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Long-Term Culture of Isolated Pancreatic Islet Cells (38816)

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(Introduced by Fumio Nakayama)

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There have been several reports on the prolonged culture of islet cells using neonatal or fetal mammalian pancreas (1–4). However, these cultures were prepared from the whole pancreas by enzymatic treatment and thus were most probably composed of mixed populations of both endocrine and exocrine pancreatic cell origin. Anderson *et al.* (5) isolated the pancreatic islets of guinea pigs up to 4-wk old and succeeded in cultivating them for 3 wk. An attempt has been made in our laboratory to cultivate pure pancreatic islet cells with the purpose of studying the regulatory mechanism underlying the endocrine function (6). In the present communication we described procedures used for the successful long-term culture of the isolated islet as well as the dissociated islet cells from young adult rat pancreas with apparently well-preserved endocrine function.

Materials and Methods. Under ether anesthesia, the pancreatic tissue of 6-wk-old WKA rats were removed aseptically after injecting 10 ml of Hanks' Balanced Salt Solution (HBSS) through the common bile duct (7). The tissue was cut with a scissors into the smallest fragments possible, washed three times in HBSS, and treated with collagenase (Worthington, Freehold, NJ). The collagenase solution was prepared by dissolving 35 mg of collagenase in 8 ml of HBSS and sterilized through a membrane filter (pore size 0.45 μm, Sartorius, Göttingen, West Germany). Fetal calf serum was added to the solution, 1:4 v/v, prior to digestion (8). The isolated islets suspended in the medium were picked up manually using a stainless-steel wire loop of approximately 200 μm in diameter under a dissecting microscope. For the culture of isolated pancreatic islets as a whole, two islets were explanted into each well of Microtest II (Falcon Plastics, Los Angeles, CA) trays containing 0.1 ml of the culture medium. For islet cell culture, i.e., culture of cells dissociated from the isolated pancreatic islet, 40 islets collected were treated with a mixture of 200 HUM of crystalline trypsin (Trypsilin, Mochida, Tokyo, Japan) and 0.02% EDTA (Difco Lab., Detroit, MI) in PBS at 37°C for 15 min. The dissociated cells, 10^4 in number, were inoculated into each well with 0.1 ml of the medium. The culture medium consisted of 80% (v/v) modified Eagle's medium and 20% fetal calf serum. The modified Eagle's medium (9) contained 17 amino acids, eight vitamins, 4 mg of hypoxanthine, and 7.2 mg of thymidine per liter. Glucose was added to a final concentration of 1 mg per ml. The culture was incubated at 37°C in a CO_2 incubator, gassed with air containing 5% CO_2, and refed every 4th day. For light and electron microscopy, the cultured islet or islet cells were fixed in 2% glutaraldehyde buffered with 0.075 *M* cacodylate at pH 7.4 for 1 hr, postfixed in 1% cold osmium tetroxide in the same buffer for 1 hr, dehydrated in a series of graded ethanol and propylene oxide, and embedded in epoxy resin. The sections were cut with glass knives on a Porter–Blum microtome. The sections, 2 μm in thickness and stained with toluidine blue, were examined by light microscope. Thin sections were stained with lead tartrate and examined by a JEM-T8 electron microscope. The content of immunoreactive insulin (IRI) in the medium harvested every 4th day was assayed by the double-antibody method (10).

Results. Culture of isolated islets. In the majority of the cultures, the islets were found to be loosely adherent to the bottom of the well throughout the culture period. In a few instances, the proliferation of fibroblasts resulted in an anchoring of the islet to the bottom after several days. No noticeable

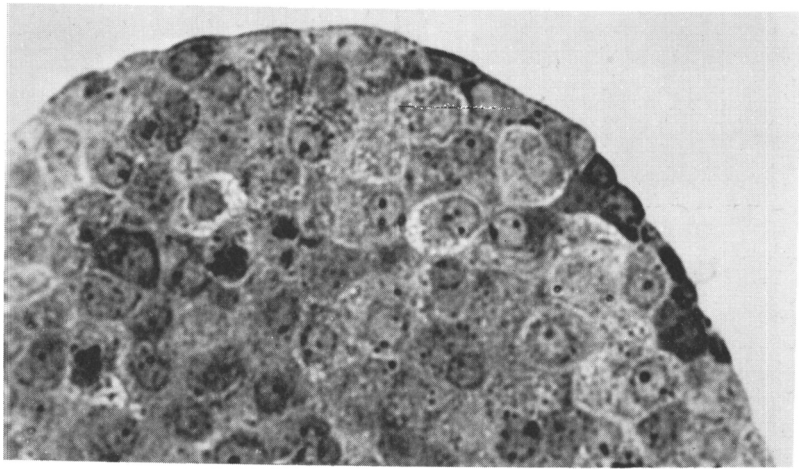

FIG. 1. Thirty-day culture of the isolated islet of a 6-wk-old rat pancreas stained with toluidine blue. Note the good preservation of islet cells (×750).

change in the appearance of the islets was detected during the 75-day culture period when observed under an inverted phase microscope. The sections of the islets taken on the 30th day, stained by toluidine blue, demonstrated excellent preservation of their architecture (Fig. 1). The ultrastructure of B cells at the 30th day was almost identical to that of the intact cells. There were abundant secretory granules and an increased number of lysosomes scattered in the cytoplasm (Fig. 2). The B cells of the islet maintained their function throughout a 75-day period as demonstrated by assay of immunoreactive insulin in the medium (Fig. 3). IRI content of the medium harvested every 4th day ranged from 13,000 to 19,000 μU per ml throughout the culture period.

In one series of experiments, the islets were first exposed to 1 mg per ml of glucose for 20 hr followed by the exposure to 3 mg per ml of glucose for 20 hr starting from the 55th day of culture. Average amount of insulin released into the medium in each 20-hr period was 3,000 μU per ml and 6,400 μU per ml respectively, indicating that cultured B cells were maintaining their insulin-secreting function and were responding to the exposure to the increasing amount of glucose.

Islet cell culture. The rate of single cells per total number of particles, i.e., single cells plus cell aggregates, was 80–90% after the treatment of isolated islets with trypsin–EDTA. The cells were spherical in shape and loosely adhered to the bottom of the well after several days (Fig. 4). They did not spread out to form a monolayer throughout the 44-day culture period. In the majority of the cultures, cell aggregates appeared simulating small pancreatic islets which was noted after a few days. Some gradually increased their size as the culture time progressed. A section of the cell aggregates at 2 wk, stained by toluidine blue, demonstrated almost identical structure to that of the intact islets (Fig. 5). An electron-microscopic study revealed that they consisted of well-preserved A and B cells containing numerous secretory granules in the cytoplasm, although there were more intercellular spaces present toward which cytoplasms of adjacent cells protruded, as compared with the intact islets (Fig. 6). IRI content of the culture medium was 17,000 μU per ml for the 1st week, which gradually decreased to 8,000 μU per ml in the 6th week (Fig. 3).

Discussion. Moskalewski (8) first maintained isolated pancreatic islets from adult guinea pig on plasma clots for about 2 wk. Anderson *et al.* (5) also described the successful cultivation of the isolated pancreatic islet by the use of tissue culture chambers for up to 3 wk. However, no report is yet available on the successful cultivation of isolated pancreatic islets for more than 2 mo with

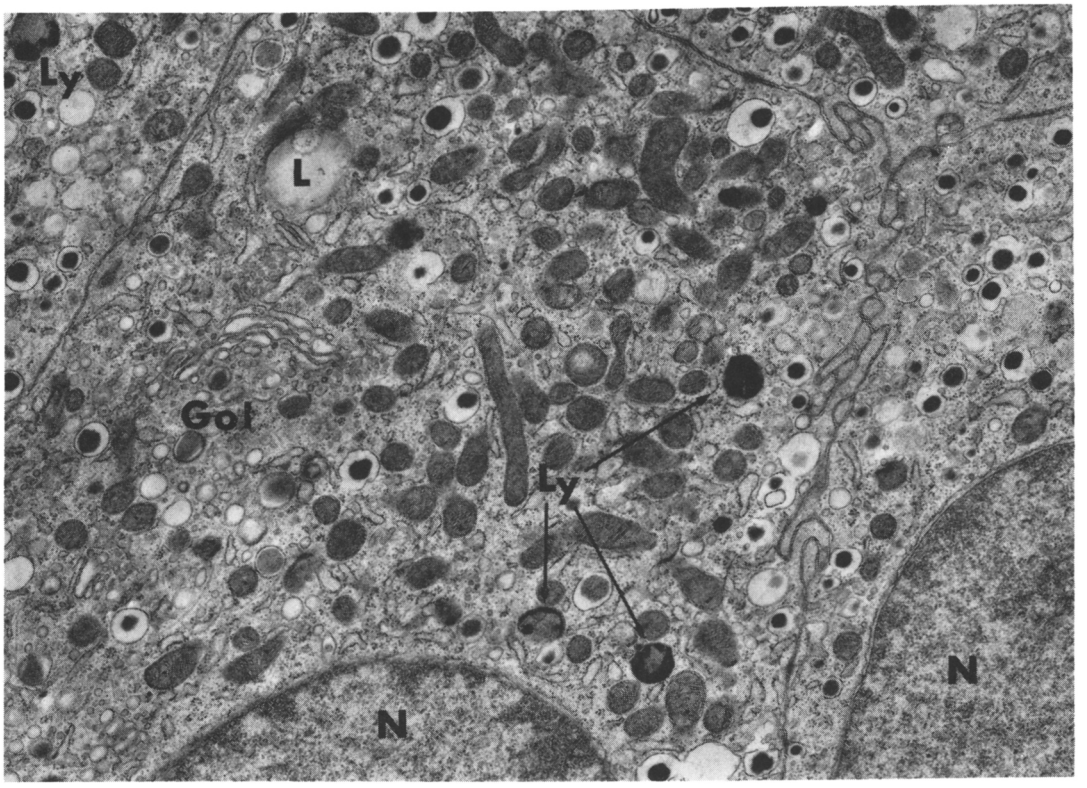

FIG. 2. Electron micrograph of B cells on the 30th day of culture of isolated islets containing abundant secretory granules, mitochondria, and increased numbers of lysosomes. (×12,000). Gol: Golgi complex, L: Lipid, Ly: Lysosome, N: Nucleus.

well-preserved islet cells as demonstrated by electron microscopy and radioimmunoassay of insulin in the medium. Furthermore, this is the first report on the prolonged culture of the dissociated pure islet cells which were inoculated quantitatively by counting the cells. Trypsin–EDTA digestion of the islets indicated that the pancreatic islets of a 6-wk-old rat were composed of approximately 1.2–1.5 × 10^3 cells. The importance of conditioned medium not only for cellular differentiation (11) but also for cell survival and multiplication (12) in culture cannot be overemphasized at the present state of the improvement in medium formulation. For the purpose of cultivating only two islets, composed of 2.4–3.0 × 10^3 cells, or 10^4 dissociated islet cells *in vitro*, Microtest II trays provide the very small amount of medium that can be easily conditioned.

In the culture of isolated islets, the histo-

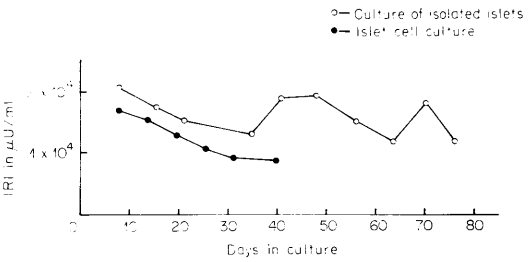

FIG. 3. IRI in the medium assayed every 4th day. ○——○, culture of isolated islets, ●——●, islet cell culture.

logical integrity of pancreatic islets was well retained throughout the 75-day culture period with vigorous secretion of insulin into the medium. Good response of the cultured islets to the exposure to glucose in secreting increasing amount of insulin is in accord with the well-preserved ultrastructure of the islets

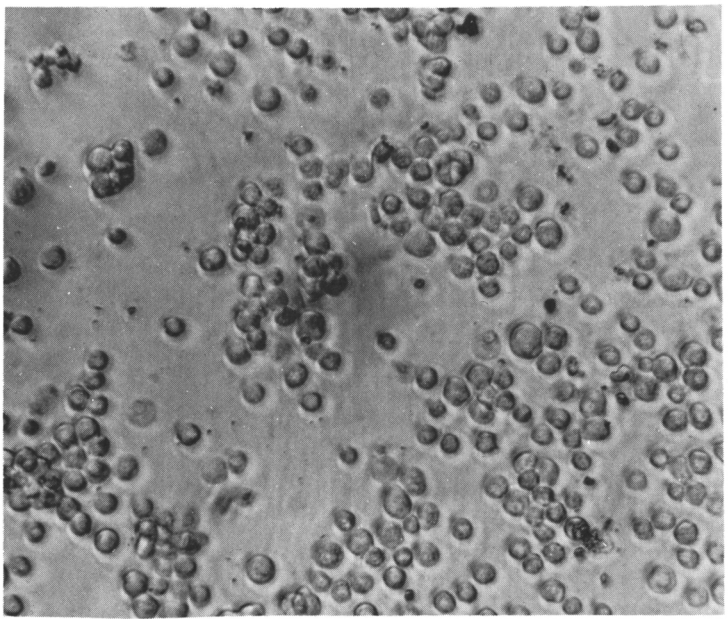

FIG. 4. Phase-contrast micrograph of the islet cells dissociated by trypsin–EDTA treatment on the 4th day of culture (×100).

observed on 30th day, showing perfect preservation of the morphology of B cells. In islet cell culture, the pancreatic tissue of young adult rats was first digested with collagenase and subsequently treated with trypsin–EDTA until the complete dissociation of islet cells was attained. Thus, a definite number of cells could be inoculated, if desired, into the well by cell counting. Hence, the present culture technique permits quantitative evaluation of B cell function.

Aggregation of dissociated mammalian cells was well documented by Moscona (13). However, to our knowledge, the phenomenon of self-aggregation was observed only when the embryonal cells were dispersed in a liquid medium. Hilwig *et al.* (1) described the self-aggregation of pancreatic cells from a human fetus in monolayer culture in which the epithelial cells gave rise to aggregates in which A and B cells could be recognized. In the present culture system, the dissociated cells obtained from isolated islets of young adult rat pancreas became aggregated to form so-called pseudoislets. With respect to the increment of aggregate in size, two possibilities exist, i.e., cell proliferation on the one hand and accretion and merger of islet cells on the other. The gradual decrease in number of dissociated cells with concomitant emergence of aggregates with time favors the latter possibility, although participation of the proliferation of islet cells may well occur.

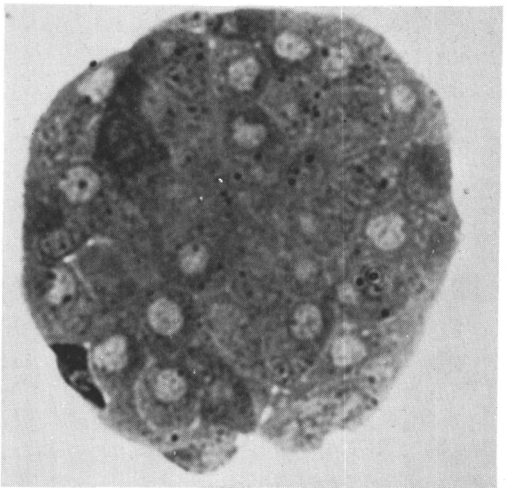

FIG. 5. Two-micron section of "pseudoislet" on the 14th day of islet cell culture stained with toluidine blue. Note the similarity of its structure to that of the intact islet.

Summary. Successful long-term culture of

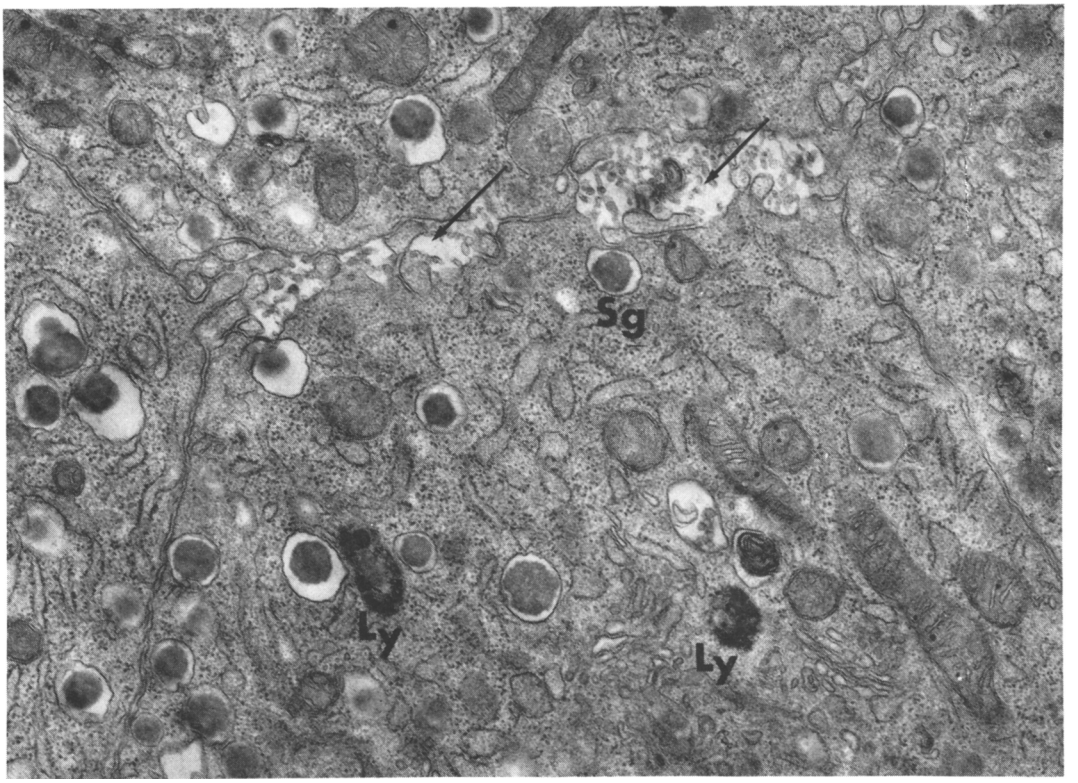

FIG. 6. Electron micrograph of B cells in a "pseudoislet" on the 14th day of islet cell culture. Note wider intercellular spaces (arrows) in the "pseudoislet" as compared with the intact islet (×25,000). Ly: Lysosome, Sg: Secretory granule.

isolated pancreatic islet as well as dissociated islet cells was accomplished with the use of Microtest II trays which provides the very small amount of culture medium that is easily conditioned. In the culture of isolated islet, the ultrastructure of the islet cells was completely preserved up to the 30th day and vigorous secretion of insulin was maintained even on the 75th day. While in the islet cell culture, insulin secretion gradually decreased from the 12th day. The latter procedure makes inoculation of a definite number of dissociated pure islet cells possible, thus opening the way to the quantitative evaluation of B cell function *in vitro*.

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