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EFFECT OF PERFUSION WITH NUTRIENT MEDIUM ON SECRETORY ACTIVITY OF RAT HEPATOCYTES AND PANCREATIC ISLET CELLS

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Controlled cell culture has attracted ever-increasing attention of research workers in recent years. Both the systems of continuous-flow culture and the various semipermeable synthetic materials suitable for culture of specialized cells, including hormone-secreting cells, have been improved [1, 2, 5, 7, 10, 12].

The writers showed previously that under the conditions of a continuous-flow system of culture cells of the adenohypophysis and the pancreatic islets of Langerhans, when placed on porous membranes, become attached, grow, and secrete their specific hormones [1, 5]. Of the many synthetic semipermeable membranes studied, membranes of sodium borosilicate glass with an effective pore radius of 5-10 nm and a thickness of 1 mm were selected. Pituitary cells placed on such a membrane secreted hormones which, depending on their molecular weight, penetrated selectively into the culture medium.

The present investigation was conducted on an apparatus for continuous-flow cultures by an improved system. Hepatocytes, secreting serum albumin, and pancreatic islet cells, actively producing the hormone insulin, were chosen as biological models. The aim of the investigation was to study the viability and functional activity of these cellular structures during perfusion with nutrient medium.

EXPERIMENTAL METHOD

The basic scheme of the apparatus for cell culture in a chamber with a porous membrane was fully described previously [1]. In this system the gas mixture, containing air and suf-

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ficient CO_2 to maintain the assigned pH value, was passed through sterilizing filters on the "air life" principle into the cultivator chamber (Fig. 1). The chamber, measuring 18 cm^3, had top and bottom compartments, separated by a semipermeable membrane, on which the cells were placed. Communication between the compartments was effected only through the pores of the membrane. The medium was incubated in the system as it passed through the bottom compartment. The temperature in the cultivator was maintained constant at the assigned temperature by means of water which was passed through a special jacket by means of an ultrathermostat. The experiments were carried out on 2-day-old primary cultures of hepatocytes and pancreatic islet cells of Wistar rats. The methods of obtaining the cell cultures were described in detail previously [4, 6].

In each experiment two isolated hepatocytes or β-cells were seeded at the rate of 1.5-2 million cells to 1 ml nutrient medium. The hepatocytes were cultured in Eagle's medium, the islet cells in medium 199. The nutrient medium contained 10% embryonic calf serum (from Serva, West Germany). After seeding with the cells the chamber was incubated for 18-20 h. The medium was then completely replaced by an identical medium, in which the concentration of embryonic calf serum was reduced to 1% for β-cells or was replaced by 0.1% bovine serum albumin for hepatocytes. The control chamber was again incubated whereas the experimental chamber was connected to the apparatus.

Insulin diffused into the circulating medium through pores in the sodium borosilicate glass, and albumin accumulated in the top compartment of the chamber. As specific stimulator of insulin secretion glucose was used in a concentration of 20 mM. Glucose was added to the bottom compartment in the control chamber. Samples of 0.2 ml were taken to determine the hormone concentration under sterile conditions through the top or bottom side tube. The first sample was taken 1 h after the last change of medium and the chamber was connected to the apparatus.

Albumin secreted by the hepatocytes was determined by radioimmunoassay [3]. Immunoreactive insulin was determined with ready-prepared RIA kits (Institute of Isotopes, Hungarian Academy of Sciences). Radioactivity was counted on an instrument from Searle (Holland).

To determine the cell protein content, the cells were removed from the glass after incubation mechanically and sedimented by centrifugation in 0.9% NaCl for 15 min at 3000 rpm. The resulting sediment was hydrolyzed in 1 ml of 0.5 N NaOH for 24 h at 37°C. The protein concentration in aliquots of the digest was determined by Lowry's method [8].

EXPERIMENTAL RESULTS

Continuous circulation of nutrient medium had a beneficial effect on the secretory capacity of both hormone-secreting and hormone-sensitive cells. Circulation of the medium led to a marked increase in albumin secretion by the hepatocytes (Fig.2). Under these conditions saturation of the medium with albumin in the top compartment of the chamber took place after only 5 h of perfusion, and after 24 h the albumin concentration in the medium was virtually not increased.

When calculated relative to cell protein, the albumin concentration during perfusion of the cells with medium was significantly higher than that in the control chamber (450 and 220 μg/g protein/h, respectively). On the basis of these results it is evident that the rate of albumin secretion in a dynamic incubation system was higher than in a static system. Comparison of the results of these experiments with data in the literature [11] showed that the rate of albumin secretion during perfusion of the hepatocytes was comparable with values obtained in experiments *in vivo*.

Dependence of the rate of insulin secretion by the islet cells on perfusion was revealed with two concentrations of glucose in the medium: 5.5 and 20 mM. The results are evidence that basal insulin secretion (glucose concentration in the medium 5.5 mM) was significantly higher when the nutrient medium circulated continuously than in the control. Significant differences in hormone secretion were discovered as early as after 2 h, and after 24 h the quantity of insulin secreted in the perfusion chamber was almost three times greater than in the control (Fig. 3a).

Similar results were obtained during stimulation of insulin secretion by glucose (20 mM). In this case also definite differences were found only 2 h after addition of glucose to the circulating medium (Fig. 3b). This time is evidently necessary for glucose to diffuse to the cells in the top compartment of the chamber. It is stated in the literature that continuous perfusion of the medium may be used for work with isolated liver cells [9]. The authors cited

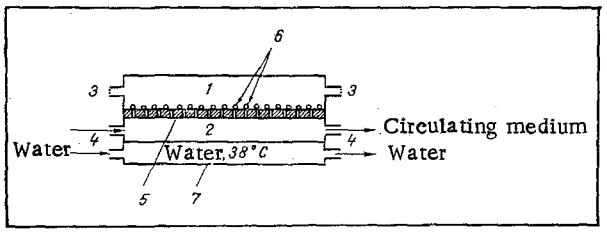

Fig. 1

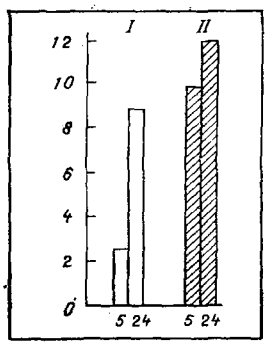

Fig. 2

Fig. 1. Diagram of cultivator chamber: 1) Top compartment; 2) bottom compartment; 3) top connecting pipes; 4) bottom connecting pipes; 5) porous membrane; 6) cells on membrane surface; 7) constant-temperature jacket.

Fig. 2. Effect of perfusion with nutrient medium on time course of albumin secretion by rat hepatocytes. Abscissa, incubation time (in h); ordinate, albumin concentration (in μg/mg protein). I) Control; II) with circulation.

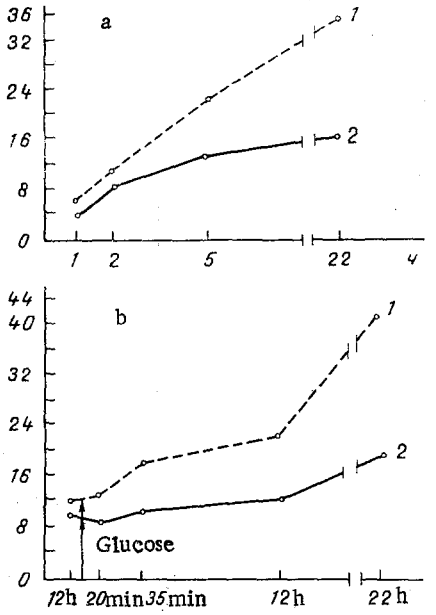

Fig. 3. Effect of perfusion with nutrient medium on time course of insulin secretion in culture of rat pancreatic islet cells. Abscissa, incubation time (in min); ordinate, insulin concentration (in milliunits/mg protein). a) Glucose concentration in medium 5.5 mM; b) glucose concentration 20 mM. Continuous lines — control; broken lines — circulation.

stated that the viability of the hepatocytes was improved under these circumstances and that carbohydrate metabolism was intensified.

The results of the present investigation, obtained on two different cell systems, show convincingly that perfusion of the cells with nutrient medium has a beneficial action on their function. This phenomenon can probably be explained by intensification of cell metabolism due to the better supply of nutrients to the cells and better removal of metabolic products (by dilution) when the medium circulates continuously. All in all these factors create favorable conditions for the vital activity of cells in culture.

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PROLIFERATION OF HEMATOPOIETIC STEM CELLS AND THEIR MIGRATION INTO THE MOUSE SPLEEN AFTER A SINGLE INJECTION OF *Mycoplasma arthritidis*

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The continuing spread of mycoplasmas and broadening of the spectrum of pathology for which they are responsible has increased interest in these microorganisms. Contaminating mycoplasmas, as intracellular and membrane parasites, can not only induce pathological cellular reactions, but can also have an effect on the results of experimental research. Despite induction of cellular or humoral immune protective responses, many mycoplasmas are capable of long persistence in the tissues of the carrier organism, and in particular, in the hematopoietic and lymphoid tissues, as has been shown for *Mycoplasma arthritidis* [4].

The aim of this investigation was to study the reaction of the hematopoietic tissue of mice and their hematopoietic stem cells (CFU) in response to a single injection of living *M. arthritidis*. The results of the initial experiments on this problem were published previously [2, 5].

EXPERIMENTAL METHOD

Male CBA/Ca ($H\text{-}2^k$) and BALB/c ($H\text{-}2^d$) mice aged 8-12 weeks, obtained from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR, were used in the experiments.

Strain PG6 of *M. arthritidis* was obtained and kept as described previously [2]. The mycoplasmas were injected intraperitoneally into the mice in a dose of 10^8 CFU/mouse. Growth medium for mycoplasmas was injected into the control mice.

To determine the number of CFU-s, hydroxyurea was injected intraperitoneally into the mice in the S phase of the cell cycle in a dose of 500 mg/kg body weight. After 1 h the mice were killed by cervical dislocation, the bone marrow was removed from the femora, and the number of CFU-s was determined by exocolonization of the spleen of lethally irradiated syngeneic recipients [14]. The number of CFU-s in the phase of DNA synthesis was determined by the formula:

$$A = \frac{a-b}{a} \times 100$$

where A is the number of CFU-s in the S phase (in percent), a, the number of colonies formed by CFU-s of intact mice, and b the number of colonies formed by CFU-s of mice receiving hydroxyurea.

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