COMPENSATION OF ALLOXAN DIABETES IN RATS BY TRANSPLANTATION OF EMBRYONIC PANCREATIC TISSUE INTO THE ANTERIOR CHAMBER OF THE EYE AND CEREBRAL VENTRICLES

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To compensate experimental diabetes in animals isolated islets of Langerhans [7-9], dissociated and previously cultured β-cells of islets of Langerhans [4], and embryonic pancreas have been transplanted. Tissue is transplanted into the portal vein, the anterior lobe of the liver, or beneath the internal capsule of the kidney [4, 6-8, 10]. During these procedures the main problem of transplantation has been encountered, namely tissue incompatibility between graft and recipient. For successful transplantation preliminary treatment of the recipient animal with immunodepressants is essential in every case. Isolation of the nervous system from the general immune system of the body by the blood–brain barrier makes it possible for transplantation of tissues of nervous and endocrine nature to be carried out into the brain or anterior chamber of the eye (ACE) without any immunosuppression [1, 5, 11-13]. It thus becomes possible to correct some defects of neuroendocrine and endocrine nature by allografting and xenografting of the corresponding tissue [5, 12].

In the present investigation the possibility of compensating a defect of endocrine nature (alloxan diabetes in rats) by transplantation of the embryonic pancreas into ACE and the cerebral ventricles was studied.

EXPERIMENTAL METHOD

Experiments were carried out on 43 noninbred rats weighing 180-220 g. The animals were divided into four groups: 1) normal (n = 10), 2) control group with diabetes (n = 10), 3) rats with diabetes into whose ACE (n = 13) or cerebral ventricles (n = 5) embryonic pancreatic tissue was transplanted, and 4) the active control (n = 5), i.e., rats with diabetes into whose ACE pieces of kidney from 18-day embryos, measuring 0.8-1.0 mm^3 in volume, were transplanted. Diabetes was induced in the rats by intraperitoneal injection of 200 mg/kg of alloxan hydrate (from Chemapol, Czechoslovakia). The appearance of diabetes was verified by determining the glucose concentration in the urine by the orthotoluidine method and recording the color on an SF-26 spectrophotometer at a wavelength of 600 nm. Animals in which the glucose concentration in the urine exceeded 4000 mg% for 1-2 weeks were chosen for transplantation. Tissue transplantation was carried out under sterile conditions. Under pentobarbital anesthesia caesarean section was performed on a donor rat on the 17th day of pregnancy (the embryos were removed, washed in distilled water and alcohol, and transferred to sterile Eagle's solution). The pancreas was removed from the enbryos under a stereoscopic microscope. One or two pieces, each 0.8-1.0 mm^3 in volume, were excised from the tail of the pancreas and transferred to a Petri dish containing sterile Eagle's solution. Transplantation into ACE was based on methods described in the literature [3, 10]. A drop of 0.1% atropine solution and a drop of 0.5% procaine solution were instilled into the eye of the recipient rats. Transplantation was carried out under ether anesthesia. By means of a screw-operated tuberculin syringe, to which a glass tip was fitted (internal diameter 0.8-1.0 mm), the graft was taken from the Petri dish and introduced through an incision in the cornea into ACE. Transplantation into the cerebral ventricles was carried out by the method in [1]. No immunodepression was used either during or after the operation. After the operation the rats were kept on a standard diet worked out for laboratory animals. Once a week the glucose concentration in

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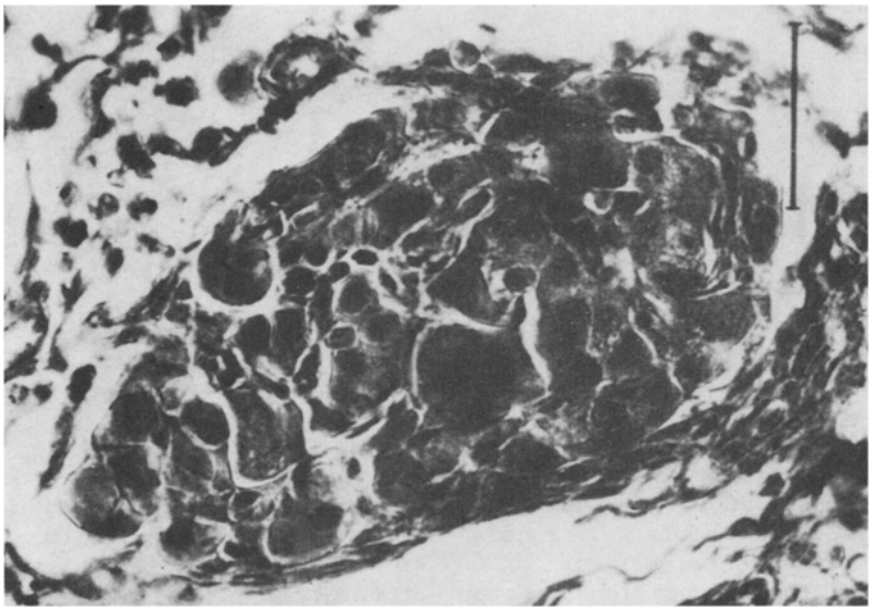

Fig. 1. Group of pancreatic islet cells from 17-day rat embryo ten weeks after transplantation into anterior chamber of the eye. Stained by Mallory's method. Scale 30 μ.

their urine was determined. In animals in which transplantation was carried out into ACE, after a specified time the eye with the graft was enucleated and the glucose concentration tested. At the end of the experiments the graft was subjected to histological analysis with staining by Mallory's method and with hematoxylin-eosin. The results were subjected to statistical analysis on the "Elektronika-60" computer. The mean value of the urinary glucose concentrations, standard deviation, and confidence limits for normal (group 1) and diabetic animals (group 2) were calculated. Data for the other groups were compared with these values. The significance of differences was determined by Student's t test. Diabetes was considered to be fully compensated if the data for the animal concerned did not differ significantly from normal, and to be partially compensated if the data differed significantly from those of groups 1 and 2.

EXPERIMENTAL RESULTS

Visual observation on the grafts in ACE showed that after 2-3 days blood vessels began to grow out toward them. Their number and diameter increased for approximately 10-15 days, and and after two weeks some of the grafts were completely hidden beneath blood vessels. Histological analysis of the grafts 6-20 weeks after transplantation revealed islet cells incorporated into a connective-tissue stroma (Fig. 1).

Of the 13 rats with pancreatic tissue transplanted into ACE, complete compensation of the diabetes was observed in 8 animals, partial compensation in 3, and in the other two the glucose concentration remained at its previous level. Compensation usually began in the second week after transplantation (Fig. 2). In the third week the glucose concentration in the urine fell to normal and remained at that level for 20-30 weeks (the usual period of observation). In two rats compensation was observed for 11 months (maximal period of observation). After enucleation of the eye containing the graft the glucose concentration in the rat's urine rose again (Fig. 2, squares at 7th and 10th weeks). Of five rats with the pancreas transplanted into the cerebral ventricles complete compensation of the diabetes was observed in three, partial compensation in one, and no compensation in one rat. The time course of compensation was the same as in the rats with the graft in ACE. No compensation of diabetes was observed in any animal in the active control group.

The results point to the possibility of successful compensation of alloxan diabetes in rats by transplantation of embryonic pancreas into ACE and into the cerebral ventricles. The compensation lasted throughout the period of observation (11 months) with no sign of an immune response of rejection by the recipient.

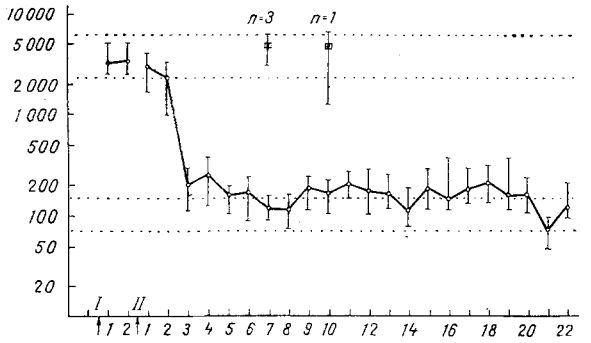

Fig. 2. Time course of urinary glucose level in rats after transplantation of pancreas from 17-day rat embryos into anterior chamber (averaged data for 8 rats). Top two broken lines indicate confidence levels for the state of diabetes. Two bottom broken lines denote confidence levels for normal state. I) Time of injection of alloxan hydrate; II) time of transplantation. Squares at 7th and 10th weeks indicate return of diabetes after enucleation of the eye; n) number of animals. Abscissa, time (in weeks); ordinate, sugar concentration (in mg%), logarithmic scale.

A special feature of this investigation is that compensation of diabetes took place after transplantation of a minimal quantity of tissue compared with other investigations of which the writers are aware. For instance, in the work of Mullen et al. [10], from one to three whole pancreases were required for transplantation beneath the internal capsule of the kidney in order to compensate diabetes. In other investigations [7-9], in which transplantation was carried out into the portal vein and anterior lobe of the liver, compensation was achieved when the islets of Langerhans were transplanted from 3 to 5 adult rats. In the present experiments the volume of tissue transplanted was about the same in all cases, namely about one-quarter of one embryonic pancreas.

Compensation of diabetes by a small volume of tissue is not unexpected. We know that diabetes does not develop in animals if only 15-20% of the islet cells remain intact. To compensate diabetes in man 200 mg of normally functioning islet tissue is sufficient [2]. Our data show that 0.8-1.0 mg of islet tissue is sufficient to compensate diabetes in a rat. A stable positive effect is obtained under these circumstances without immunodepression. We know, however, that the blood–brain barrier is impermeable to insulin. Evidently during vascularization of the grafts in ACE and the cerebral ventricles a tissue–blood barrier specifically permeable for insulin is formed.

In no case of compensation was hypoglycemia observed, evidence that the function of the graft was regulated by the recipient, as a result of which the glucose concentration was established at the normal level.

The experiments thus showed that experimental diabetes can be compensated by transplantation of embryonic pancreatic tissue into a region of the body protected against the action of the immune system.

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EFFECT OF CYCLIC NUCLEOTIDES ON PROLIFERATIVE ACTIVITY OF PRIMARY CELL CULTURES FROM THE ATHEROSCLEROTIC HUMAN AORTA

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Artificially induced atherosclerosis in animals is characterized by intensification of proliferative activity of the cells in the intimal layer of arteries [4, 9]. It has been suggested that intensification of proliferation also takes place in a region of spontaneous atherosclerosis of blood vessels in man. Cells isolated from zones of initial atherosclerotic lesions, in primary culture, have been shown to have greater proliferative activity than cells of the undamaged intima [1, 2]. Abundant facts have now been gathered to show that proliferation and differentiation of certain types of cells in eukaryotes are under the control of the cyclic nucleotide system and that elevation of the intracellular cAMP level inhibits cell division [11]. It has been suggested that cAMP derivatives and compounds which raise the cAMP concentration may be effective preparations inhibiting cell proliferation. However, there is evidence in the literature that cAMP may have the opposite action on proliferative activity of certain types of cells [6]. Hence the need to investigate the role of cyclic nucleotides in the processes of cell division and differentiation for each cellular system.

The object of this investigation was to study the effect of cyclic nucleotides on incorporation of [^{3}H]thymidine into cells of the intima from unaffected and atherosclerotic regions of the human aorta.

EXPERIMENTAL METHOD

A culture of intimal cells from atherosclerotic and nonatherosclerotic regions of the human aorta was obtained as described previously [7]. The cells were cultured in plastic Petri dishes 35 mm in diameter (Corning, USA) with seeding density of 10^4 cells/cm^2 (plating efficiency 50-60%) in 1.5 ml of medium 199 containing 10% embryonic calf serum, 2.5 μg/ml fungizone, 100 μg/ml kanamycin, and 2 mM glutamine (all reagents from Gibco, USA) at 37°C in an atmosphere of 5% CO_2 and 95% air, saturated with water vapor. Every 3 days the medium was changed. On the 10th day dibutyryl-cAMP (dbcAMP), dibutyryl cGMP (dbcGMP), or sodium butyrate (Sigma, USA) was added to the cultures. Next day the compounds were added again, together with 1 μCi/ml of [^{3}H]thymidine (21 Ci/mmole, from Amersham Corporation, England). After one day the cultures were washed twice with isotonic Dulbecco's phosphate buffer and the cells were suspended by 0.25% trypsin with 1 mM EDTA (Gibco, USA). To determine incorporation of labeled thymidine 2 ml of 15% TCA was added to 1 ml of the cell suspension. The residue after centrifugation (6000g, 20 min) was washed twice with 2 ml of 10% TCA and dissolved in 200 μl of 0.5 N NaOH. The solution was neutralized with 0.5 M HCl and radioactivity was measured on a 1215 RackBeta II liquid scintillation counter (LKB, Sweden), using Bray's scintillation solution [3]. Liposomes containing cAMP were prepared from ovolecithin, stearylamine, and cholesterol (Gibco, USA) in molar proportions of 5:1:3 by Papahadjopoulos' method [8]. The liposomes (0.2 mg/ml of total lipid and 10^{-5} M cAMP in the final volume) were added to the cells on the 10th day of culture. [^{3}H]Thymidine was added to the medium 24 h after the second addition of liposomes and its incorporation was determined as described above. The experimental results were subjected to statistical analysis. The significance of differences was estimated by Student's t test.

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