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FORMATION OF THE ENDOCRINE FUNCTION OF β-CELLS OF THE ISLETS OF LANGERHANS IN CULTURE

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It has now been shown that β-cells of monolayer cultures of the islets of Langerhans preserve their morphological structure and insulin-producing properties for a definite time [5, 6, 9], although the dynamics of formation of their endocrine function in culture has not yet been adequately studied. The writers showed previously that definite stages appear in the development of cell cultures of endocrine glands [2].

The object of this investigation was to study the connection between biosynthesis and secretion of insulin by β-cells and the stage of development of the culture.

EXPERIMENTAL METHOD

Cultures were obtained from the pancreas of 19-day-old guinea pigs and 3-3.5-month pig fetuses. The minced pancreatic tissue was distintegrated on a magnetic mixer in a solution consisting of equal parts of a 0.3% solution of collalitin and a mixture of equal volumes of 0.3% trypsin solution and 0.02% versene solution at 25-27°C. The detached cells were covered with cold nutrient medium, washed twice in it, and centrifuged for 10-15 min at 800 rpm. The cell residue was suspended in growth medium consisting of equal volumes of medium No. 199 and 0.5% lactalbumin hydrolysate solution, 20% bovine serum, 100 mg% glucose, and 100 i.u./ml penicillin. The cell suspension, enriched with conglomerates of islet cells [1], was poured into test tubes with mica disks measuring 0.5-1.5 mm. The medium with unattached cells was removed after 24 h and subsequently changed at intervals of 2 days. The cells were labeled with ^{3}H-thymidine (1 μCi/ml, 1 h; specific activity 96 μCi/mmole), ^{3}H-uridine (1 μCi/ml, 0.5 h; specific activity 87 μCi/mmole), and ^{3}H-leucine (20 μCi/ml, 1 h; specific activity 79 μCi/mmole), which were added to the tubes 24 h after the medium was changed. After the end of incubation the preparations were washed in physiological saline, fixed, and dehydrated in 80 and 96% ethanol, glued to slides, dried, and washed in cold 3% $HClO_4$ for 10 min. Autoradiographs were prepared with type R (Photographic Chemical Research Institute) liquid photographic emulsion. Exposure lasted 10 days at −4°C. After development, the preparations were stained with aldehyde-fuchsin. Labeled β-cells were analyzed by the MBI-3 microscope (ocular 10 ×, objective 90 ×). The labeling index for ^{3}H-thymidine or ^{3}H-leucine was determined after examination of 1000 cells in 2-3 preparations in each layer. To analyze the intensity of incorporation of ^{3}H-uridine and ^{3}H-leucine, granules of reduced silver in the emulsion were counted above nuclei and cytoplasm of 500 β-cells. The concentration of insulin secreted into the culture medium was determined by a radioimmunologic method, using kits from Cea-Ire-Sorin (Italy). Student's and Fisher's tests were used for statistical analysis [3].

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TABLE 1. Distribution of β-Cells (in % of number tested) by Intensity of Incorporation of ^{3}H-Uridine at Different Stages of Growth of Monolayer Cultures of Islets of Langerhans (M ± m)

Day of culture	n	Number of grains of silver above nucleus				
		5—15	16—25	26—35	36—45	46—55
2	4	80±4,8*	17±0,9	4±1,3	—	—
	4	72±5,1**	20±2,1	8±2,7	—	—
5		9±3,5	31±2,2	32±2,9	27±2,4	10±1,6
		3±1,8	29±2,8	42±2,8	30±3,2	14±2,2
15		50±5,8	20±2,4	15±3,4	5±1,8	—
		43±3,6	15±29	20±3,6	7±2,1	

Legend. Here and in Table 2, one asterisk denotes guinea pigs, two asterisks pig fetuses; $P < 0.001$ in all intervals.

TABLE 2. Distribution of β-Cells (in % of number tested) by Intensity of Incorporation of ^{3}H-Leucine at Different Stages of Growth of Monolayer Cultures of Islets of Langerhans (M ± m)

Day of culture	n	Number of grains of silver above cytoplasm					
		single grains			conglomerates		
		2—5	5—10	11—16	1—3	4—6	7—10
2	4	7±2,8*	—	—	11±4,5	5±0,8	—
	4	20±4,2**	12±2,3	—	27±4,8	6±2,1	—
5		45±3,1	10±2,6	12±5,2	58±5,7	10±2,3	5±0,8
		42±4,1	23±5,4	14±3,2	63±3,7	18±2,9	14±1,4
15		17±2,1	4±0,8	6±3,7	30±4,8	7±2,0	2±0,8
		16±3,2	15±5,7	10±2,6	32±3,6	20±2,9	6±2,3

EXPERIMENTAL RESULTS

The cultures were tested on the 2nd-3rd, 5th-6th, and 15th-16th days, which corresponded to stages of intensive growth, stabilization, and physiological death of the cultures. Depending on the character of radioactive labeling the β-cells were distributed among those containing incorporated ^{3}H-leucine in their cytoplasm (sometimes only at the periphery of the cytoplasm) and those containing ^{3}H-thymidine incorporated in the nucleus. Those which stained deeply with aldehyde-fuchsin and incorporated ^{3}H-leucine were classed as insulin-synthesizing, highly differentiated β-cells. β-Cells staining weakly with aldehyde-fuchsin and incorporating ^{3}H-thymidine were classed as less highly differentiated cells, which had not yet lost their ability to proliferate. Among the cells synthesizing insulin, most were β-cells with a low intensity of incorporation of ^{3}H-uridine and ^{3}H-leucine (Tables 1 and 2). On incubation for 30 min with ^{3}H-uridine, the label was distributed mainly in the nuclei, and in the stage of intensive growth of the cultures, it was located in the nucleoli. On transition to the stage of stabilization, the proliferative activity of the β-cells fell considerably, but their insulin-synthetic activity increased. Incorporation of ^{3}H-thymidine was reduced by about two thirds, and incorporation of ^{3}H-leucine was increased about threefold (Fig. 1). ^{3}H-uridine and ^{3}H-leucine were incorporated more intensively, indicating increasing insulin-synthetic activity of the β-cells (Tables 1 and 2; Fig. 2). Under these circumstances incorporated radioactive leucine was distributed not only as separate granules, but also as conglomerates of granules of silver. In some cells radioactivity of ^{3}H-uridine was located in both nucleus and cytoplasm. This is evidence of an increase in the rate of RNA transport from nucleus into cytoplasm, and it also confirms the increased insulin-synthetic activity of the β-cells. Together with an increase in insulin biosynthesis, the secretory activity of the β-cells also was increased. Determination of the concentration of immunoreactive insulin (IRI) in the culture medium showed a significant increase in its concentration at this stage of growth of the cultures (Table 3). These results are in agreement with those obtained by other workers, showing that the quantity of insulin secreted correlated positively with the increase in the number of β-cells in the course of culture [4, 7, 8]. During physiological aging of the cells and the development of destructive changes in them

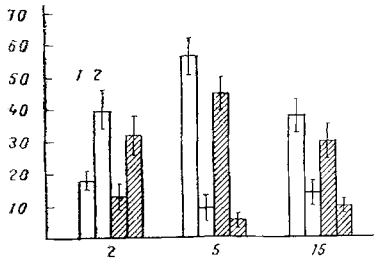

Fig. 1. Changes in indices of labeled β-cells depending on stage of growth of monolayer cultures of islets of Langerhans. Abscissa, period of culture (in days); ordinate, index of labeled β-cells (in %). I) Index of cells incorporating ^{3}H-thymidine; II) index of cells incorporating ^{3}H-thymidine [As in Russian original — Consultants Bureau]. Unshaded columns — pig fetuses, shaded columns — guinea pigs (n = 4; P < 0.001 for all intervals).

TABLE 3. Secretion of IRI at Different Stages of Growth of Monolayer Cultures of Islets of Langerhans (M ± m)

Day of culture	IRI concentration, microunits/ml	n	P
3—4	63±11* 870±158†	8 8	<0,05
5—6	74±8 967±118	8 8	
15—16	46±5 328±112		<0,001

*Guinea pigs.
†Pig fetuses.

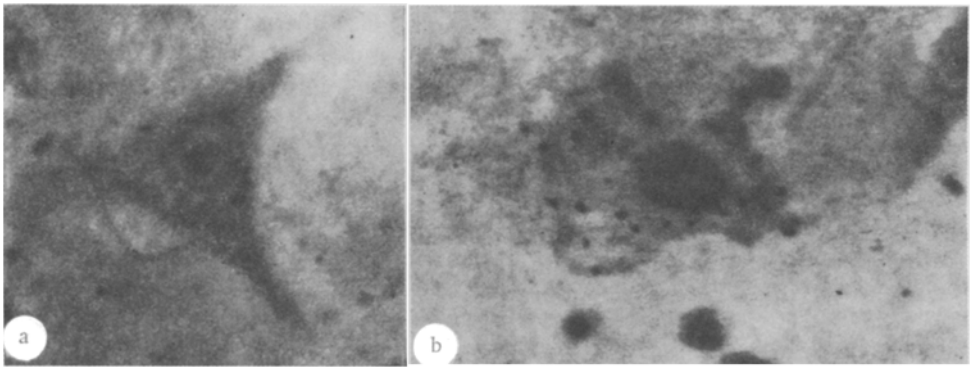

Fig. 2. Incorporation of ^{3}H-leucine into β-cells of monolayer cultures of islets of Langerhans from guinea pig pancreas: a) stage of intensive growth; b) stage of stabilization. Stained with aldehyde-fuchsin, 630 ×.

the integrity of the cultural layer was disturbed. Proliferative processes aimed at making good the physiological loss of cells were resumed, but by a much lesser degree than in the stage of intensive growth of the cultures (Table 1). The intensity of the insulin-synthetic processes showed a parallel decrease, manifested as weakening of the ability of the β-cells to incorporate labeled amino acids (Tables 1 and 2). Whereas in the stabilization stage maximal incorporation of ^{3}H-leucine took place during 1 h of incubation, in the stage of physiological death maximal incorporation of ^{3}H-leucine was observed after 2-2.5 h. Corresponding to the reduction in the number of β-cells and in their insulin-synthetic activity, the level of insulin secretion also was lowered (Table 3).

The results of this investigation thus showed that in stages of intensive growth and physiological death of the cultures β-cells can be divided into insulin-synthesizing and proliferating. The insulin-synthetic and secretory activity of monolayer cultures of islets of Langerhans is increased during their transition into the stage of stabilization.

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