of Health). Suitable controls were included. Sections were deparaffinized and hydrated; then coated with the antiseraum for 1 hr. Then they were given two 10-min rinses in saline and further incubated for 45 min with fluorescein isothiocyanate-conjugated 7S fraction of rabbit antiship IgG (Nutritional Biochemicals Corp.). The sections were rinsed twice more and mounted in saline for observation on a Zeiss photomicroscope II, using an FITC excitation filter-490 cutoff and a K 530 barrier filter with UV illumination and reflected fluorescence.

Beta cells were stained with a similar technique using guinea pig anti-insulin serum ((526,625),(984,995) and an FITC conjugated 7S fraction of rabbit antiguinea pig IgG (Nutritional Biochemicals Corp.). Gomori’s aldehyde fuchsin (8) with pon- ceau de xylidine counterstain was also used.

Delta cells were stained with pseudoisocyanin after acid hydrolysis (9) and examined on the Zeiss UV fluorescence microscope using a BG 3 excitation filter and a K 530 barrier filter and reflected fluorescence. In order to stain D cells in culture it was necessary to leave the sections in pseudoisocyanin overnight, as is the case with fetal material (3). The alcoholic silver nitrate method of Hellerstrom and Hellman (10) was also used for D cells.

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**Figure 1.** Fetal pancreas collected at 13 days of gestation, just 2 days after formation of the pancreatic bud, and cultured for 10 days. a. Islet composed mostly of B cells stained with the fluorescent antibody technique for insulin (X 460). b. Two A cells in a duct wall, stained with the fluorescent antibody technique for glucagon. Dark area is the duct lumen (X 640). c. Fluorescent D cell in the wall of a small duct, stained with pseudoisocyanin (X 640). d. Fourth cell type stained with silver. Several black cells around the periphery of a small islet (X 640).