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HOMO- AND HETEROSPECIFIC TRANSPLANTATION OF EMBRYONIC NERVE TISSUE

A. G. Bragin and O. S. Vinogradova

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Attempts to transplant embryonic nerve tissue into the brain of an adult animal of the same species were made several decades ago [1, 6, 8]. It was then shown by morphological methods that the grafts may take and differentiate in the recipient's brain. A long time after these investigations the second stage began: investigation of grafts with the combined use of morphological, histological, and autoradiographic methods [2, 3, 7, 9, 11, 12]. The results of these investigations showed that blood vessels which provide for normal nutrition grow into the graft, neurons differentiate from neuroblasts, and the architectonics of the structure which was transplanted is formed, with preservation of its biochemical properties. For example, grafts of the raphe nuclei synthesized serotonin, grafts of the septum synthesized acetylcholine [2, 3]. Fibers from the graft can grow toward neighboring structures of the recipient's brain and can also receive afferent connections from them [4, 10, 12].

These facts suggest that the function of injured structures of the recipient's brain can be restored and replaced by a graft. Despite obvious specific difficulties, transplantation into the brain does not meet with the main fundamental difficulty, the problem of rejection of foreign tissue through the action of immune mechanisms. The presence of the blood–brain barrier in transplantation into the brain theoretically allows transplantation of the tissue not only of another individual, but also of another biological species. The object of the present investigation was to make a comparative experimental study of homo- and heterospecific transplantation of nerve tissue.

EXPERIMENTAL METHOD

The operation was performed under sterile conditions. The recipient animals (rats and rabbits) were scalped under pentobarbital anesthesia (40-50 mg/kg body weight) and fixed in SEZh-2 or STM-3 stereotaxic apparatuses in accordance with coordinates taken from the atlas of Fifkova and Marsale [5]. A hole 5 mm in diameter, with coordinates of its center AP = 0, L = 0.5 mm for rabbits and AP = +1, L = 0.5 mm for rats, was then made in the cranial bones

TABLE 1. Transplantation of Embryonic Rat Septal Tissue into Brain of Rats and Rabbits

Recipient rats			Recipient rabbits		
No. of animal	duration of expt., days	graft	No. of animal	duration of expt., days	graft
1*	50	Glia	1	150	Glia
2	60	"	2	30	Neurons
3*	40	Neurons	3	25	"
4	40	"	4	40	"
5	180	No graft	5	45	Glia
6*	190	Neurons	6	60	No graft
7	190	No graft	7	120	Neurons
8	200	Neurons			
9	200	"			

*Noninbred rats were used as recipients.

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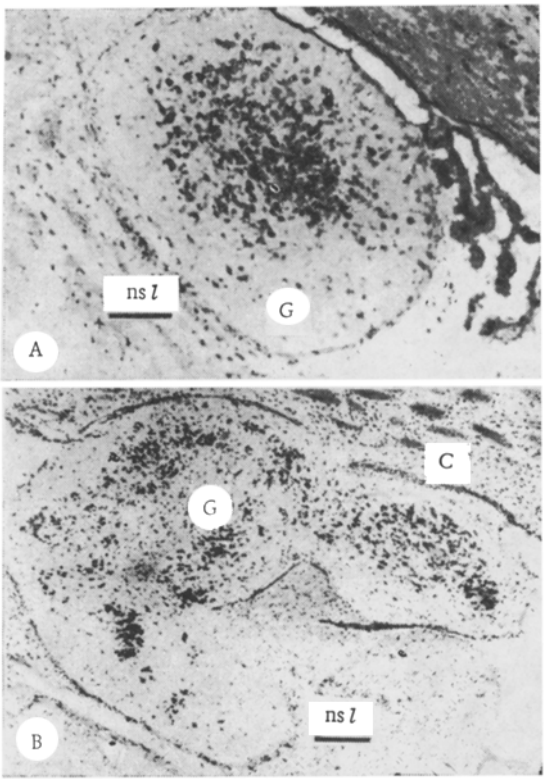

Fig. 1. Examples of homospecific grafts from tissue of septal nuclei of rat embryos transplanted into ventricle (A) and between caudate nucleus and septum (B). G) Graft, ns1) lateral septal nucleus; C) caudate nucleus. Scale: A) 100 μ, B) 200 μ.

above the septofimbrial nucleus. Bleeding was carefully stopped after this procedure.

On the 16th-17th days of pregnancy caesarian section was performed on the donor rat under deep pentobarbital anesthesia and the embryos removed. They were washed in two portions of sterile Hanks' solution, after which the membranes covering the brain were divided under a binocular loupe on a watchglass in a third solution. The principal instrument used to remove and transplant the grafts was a tuberculin syringe with a set of replaceable glass tubes, the internal diameter of which was 0.5-0.8 mm, external diameter 0.6-1.0 mm, and length 20-40 mm. The tubes were first secured by means of acrylic glue to the base of the injection needle. To make the system watertight, it was coated with supercement or Viniflex varnish. Tissue from the septum, in a volume of 0.5-1 mm^3, was taken by means of a tuberculin syringe with glass tip, filled with Hanks' solution. The graft was washed in sterile Hanks' solution. The syringe was then fixed to a stereotaxic apparatus, the end of the glass tip was inserted to the required depth, and the graft was slowly advanced into the recipient's brain. Bleeding was then stopped again and the brain surface was washed with Hanks' solution and the burr-hole was flooded with warm agar-agar made up in physiological saline containing 1% streptocide.

For animals on which electrophysiological investigations were to be conducted, a holder for a micromanipulator, hermetically sealed with a cork, was mounted on its skull. The electrophysiological investigations began 3-4 weeks after transplantation. Unit activity was recorded from the graft extracellularly in waking rabbits in a special frame which restrained movement a little. After the electrophysiological experiments a coagulation tag was left at the point of recording from the neurons, and it was subsequently confirmed in histological sections that the electrode had entered the graft. Morphological studies of survival of the grafts (stained by Nissl's method) were carried out 3-28 weeks after transplantation.

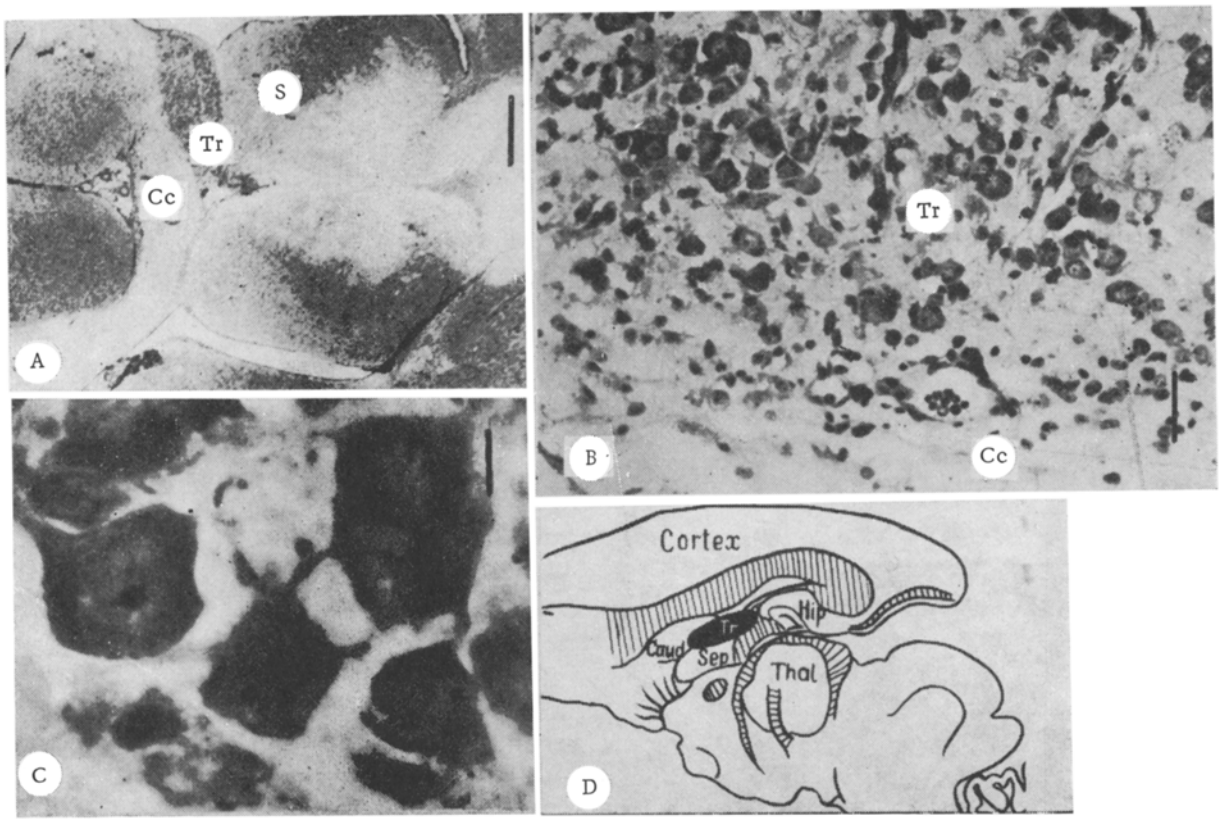

Fig. 2. Heterospecific transplantation of embryonic rat hippocampus into rabbit. Scale: A) 1000 μ, B) 70 μ, C) 10 μ, D) diagram of location of graft in sagittal plane. S) Septum; Tr) transplant; Cc) cerebral cortex. Legend as in Fig. 1.

EXPERIMENTAL RESULTS

Grafts from the septal region of 16-17-day Wistar rat embryos were transplanted into three adult noninbred rats, six Wistar rats (weighing 200-250 g), and seven Chinchilla rabbits (weighing 2.5-3.0 kg). The results are summarized in Table 1.

It will be clear from the data in Table 1 that in two of nine recipient rats the graft did not take and a cavity filled with CSF was observed at the site of transplantation. This occurred in cases when the graft was introduced into the fibrous part of the brain, into the corpus callosum, for example. In two cases proliferation of glia was observed and in five cases the grafts took successfully and neurons differentiated in them. This was observed in those animals in which the graft was introduced into the cerebral ventricles or between brain structures so that it was partly in contact with the vascular plexuses of the ventricle or the pia mater. Well-developed grafts could be seen in the period between the 1st and 6th months after transplantation (later periods were not investigated).

The form of the graft depended on where it was transplanted. For instance, grafts introduced into the cerebral ventricles were spherical in shape. The volume of these grafts in our experiments varied from 0.5 to 2.0 mm^3 and it was proportional to the quantity of tissue transplanted (Fig. 1). Grafts transplanted into an incision between structures had a more complex shape. A graft of the septal region, transplanted into the region of the septofimbrial nucleus, is shown in Fig. 1B. Lying between the lateral septal nucleus and caudate nucleus, it is curved in shape, with constriction bands. In some cases the graft was separated from the recipient's brain by a thin glial sheath, in other cases this membrane was not found. A mixed case is illustrated in Fig. 1B: A glial sheath is present between the graft and the lateral septal nucleus on the right, whereas on the left the graft tissue is directly continuous with the recipient's tissue. In most grafts the density of the neurons was higher in the center than at the periphery. In some grafts (especially large ones — 1 mm^3 or more) the neurons formed groups in which the number of well-differentiated neurons

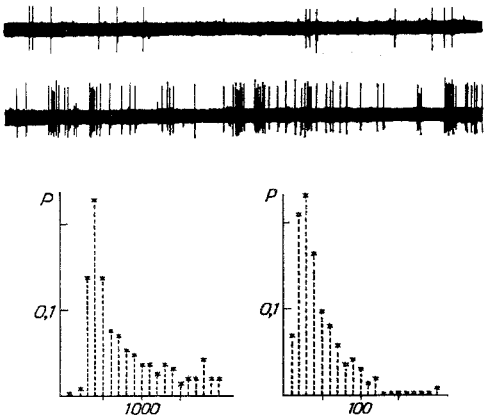

Fig. 3. Unit activity in graft of rat nerve tissue transplanted into rabbit (rabbit No. 3, 25th day after transplantation). Top: traces of spontaneous activity. Time marker 200 msec. Bottom: corresponding histograms of interspike intervals: for neuron A1 on left, for neuron A2 on right. Abscissa, time (in msec); ordinate, probability of interspike interval.

ranged from several dozens to several hundreds. As a rule cross sections of blood vessels running through the graft could be seen in the sections. No difference was found after transplantation into the brain of inbred and noninbred animals.

As Table 1 shows, grafts of nerve tissue from rat embryos can also take when transplanted into rabbits. Their survival and differentiation were found in four of seven cases. No differences in principle could be detected from homospecific transplantation. A graft of the rat septum transplanted into a rabbit is shown in Fig. 2 (No. 3 in Table 1) 25 days after transplantation. It differs sharply from the adjacent structures in the density of its component cells (Fig. 2A). Under higher power (Fig. 2B and C) it will be clear that the base of the graft is formed by neurons with a well-formed nucleus, and with one or two nucleoli. The beginnings of processes of some neurons can be distinguished. Small, deeply stained cells of glial origin are present among the neurons.

In two rabbits (Nos. 3 and 4) unit activity was recorded in the graft extracellularly. Of the 14 neurons recorded three did not have spontaneous activity and generated spikes only during movement of the microelectrode near them. The remainder had spontaneous activity of between 0.1 and 20 spikes/sec (mean frequency 4 spikes/sec). Examples of spontaneous unit activity of two rat septal neurons transplanted into a rabbit's brain, and the corresponding histograms of interspike intervals are illustrated in Fig. 3. Trace A2 shows grouping of the spike discharge into bursts with a frequency of 2-4 spikes/sec.

This investigation thus showed for the first time that heterospecific transplantation of embryonic nerve tissue from rats into rabbits is possible, and it also demonstrated that unit activity of the transplanted neurons is preserved. The further development of this technique will provide much new data relating to the elucidation of the principles of ontogenetic development of brain structures. One possible application of this technique in practical medicine is the reconstruction of certain brain structures which have lost their intrinsic functions.

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

V. P. Komissarenko, I. S. Turchin, and N. P. Lazareva

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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].

Laboratory of Pathophysiology, Institute of Endocrinology and Metabolism, Ministry of Health of the Ukrainian SSR, Kiev. Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 92, No. 10, pp. 489-491, October, 1981. Original article submitted February 18, 1981.

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