MICROFILM STUDY OF CULTURES OF ISLET CELLS OF THE BOVINE FETAL PANCREAS

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UDC 612.64'349.7

The dynamics of growth of a culture of bovine fetal pancreatic islet cells was studied by time-lapse microfilming. The monolayer cultures studied were purely epithelial in character. Under the influence of an increased glucose concentration in the growth medium (up to 300 mg %) the mitochondrial system of the islet cells was activated, and cytogranulokinesis and the accumulation of secretory granules were intensified. Later degranulation of the islet cells was observed. The results were compared with those of the control cytological investigation of fixed and stained cultures.

KEY WORDS: microfilming; cell cultures; islet cells.

Details of the possible preparation of monolayer cell cultures from the bovine fetal pancreas with the aid of collalytin – a substance with collagenase action, have recently been published [1, 2]. Cell complexes settling in the course of 20 min to the bottom of culture flasks have been shown to contain up to 60-70% of epithelioid cells with (aldehyde–fuchsin)-positive granules in their cytoplasm. Under the influence of an increased glucose concentration in the medium (300 mg %) degranulation of these cells was observed after 24 h. It was concluded that the cells described correspond in their morphological and functional characteristics to the B cells of the islets of Langerhans.

One of the most important factors controlling the level of secretion in the B cells of the pancreatic islets is a change in the glucose concentration in the blood plasma (in vivo) or in the nutrient medium (in vitro) [3-9]. It was accordingly interesting to study cultures of islet cells in gross medium containing a normal and increased glucose concentration, by means of a method capable of revealing details of the dynamics of the secretory process that cannot be recorded by the ordinary microscopic study of "static" preparations of fixed and stained cultures. Time-lapse cinemicrography is one such method.

This paper describes the results of a microfilming study of monolayer cultures of bovine fetal pancreatic islet cells when cultured in the presence of a normal (100 mg %) and an increased (300 mg %) glucose concentration.

EXPERIMENTAL METHOD

Cell cultures were obtained from the pancreas of bovine fetuses (4th month of intrauterine development). Fragments of the pancreas measuring 1-2 mm were treated with a mixture of equal volumes of a 0.3% solution of collalytin and a 0.25% solution of trypsin [1]. In the course of 15-20 min the tissue suspension was disturbed on a magnetic mixer at room temperature (20-22°C), centrifuged at 8000 rpm for 10 min, and resuspended in medium 199 with 10% bovine serum. The suspension was introduced into microfilming chambers (LOMO) with specially mounted coverslips. After the cells had settled for 20 min the supernatant with cells remaining suspended was carefully removed and fresh growth medium of the same composition was added to the chamber. The microfilming began after 24-36 h – during which time adhesion of most cells was completed. Time-lapse cinemicrography was carried out under phase contrast by means of the KSR-1 motion picture camera, connected to the MBB-1 microscope, on KN-3 film. The magnifications used were: objective 20×, objective 90× (oil immersion), and ocular 5×, homal. The intervals between the frames were 15 sec, so that by projection

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0007-4888/78/8502-0204$07.50

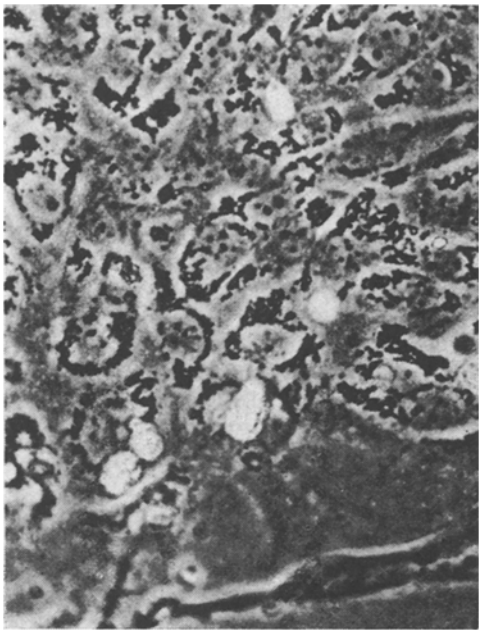

Fig. 1. Monolayer culture of pancreatic islet cells of bovine fetus. Peripheral part of zone of growth (right), 72 h in culture. Magnification 100×, phase contrast (frame from microfilm).

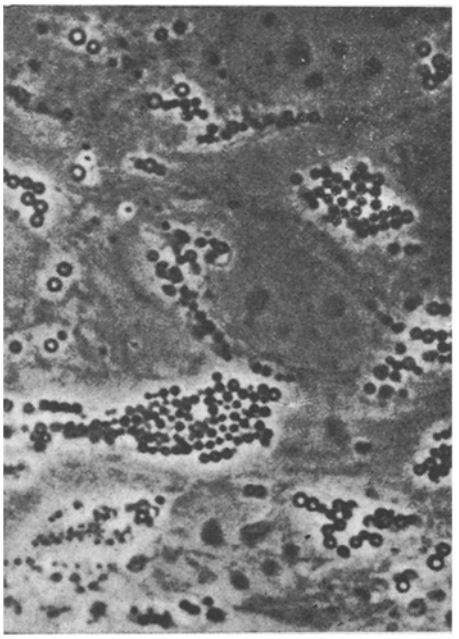

Fig. 2

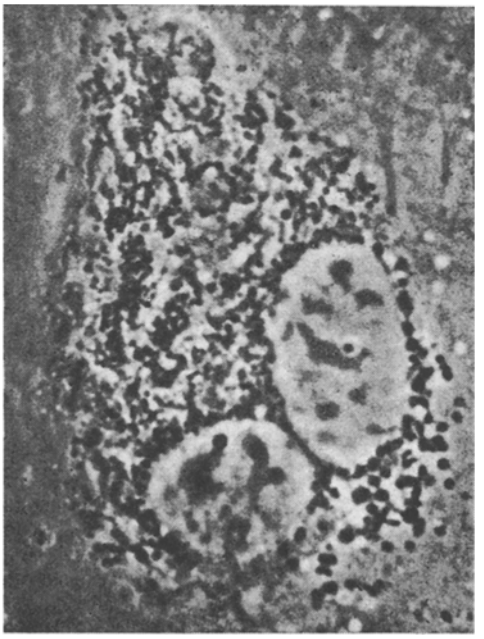

Fig. 3

Fig. 2. Monolayer culture of pancreatic islet cells of bovine fetus. Region of culture growing in normal glucose concentration (100 mg %). Many lipid droplets present in cytoplasm of epithelial cells. Here and in Fig. 3: magnification 450×. Phase contrast (frame from microfilm).

Fig. 3. Region of culture 1 h after increase in glucose concentration in growth medium to 300 mg %. Activation of mitochondrial apparatus, increased accumulation of secretory granules in cytoplasm of binuclear epithelial cell.

of the film the process could be speeded up 360 times. A control cytological study was made of cultures of islet cells grown on cover slips in penicillin flasks, fixed with 96° ethanol at the corresponding times and under the same experimental conditions. The preparations were stained with hematoxylin–eosin and aldehyde–fuchsin.

EXPERIMENTAL RESULTS

The microfilming data showed that by 36 h after seeding of the tissue suspension islets of cells measuring up to 1 mm had formed. Their central part appeared stratified and refracted light strongly. The middle and peripheral parts of the islets consisted of a single layer of polygonal cells of epithelial character with pale, spherical nuclei containing 1 to 3 nucleoli. Rotational activity of the nuclei was low. By 72 h (Fig. 1) the middle and peripheral parts of the culture had grown considerably in extent both by centrifugal migration of the epithelial cells and as a result of their continued spreading. In the zone of growth only a few mitoses could be observed. In the cytoplasm of the epithelial cells rod- and thread-like mitochondria and a few perinuclear dark secretory granules were detected. Cells located at the periphery were larger than the rest (50-80 μ) and sometimes had a triangular or irregularly branched shape. By their arrangement and shape these cells corresponded to the "special" or fibroblast-like islet cells described previously in the literature [1]. Their perinuclear granules were more numerous. A wide ectoplasmic homogeneous zone, bounded by a mobile undulating membrane, could be distinguished in these cells. In all the islet cells many lipid droplets could be observed in the zone of growth; in some cells they masked the mitochondria and secretory granules. Mitochondria, lipid droplets, and secretory granules in the epilethial cells were highly mobile and moved haphazardly about the cytoplasm (cytogranulokinesis).

After a longer period of microfilming (4-5 days) further widening of the zone of growth of the culture was observed; the epithelial character of the monolayer remained completely undisturbed, whereas differences between the size of the epithelial cells had increased. In the largest cells, located nearer to the periphery, the lipid droplets had become large but their total number was reduced (Fig. 2). As before the secretory granules were distributed mainly in the perinuclear region of the cytoplasm. The edge of the zone of growth became increasingly homogeneous and sharply outlined. Active proliferation of fibroblasts was not observed close to the epithelial islets.

On the 5th day after seeding the cells glucose was added to the culture medium to give a final concentration of 300 mg %. Substantial changes were observed in a high proportion of epithelial cells after only 15-30 min. The cytogranulokinesis in the cells became more pronounced and the mobility of the mitochondria increased. During the 4-5 h after addition of glucose the number of mitochondria increased and most mitochondria were thread-like in shape. The number of secretory granules accumulating between the mitochondria rose particularly sharply (Fig. 3). The intensification of this process continued for about 12 h, after which the number of secretory granules fell gradually and the cytoplasm of the epithelial cells became more homogeneous. Large lipid drops remained in the cytoplasm.

Control cytological investigation of preparation of cultures of islet cells grown on coverslips in penicillin flasks showed that during the first 4-5 h after a change from medium with the normal glucose concentration (100 mg %) to medium with an increased glucose concentration (300 mg %) the number of epithelioid cells with (aldehyde–fuchsin)-positive granules in their cytoplasm increased considerably. However, after 20-24 h complete degranulation of the islet cells was observed, just as in the case described previously [1]. Meanwhile, large quantities of lipids still remained in the degranulated islet cells and, for that reason, after treatment of the preparations with alcohol and xylol, the region of the lipid droplets appeared to consist of large empty spaces.

The results of the microfilming study of the behavior of the pancreatic islet cells of bovine fetuses when grown in normal and increased concentrations of glucose in the culture medium not only confirm the earlier hypothesis that these cultures contain large numbers of B cells, but also give a clearer idea of the structural dynamics of the secretory process effected by the B cells in response to the stimulating action of glucose.

LITERATURE CITED

1. R. A. Babikova, V. N. Blyumkin, B. I. Shal'nev, et al., Byull. Éksp. Biol. Med., No. 3, 350 (1977).
2. V. I. Shumakov, V. N. Blyumkin, R. A. Babikova, B. I. Shal'nev, et al., in: Collected Transactions of the Institute of Transplantation of Organs and Tissues, Ministry of Health of the USSR [in Russian], Moscow (1977), pp. 63-67.
3. S. J. Ashcroft, J. M. Bassett, and P. J. Randle, Lancet, 1, 888 (1971).

4. W. L. Chick, V. Lauris, J. H. Flewelling, et al., Endocrinology, 92, 212 (1973).
5. W. L. Chick, A. A. Like, and V. Lauris, Science, 187, 847 (1975).
6. C. Hellerström, A. Andersson, and R. Gunnarsson, Acta Endocrinol. (Copenhagen), 83, Suppl. 205, 145 (1976).
7. J. Hilwig, S. Schuster, W. Heptner, et al., Z. Zellforsch., 90, 336 (1968).
8. A. E. Lambert, B. Blondel, Y. Kanazawa, et al., Endocrinology, 90, 239 (1972).
9. R. G. Larkins, Endocrinology, 93, 1052 (1973).

MECHANISMS OF REVERSIBILITY OF THE STATHMOKINETIC REACTION INDUCED BY SUBOPTIMAL TEMPERATURES

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UDC 612.014.3:612.6].014.43

Autoradiographic studies using [^{3}H]leucine showed that the reversibility of the stathmokinetic reaction induced by a suboptimal temperature (21°C) does not require additional protein synthesis and, consequently, is not connected with the formation of new microtubules. Normalization of the mitotic regime was delayed in the presence of copper ions, which prevent polymerization of the microtubules. These data suggested that repolymerization of subunits of microtubules is the principal method of restoring mitosis after exposure to a suboptimal temperature.

KEY WORDS: mitosis; stathmokinetic reaction; suboptimal temperature.

Much evidence has been obtained to show that the stathmokinetic reaction, which can be induced by various factors such as a high hydrostatic pressure, a low temperature, and the action of antitubulins, is a reversible process. The mechanisms of restoration of the normal course of mitosis are determined by the character and degree of injury to the microtubules forming the division spindle. The reversibility of the stathmokinetic reaction may arise in two ways: through additional protein synthesis and the formation of new microtubules after treatment with colchicine [1] or repolymerization of subunits after the action of colcemid [2]. These differences in the mechanisms of reversibility of the reaction are explained by differences in the degree of binding of the subunits of the reserves by the alkaloids [5]. When the temperature falls, causing delay of division in the metaphase stage [3, 6], by contrast with the action of alkaloids of the colchicine series, the reserves are not dissipated. Despite the fact that the rapid recovery of mitotic and cytoplasmic microtubules destroyed by cooling has been described in several papers [8, 9, 12], the mechanisms responsible for reversibility of the effect of a low temperature have received little study. In this investigation an attempt was made to elucidate some of the mechanisms which lie at the basis of restoration of the normal course of mitosis after exposure to a suboptimal temperature.

EXPERIMENTAL METHOD

Experiments were carried out on a monolayer culture of Chinese hamster fibroblast-like cells, clone 237. The cells were seeded in penicillin flasks with a density of about 150,000 cells/ml. The stathmokinetic reaction was induced after culture for 24 h by cooling the cells for 2 h to 21°C. To test the possibility of formation of new microtubules, the dynamics of protein synthesis was determined by autoradiography during the period of development and reversibility of the stathmokinetic reaction. [^{3}H]Leucine, in a dose of 10 μCi/ml (specific activity 2.5-10 mCi/mmole), was added to the culture medium for 10 min before cooling (control), after cooling, and every 10 min after the cultures had been returned to optimal temperature conditions (37°C). The material was processed by the usual methods for autoradiography. The level of protein synthesis was judged from the number of tracks above the metaphase cell. To study the role of repolymerization in the reversibility of the stathmokinetic reaction, the effect of copper ions, which prevent polymerization of micro-

Laboratory of Cytology, Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 2, pp. 205-207, February, 1978. Original article submitted July 13, 1977.

0007-4888/78/8502-0207$07.50