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INSULIN-FORMING ACTIVITY OF MONOLAYER CULTURES OF BOVINE FETAL PANCREATIC ISLET CELLS

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UDC 612.647.349.7-085.23

The insulin concentration in the growth medium of primary monolayer cultures of bovine fetal pancreatic islet cells grown in the presence of a normal and increased (300 mg%) glucose content, was determined by a radioimmunologic method. A high glucose concentration led to increased secretion of insulin. The results of the cytological study showed definite correlation between mitotic activity of the cells of the culture and the intensity of insulin secretion into the medium. KEY WORDS: monolayer culture; islet cells; insulin secretion; mitotic activity.

A method of obtaining and growing monolayer primary cultures of bovine fetal pancreatic islet cells was described previously [1, 2]. It was shown that islets and more extensive areas of monolayer, consisting of epithelial cells, a high proportion of which contain aldehyde-fuchsin-positive granules in their cytoplasm, are formed 3-4 days after seeding of the cells. The addition of an increased concentration of glucose (final concentration 300 mg%) to the culture medium led to degranulation of the cells after 24 h. These results indicate that the cells described above correspond in their morphological characteristics to the B-cells of the islets of Langerhans.

This paper describes the results of determination of the insulin-forming activity of cultures of bovine fetal pancreatic islet cells at different times of culture and under particular experimental conditions.

EXPERIMENTAL METHOD

Monolayer cultures of islet cells were obtained from the pancreas of 4-6-month bovine fetuses with the aid of solutions of collalytin and trypsin [1, 2]. Cultures were grown in Carrel's dishes and flasks of various sizes, and also in penicillin flasks with coverslips. The medium was changed 2-3 days after seeding. The insulin concentration in the culture medium was determined by a radioimmunologic method using kits from the firm CEA-IRE-Sorin. The limit of sensitivity of the method in these experiments was 5 microunits/ml medium. The cultures of islet cells were fixed at appropriate times of the experiment with 96°C ethanol, stained with hematoxylin-eosin or aldehyde-fuchsin, and examined cytologically.

EXPERIMENTAL RESULTS

A. Hormonal Activity of the Bovine Fetal Pancreatic Cell Cultures. In the first stage of the work mixed monolayer cultures were obtained in which the contribution of the B-cells did not exceed 8-10%. In such cultures from the very beginning there were many fibroblasts, proliferation of which inhibited proliferation of the cells by the 10th-15th day after seeding. On the 3rd-5th day of culture (1-3 days after changing the medium 199 with a normal glucose concentration for the same fresh medium) the insulin concentration in the culture

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0007-4888/78/8608-1099$07.50

TABLE 1. Dynamics of Insulin Concentration (in microunits/ml) of Bovine Fetal Pancreatic Islet Cell Culture after Complete Change of Medium for Medium with Increased Glucose Concentration (300 mg%)

Experimental conditions	Before change of medium	At different times after change of medium					
		15–25 min	1 h	3 h	6 h	24 h	48 h
Seeding on Carrel's dishes	>3200	70		96	400	>3200	
Seeding on Roux flasks (1 liter)	320	14	50	120	3200	>3200	>3200

TABLE 2. Changes in Insulin Concentration (in microunits/ml) in Culture Medium of B-Cells with Daily Complete Change of Medium Containing 300 mg% Glucose

No. of expts.	Days after seeding						
	3	4	5	6	7	8	9
1	>3200	>3200	740	1520	200	—	—
2	>3200	1200	256	213	17,5	32	37
3	3200	1200	150	100	13	2520	46
4	1400	920	240	200	20	2400	132

fluid was 40-60 microunits/ml. Fractional sedimentation of the islet cell complexes in the course of 20 min led to the production of cultures in which B-cells accounted for 60-70% of the total. In such cultures there were very few fibroblasts and epithelial zones were preserved throughout the period of observation, up to one month. When cells were seeded at the rate of 10,000-20,000 cells/ml the insulin concentration in the culture fluid on the 3rd-5th day (1-3 days after the change of medium) varied from 320 to 3200 microunits/ml or more.

The present writers have recently succeeded in obtaining cultures of islet cells of a purely epithelial character. In such cultures fibroblasts were eliminated on the 3rd-4th day of culture (1-2 days after the change of medium). The proportion of B-cells in these cultures reached 80-90%. The insulin concentration in the culture fluid after seeding at the rate of 10,000-20,000 cells/ml was 6000-13,000 microunits/ml on the 3rd-4th day of culture.

B. Dynamics of Secretion of Insulin into the Culture Fluid under Various Experimental Conditions. The scheme of the experiments was as follows. To begin with purified cultures of islet cells were grown for 5 days in medium 199 with 10% bovine serum with a normal concentration of glucose in a Carrel's dish. The medium was changed 2 days after seeding. Consequently, the period of culture after the change of medium was 3 days. The insulin concentration in the growth medium at this time exceeded 3200 microunits/ml (Table 1). The medium was then completely replaced by medium with an increased glucose concentration (300 mg%). The culture medium 15 min later contained 70 microunits/ml insulin. Later its concentration in the medium rose rapidly and by the end of the first day it exceeded 3200 microunits/ml.

Similar results were obtained when cultures of bovine fetal pancreatic islet cells were grown in 1-liter Roux flasks. On the fifth day of growth (3 days after the first change of medium) the insulin concentration in the growth medium was 320 microunits/ml. After a complete change of medium for medium with an increased glucose concentration (300 mg%) the characteristic dynamics of the increase in the insulin concentration in the culture medium was observed during the next 2 days (Table 1).

In the next series of experiments, starting from the third day of culture the culture medium was completely changed for fresh medium with an increased glucose concentration (300 mg%) daily, at the same time of day (Table 2). Despite the stimulating effect of the high glucose concentration, in all four experiments a gradual decrease in insulin secretion was observed, evidently on account of aging of the culture. Meanwhile in three of the four experiments "flashes" of insulin secretion were observed (on the sixth day in the first experiment and on the eighth day in the third and fourth experiments). To study the nature of these "flashes" in one ex-

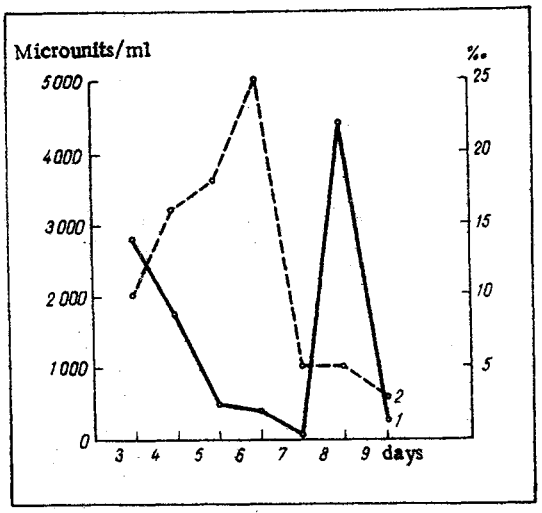

Fig. 1

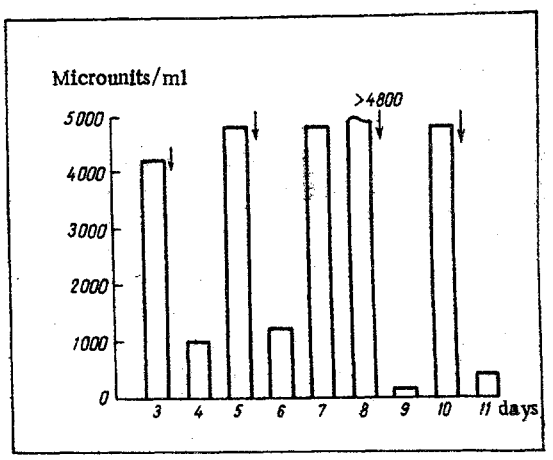

Fig. 2

Fig. 1. Correlation between level of insulin secretion (1) and mitotic activity (2) in culture of B-cells. Abscissa, days after seeding cells; ordinate: left, insulin concentration in culture medium (in microunits/ml), right, mitotic index (in %).

Fig. 2. Changes in insulin concentration in culture medium of B-cells with change of medium every 2-3 days. Arrows indicate times of change of medium (with 300 mg% glucose). Abscissa, days after seeding cells; ordinate, insulin concentration in culture medium (in microunits/ml).

periment of this series (experiment 4) the hormonal activity was compared daily with the results of cytological analysis. It will be clear from Fig. 1, in which the dynamics of insulin secretion is compared with the mitotic activity of the islet cell culture, that a wave of mitoses on the second day preceded the increase in insulin concentration in the medium. Dividing cells, passing into the next interphase, evidently were the sources of formation of the extra quantities of insulin. Conversely, on the day of the "flash" of hormonal activity the mitotic index was very low, in full agreement with modern views on relations between mitosis and specific cell function.

In the next series of experiments a complete change of medium (with 300 mg% glucose) was carried out once every two or three days. The results, shown in Fig. 2, are evidence that with a less frequent change of medium the insulin-secretory activity of the islet cell culture was maintained, actually at a rather higher level than after daily changes of medium. One possible explanation could be the less trauma inflicted on the cells of the culture.

The results point to high functional (insulin-forming) activity of monolayer cultures of bovine fetal pancreatic islet cells. This model, tested for the first time by the present writers [1, 2], can be recommended for various purposes in experimental endocrinology and transplantology. The results add to information in the literature on hormonal activity of islet cell cultures obtained from the pancreas of other animals [3-6].

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