The use of sodium citrate as diluent (Fig. 3) changes the character of dependence of activity of the suspension on time. Since sodium citrate is a buffer, keeping the pH of the medium constant [2], the action of lactic acid is weakened. As a result of this, activity rises slightly for some time, then falls with a longer "tail".

The suggested method and the apparatus used for it enable activity of a cell suspension due to intrinsic motility of the cells to be estimated, allowing for the relative proportion of motile cells and their average velocity. The simplicity of the technical solution means that the apparatus as designed can be used for automated express monitoring of cell motility in the diagnosis of human infertility and for quality control of the sperm of livestock used for artificial insemination and of bacteria used in the manufacture of fertilizers.

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# FLOATING CULTURES OF FETAL PANCREATIC CELLS

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Cultures of pancreatic islet cells (IC) from human and mammalian fetuses are nowadays widely used in experimental endocrinology and transplantology. Such cultures have begun to be used also in recent years for clinical transplantation into diabetic patients [3, 7, 8, 10, 11, 15]. For this reason, the development of reliable methods of obtaining insulin-producing cultures from the fetal pancreas of man and animals has assumed great importance. Previously [1, 6] the writers described a method of obtaining monolayer focal cultures of IC by enzyme treatment of material with collalytin and trypsin combined with microdissection and differential sedimentation. Monolayer cultures prepared in this way contained many B cells, actively secreting insulin [4, 5]. Cultures of this kind have been used for xenografting into rats with alloxan diabetes [2, 9]. However, a drawback of this method was the loss of large numbers of IC during multistage processing of the material, especially when tissue microfragments were being processed on the magnetic mixer and during centrifugation.

The aim of this investigation was to develop a new, simpler, and more rational method, not using centrifugation to be free from the disadvantage described above, capable of yielding cultures of IC in sufficient numbers for producing a therapeutic effect on clinical transplantation.

## EXPERIMENTAL METHOD

The cadaveric pancreas from human fetuses at the 16th–25th week of intrauterine development (abortions, lower-segment caesarian section) and from pig fetuses at 2.5–3 months of intrauterine life were used as the source of the cell cultures. The pancreas was removed under sterile conditions, washed thoroughly in Hanks' solution with antibiotics, and freed from capsule and large connective-tissue bands containing blood vessels. The pancreas was then cut into fragments measuring 2–3 mm and immersed in 0.2% collalytin solution in Hanks'

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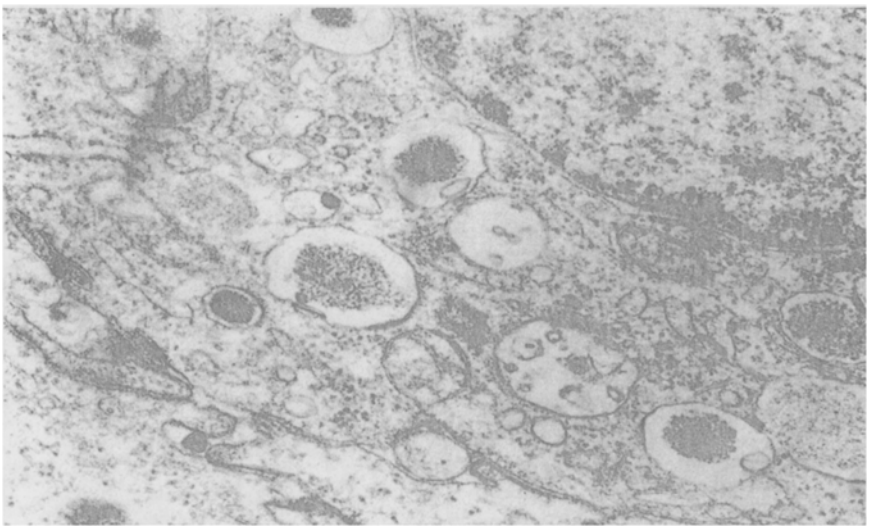

Fig. 1. Floating 5-day cytotypical culture obtained from pancreas of 2.5-month pig fetus. Area of B cell. B granules in different stages of development. 55,000 ×.

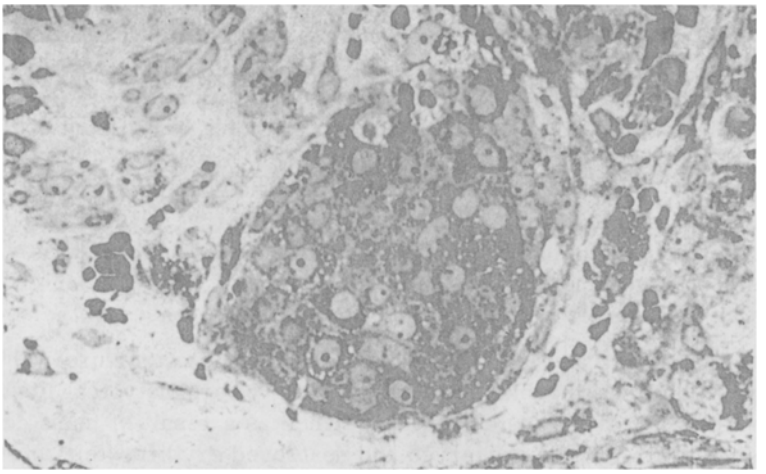

Fig. 2. Floating 5-day organotypical culture obtained from pancreas of 5-month human fetus. Concentration of endocrine cells in thickness of culture. Semithin section. Methylene blue. 1500 ×.

solution at room temperature (20–22°C). The tissue fragments were in contact with collalytin for 10 min. Fragments of pancreas were then washed repeatedly with medium 199 and again thoroughly minced to obtain microfragments measuring 0.1–1.0 mm. The resulting suspension of microfragments was introduced into sterile flasks of different capacity and covered with the corresponding volume of medium 199 containing 10% native bovine serum. The cultures were incubated at 37°C. At different times of culture individual foci of growth of the cultures were fixed with 1% glutaraldehyde in 0.2 M cacodylate buffer, pH 7.2–7.4, postfixed in 1% $OsO_4$ solution, and embedded in Araldite M. Semithin sections were cut to a thickness of 1 $\mu$ from the cultures on the LKB–8800 microtome, stained with methylene blue solution, and studied in the light microscope. Ultrathin sections were stained with uranyl acetate and lead citrate, and studied and photographed in the JEM–100B electron microscope with instrumental magnification of 5000–50,000. The concentrations of insulin and C-peptide in the culture medium were determined by radioimmunoassay.

## EXPERIMENTAL RESULTS

As a result of the treatment of pancreatic tissue described above, a suspension of irregularly shaped microfragments, floating freely in the culture medium, was obtained. In the course of 48–72 h after seeding,

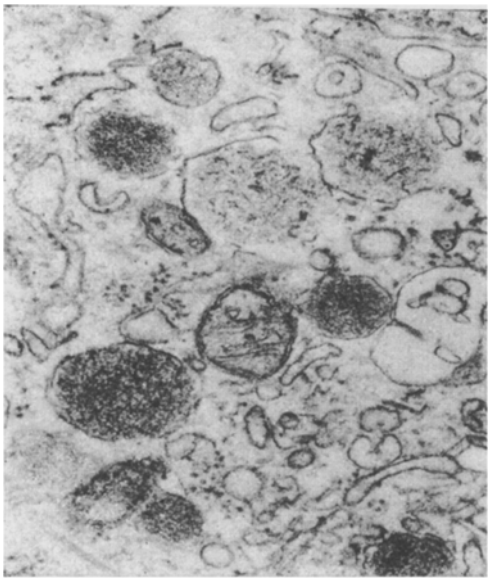

Fig. 3

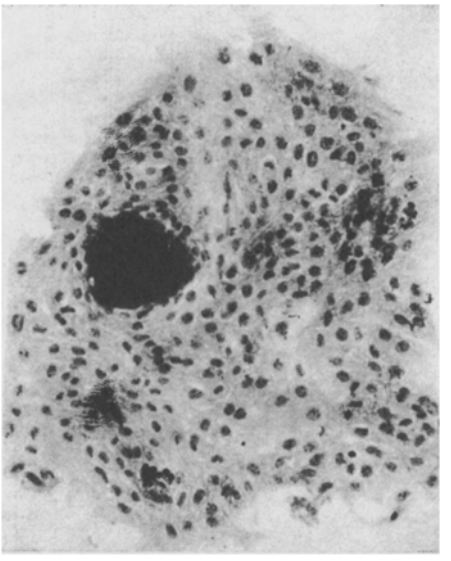

Fig. 4

Fig. 3. Area of cytoplasm of human B cell from organotypical culture (55,000 ×).

Fig. 4. Formation of monolayer zone of growth around focus of adhesion of cytotypical floating culture obtained from pancreas of 5-month human fetus (160 ×).

the microfragments became round in shape and were converted into floating cultures of regular spherical or ovoid shape with a smooth surface (diameter 0.1–1.0 mm). Depending on the size of the pancreas in human and pig fetuses (linked with the age of the fetus), the number of floating cultures in the medium varied within wide limits (from 1000 to 20,000). Even at this stage it was possible to distinguish two types of floating cultures under the light microscope: cytotypical and organotypical (the latter were the majority). Cytotypical floating cultures were compact spherical formations 0.1–0.3 mm in diameter, which consisted entirely of closely packed epithelial polygonal cells containing fine granules in their cytoplasm. Electron-microscopic study of the cytotypical cultures showed that they consist mainly of B cells in various phases of the secretory cycle (Fig. 1).

Floating cultures of organotypical character had a more complex structure. The surface of most of them was covered with a layer of fusiform fibroblasts. The "stroma" of organotypical floating cultures was formed of lysed collagen bundles and coarsely granular debris, formed as a result of mass destruction of acinar exocrine cells. In addition, single fibroblasts, preserved and destroyed erythrocytes, and also endotheliocytes of empty capillaries were sealed into the stroma. The "parenchyma" of the floating cultures consisted mainly of epithelial cells with fine granules in their cytoplasm, in groups of different sizes (Fig. 2). The electron-microscopic investigation showed that these cells were B cells in various phases of the secretion process (Fig. 3). Single groups of epithelial cells with large granules in their cytoplasm, evidently acini in the early stage of destruction, also were found in 48-h cultures.

A characteristic feature of the organotypical floating cultures was that there was no tendency to adhere to the glass. This was probably because they are covered with a layer of fibroblasts which, during growth of the developing culture, become "polarized," and their outer free surface, facing the culture medium, loses its adhesive properties.

Meanwhile individual cytotypical floating cultures, consisting mainly of B cells, preserved their ability to adhere to the substrate (the bottom of the flask, coverslips). In this case, as a study of fixed, stained sections showed, the cytotypical floating cultures became secondarily the source of focal monolayer cultures (Fig. 4): A monolayer zone of growth consisting of flat polygonal cells, spread out over the glass, many of them in a state of mitosis, formed around them. The overwhelming majority of these cells (over 90%) contained aldehyde-fuchsine positive granules in their cytoplasm. These cells, as the electron-microscopic study showed, were B cells.

Floating cultures obtained by the method described above from the human and pig fetal pancreas, were active insulin producers. During continuous culture without change of medium the insulin concentration in

100–ml flasks reached 5000–40,000 μU/ml on the 7th day. After a complete change of growth medium this concentration was re-established in the course of 3–4 days. With frequent complete changes of medium, a high insulin concentration was maintained for 4–5 weeks.

To determine the ability of single organotypical floating cultures to secrete insulin, they were grown in penicillin flasks in 1 ml of growth medium for 7–10 days. One organotypical floating culture secreted from 30 to 60 μU of insulin in this time. The active character of hormone secretion was shown by the presence of C-peptide in the same samples in a concentration of 2–6 ng/ml.

These results must be compared with data in the literature confirming the great importance of organ culture of pancreatic fragments from mammalian and human fetuses for subsequent successful grafting of IC in recipients with diabetes. Not only do partial "spontaneous" removal of acinar cells of the exocrine part of the pancreas and removal of leukocytes contaminating the freshly isolated pancreatic tissue (passenger leukocytes) take place under these circumstances, but the antigenicity of the B cells themselves also is reduced [12–14]. Culture of pancreatic tissue in vitro, as has been shown in recent years, leads to considerable prolongation of the antidiabetic effect (increased duration of survival of transplanted IC) not only in the case of allografting, but also with a xenogeneic combination of donor and recipient [16]. The present writers were able to demonstrate this fact, in particular, in experiments with xenografting of cultures of human fetal pancreatic islet cells into rats with alloxan diabetes [2, 9].

The method of obtaining floating cultures of IC from the fetal pancreas of man and animals, suggested by the present writers, considerably widens the scope for their use in experimental and clinical transplantology.

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