The formation of colonies by blood cells, settling in the embryonic lung, can be ruled out by data showing that cell suspensions of the embryonic heart of CBA mice at the same times of development, if injected into irradiated recipients, virtually never form colonies [1].

The following conclusions can be drawn from the results: 1) hematopoietic stem cells migrate in vivo into the embryonic lung, just as also into the liver and thymus; 2) the colony-forming ability of these cells diminishes with an increase in the period of embryogenesis of the lung; 3) stromal elements of the spleen of the irradiated recipient facilitate realization of the colony-forming potential of hematopoietic stem cells in the lungs and determine the pathway of their differentiation. All these finding's suggest that the lung in vivo is colonized by hematopoietic stem cells in the early stage of embryogenesis of the mouse (evidently before the 14th day of development).

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ISLET-CELL AGGREGATES: STRUCTURE AND INSULIN-PRODUCING ACTIVITY DURING IN VITRO CULTURE

V. G. Ablamunits, V. N. Blyumkin, G. M. Mogilevskii, L. A. Kirsanova, and F. S. Baranova

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With rapid developments in the subject of free transplantation of the pancreatic islet-cells (PIC) in diabetes, particular importance is attached to research aimed at obtaining cultures with high insulin-producing activity, and purified from cells carrying class II histocompatibility antigens — so-called passenger leukocytes. Different approaches have been used to solve this problem: isolation of islets followed by their culture in vitro at 24-26°C or in contact with the gaseous phase, containing 95% of oxygen, the preparation of monolayer cultures with a high PIC content and of floating cultures of organotypical character from fetal pancreas [1-4]. There have also been isolated reports of the possibility of using "neoislets," formed by aggregation of PIC during roller-tube culture [5].

The aim of this investigation was to study the possibility of obtaining neoislets during culture of rat PIC in an ordinary stationary system, and to the investigation of their structure and insulin-producing activity.

Research Institute of Transplantology and Artificial Organs, Ministry of Health of the USSR, Moscow.

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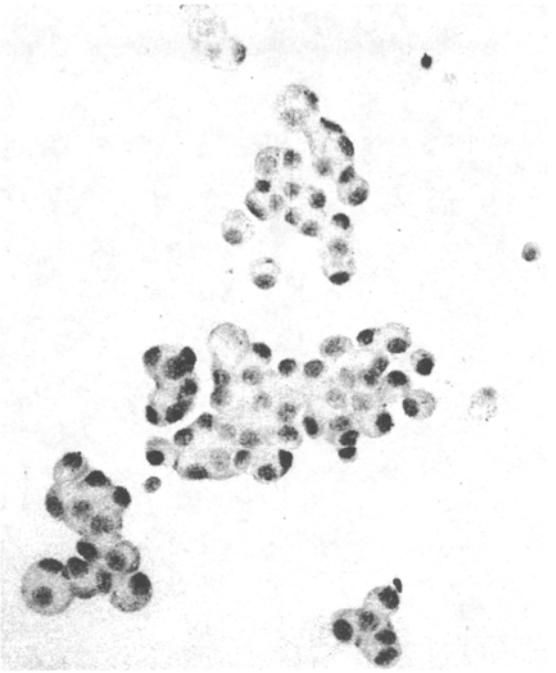

Fig. 1. Epithelial structure of aggregates after culture for 24 h. 200×. Hematoxylin.

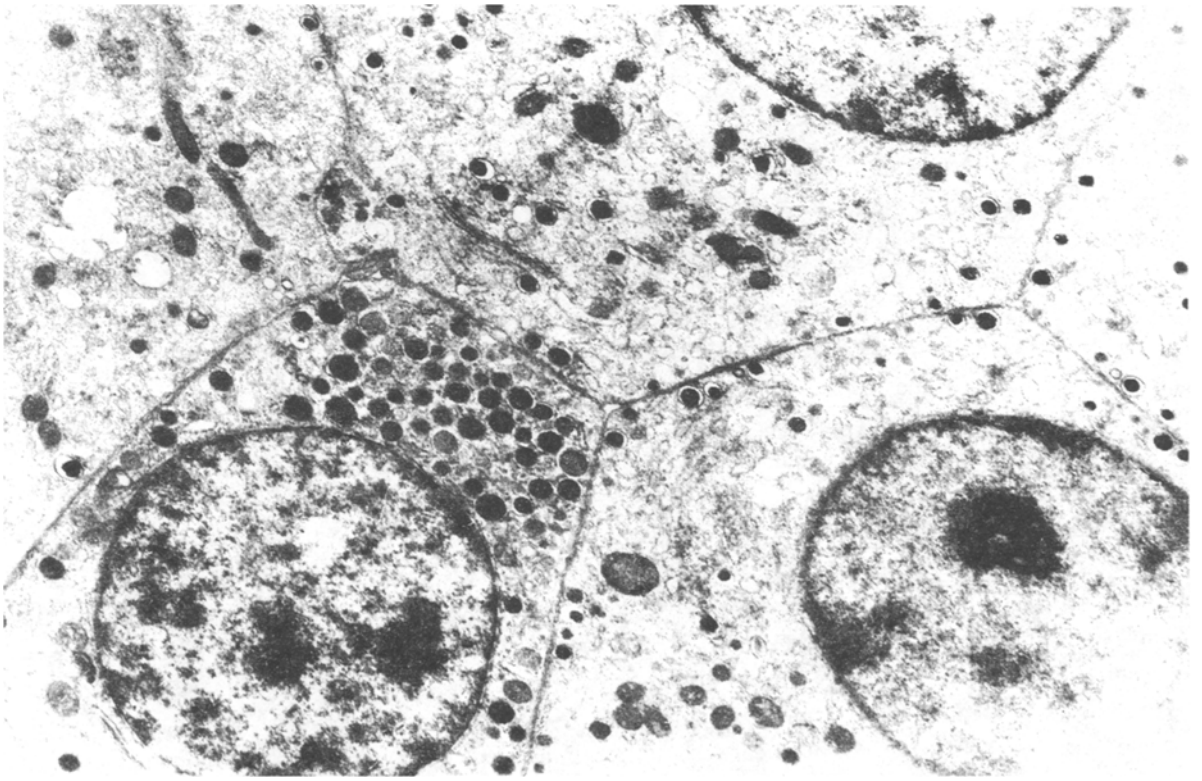

Fig. 2. Ultrastructure of a neoislet, close contact between cells. 10,000×.

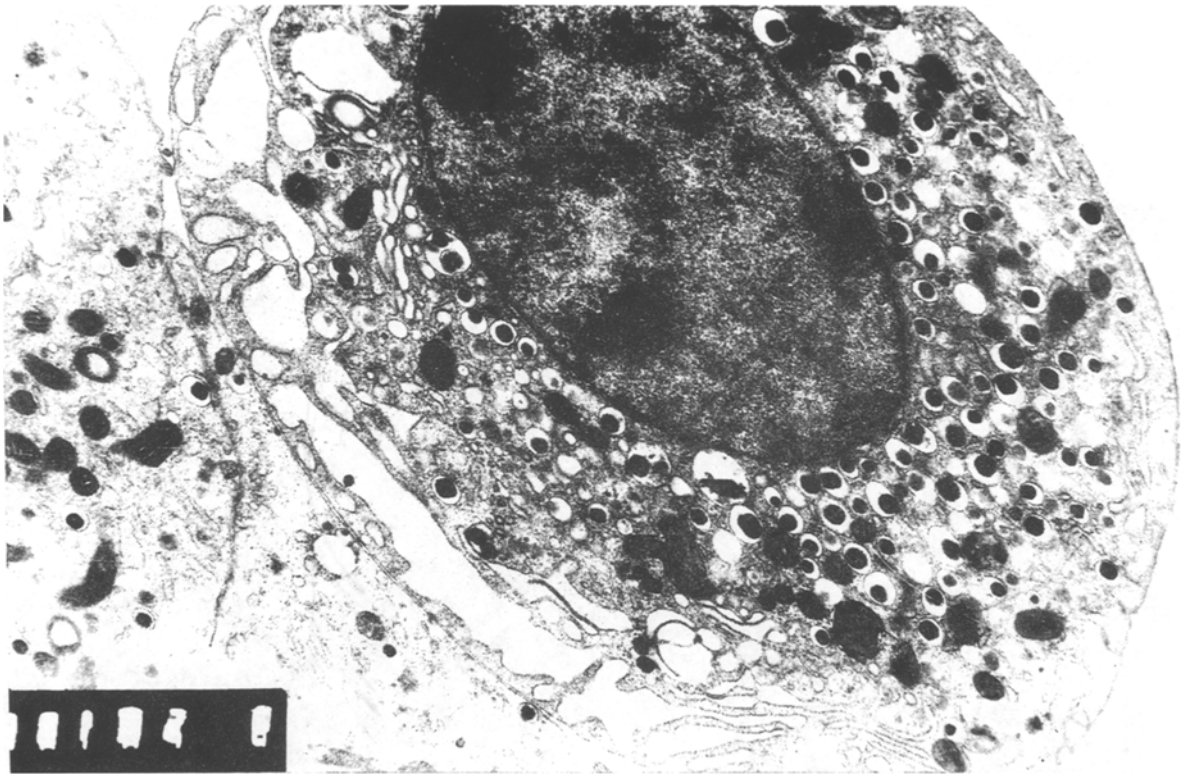

Fig. 3. β-Granules at various stages of maturation. Mature granule in lumen of cistern of endoplasmic reticulum. 18,000×.

EXPERIMENTAL METHOD

Male Wistar rats weighing 180-220 g were used. PIC were isolated by the method in [6]. After anesthesia, by intraperitoneal injection of sodium hydroxybutyrate (20%, 2 ml), the common bile duct was clamped at the point where it empties into the duodenum, and the aorta and inferior vena cava were divided. Cold (4°C) Hanks' solution (20 ml), containing collagenase ("Serva," West Germany, 20 mg, activity 150 U/mg) and HEPES (10 mM, pH 7.2) was injected into the common bile duct. The pancreas, distended by this solution, was removed and placed on a water bath (37°C). After 40 min digestion was stopped by the addition of 15 ml of cold Hanks' solution. The tissue was then vigorously shaken, filtered through Kapron gauze with pore diameter of 0.6-0.8 g, and washed 3 times with Hanks' solution during centrifugation (100 g, 10 sec). The tissue suspension was centrifuged in a four-step Ficoll—Paque density gradient ("Pharmacia Fine Chemicals," Sweden): 1.078, 1.070, 1.060, 1.045 (800g, 5 min). Islets were harvested from the boundary between the two middle layers, washed during centrifugation (100g, 10 sec) twice with Hanks' solution and once with phosphate-buffered physiological saline (PM 16, "Serva"). The islets were then placed successively in 0.02% EDTA solution (5 ml) for 5 min at room temperature and in 0.25% trypsin solution (4 ml) for 15 min at 37°C, after which they were carefully pipeted anti I conversion of the islets into a homogenous cell suspension. The cells were then washed with Eagle's medium (MEM), containing a double set of amino acids and vitamins, 10% fetal calf serum, L-glutamine (2 mM), gentamicin sulfate (80 g/liter), and HEPES (25 mM). The cells were counted in a Goryaev's counting chamber and their concentration adjusted to $5 \cdot 10^4$ in 1 ml. A suspension containing not less than 90% of living cells, as shown by staining with trypan blue, was used in the experiments. Samples of 0.2 ml of suspension (10^4 cells) were placed in wells of a 96-well flat-bottomed planchet ("Costar," USA) and cultured at 37°C in an incubator in an atmosphere containing 5% CO_2. For study under the light microscope, material cultured for different periods of time was removed from the wells, placed on a slide, dried, fixed with methanol, and stained with hematoxylin. Material for electron-microscopic investigation was fixed in 3.5% glutaraldehyde solution, postfixed with 1% OsO_4 solution, dehydrated in alcohols of increasing concentration, and embedded in Epon. Ultrathin sections were studied in the LEM 100B electron microscope. The general trend of the intravital changes was monitored by means of an inverted microscope. To study the insulin-producing function of the β-cells the cultures were placed in

MEM medium containing 5.5 mM glucose or 16.5 mM glucose and 5 mM theophylline [7]. The insulin concentration in the medium was determined by radioimmunoassay using a RIO-^{125}I-ins-M kit.

EXPERIMENTAL RESULTS

From one rat pancreas we obtained $5 \cdot 10^5$-$1 \cdot 10^6$ islet cells. Intravital observations in the inverted microscope and the study of fixed preparations stained with hematoxyl in showed that during the first 3 h of culture mass aggregation of PIC did not take place. This process increased appreciably in intensity 5-6 h after introduction of the cell suspension into the wells. Aggregates consisting of two to four cells predominated in the early stages. By 24 h, chain-like and folded aggregates, containing up to 100 cells, were formed (Fig. 1); after 3-4 h larger aggregates, round in shape with an epithelial structure, resembling the original PIC, also were found. Their diameter reached 200 μ. Cells of the central part of the neoislet were polygonal in shape and were closely packed together. The free surface of the cells facing the culture medium had arc-like outlines. The neoislets were completely free from stroma and blood vessels.

Electron-microscopic study of the neoislets showed that they consisted purely of endocrine cells, most of which were β-cells. They were in close contact with one another, and junctions were observed between neighboring cells in the form of desmosomes (Fig. 2). The nuclei of the β-cells were round or oval in shape and contained one to three nucleoli and finely dispersed chromatin. The cytoplasm of the β-cells was of average electron density, and ribosomes and polysomes were located in different parts of it. The mitochondria were elongated, with clearly visible cristae and with a matrix of average electron density. A well-developed Golgi complex and also the rough endoplasmic reticulum, forming wide cisterns in places, were noted. Secretory granules typical of β-cells at different stages of development could be seen in the cytoplasm, including mature granules with the characteristic electron-dense core and a peripheral electron-translucent zone (halo) (Fig. 3). Single mature granules could be seen in the lumen of the cisterns and in the narrow intercellular spaces. Judging from the electron micrographs, the secretion process is heterochronous in different β-cells. Besides β-cells in the composition of aggregates, several single a-cells were found. Exocrine (acinar) cells and cells of mesenchymal genesis (fibroblasts, endothel iocytes, passenger leukocytes) were not found in the composition of the neoislets.

In medium containing 5.5 mM glucose, aggregates of PIC secreted 15.2 $\pm$ 3.2 μU of insulin per hour per 10^4 cells. In response to stimulation by glucose (16.5 mM) and theophylline (5 mM) the level of insulin secretion increased to 49.6 $\pm$ 3.4 μU/h per 10^4 cells (results of a separate experiment, similar results were obtained in other experiments).

To explain the appearance of aggregates consisting entirely of PIC, the to following facts must be taken into account. Islets freed beforehand from acinar cells by centrifugation in a density gradient were subjected to trypsinization. As a result, the overwhelming majority of cells in the monodisperse suspension were PIC, and only a negligible proportion (in accordance with the original structure of the islets) consisted of cells of mesenchymal genesis. Moscona [8] showed that in roller-cell cultures cells of the same embryonic origin recognize each other and reassociate. This property is shared by PIC of adult rats [5, 9].

The results of this investigation described above are convincing evidence that reassociation of PIC also is possible when ordinary stationary cultures are used. Aggregates formed under these circumstances (neoislets) consist mainly of β-cells, they actively secrete insulin, and respond to stimulation by glucose and by theophylline. Since neoislets do not contain passenger leukocytes, they are particularly interesting for experimental and clinical transplantology.

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