MORPHOLOGICAL CHANGES IN THE CEREBRAL HEMISPHERES AND IN BRAIN TISSUE TRANSPLANTED THEREIN IN THE EARLY STAGES AFTER BRAIN CROSS-GRAFTING IN NEWBORN RABBITS

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Regeneration of nerve tissue in newborn mammals has been inadequately studied. Most investigators consider that brain neurons do not regenerate. However, some workers accept that brain neurons in mammals are capable of amitotic division during early ontogeny [1, 3].

In the investigation described below manifestations of a degenerative character arising in brain tissue of newborn rabbits after transplantation of comparatively large areas of the brain were studied.

EXPERIMENTAL METHOD

Experiments were carried out on two groups of animals. Group 1 consisted of 142 rabbits of the same breed, aged 2-4 days, in which cross-grafting of areas of the brain was carried out by an original method. A scheme of the operation of transplantation of brain areas in the newborn rabbits is given in Fig. 1. Under inhalational ether anesthesia a semilunar incision was made through the scalp in the right parieto-temporo-frontal region. The convex side of the incision faced the concha auriculae. The incision was carried deeper through the cranial bones by means of narrow-bladed scissors. The resulting osteoplastic flap was bent upward with forceps and a narrow metal spatula (0.8 cm wide) was inserted beneath it; by means of this spatula a lamina of the parieto-temporal region of the brain about $0.7 \times 0.7 \pm 0.2$ mm in area was excised and removed from the cranial cavity. The lamina of brain tissue included the cortex and underlying subcortical zones of the brain. The operation was performed under sterile conditions with an air temperature in the region of the wound of 18°C, and it was performed on two animals simultaneously. As soon as the areas of brain tissue had been removed from the cranial cavity, they were immediately replaced on the brain of the other animal, in the place from which the corresponding piece of nerve tissue had been removed. In other words homonymous areas of brain of approximately equal size were grafted from one animal to the other. As soon as the piece of brain tissue had been placed in the required position the skin and bone flaps was replaced in its usual position and the skin wound closed with a continuous silk suture.

Group 2 consisted of 122 rabbits of the same breed aged 2-4 days, on which similar operations were performed.

None of the animals died in the course of the operations.

The animals were decapitated between 1 and 45 days after the operation. The brain was removed and fixed in its entirety in 10% neutral formalin, after which a transverse incision was made through it, passing through the site of the graft. A slice of brain about 0.5 cm thick was obtained, including both the recipient's brain and also part of the graft, and this was embedded in paraffin wax. Sections were cut and stained with hematoxylin and eosin, by Nissl's, Van Gieson's and Spielmeyer's method, and for DNA by Feulgen's method.

EXPERIMENTAL RESULTS

Rejection of the graft did not occur in the animals of group 1. In group 2 the graft was rejected in three animals (2.45%).

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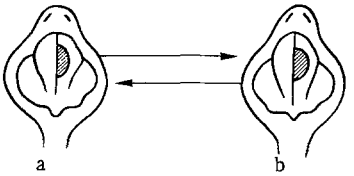


Fig. 1. Scheme of operation of cross grafting of brain areas in newborn rabbits (a and b) (shaded area).

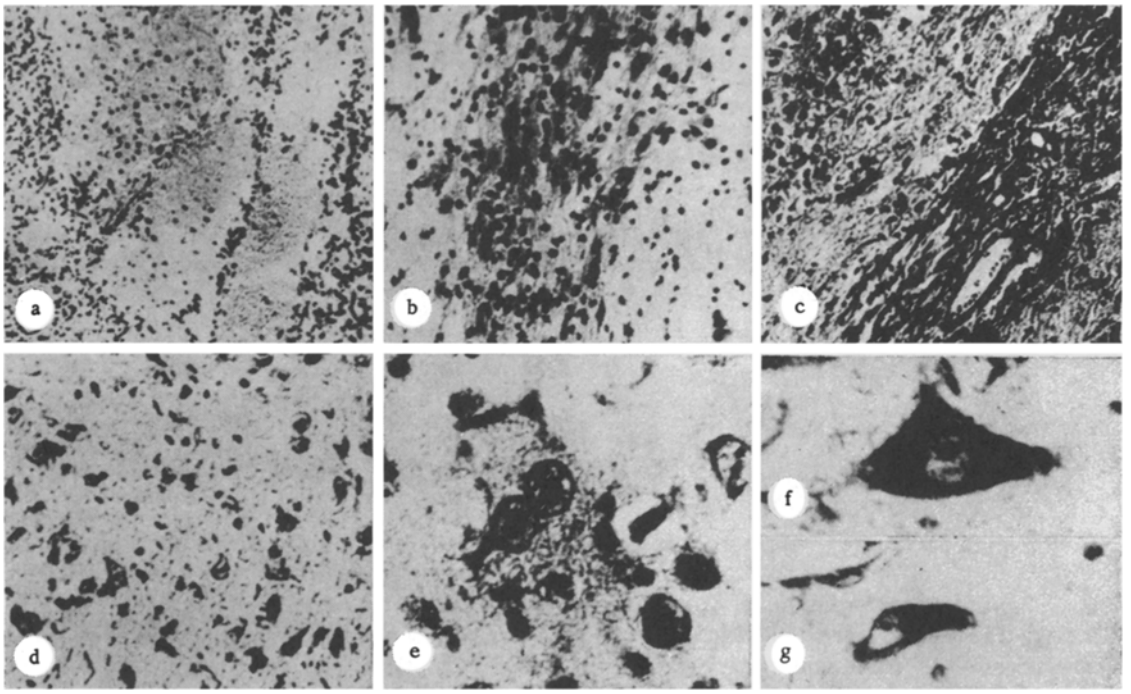
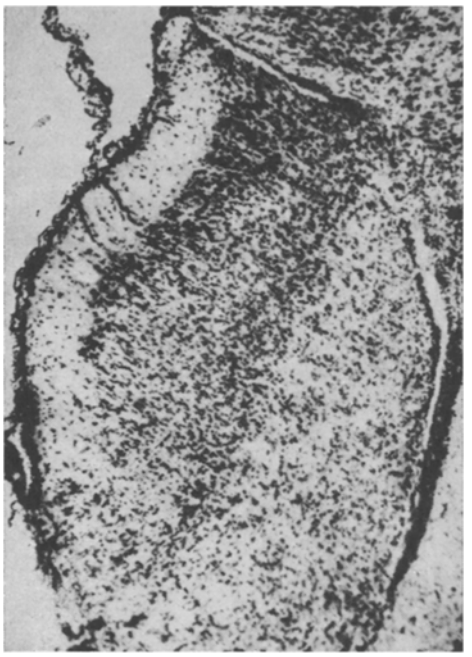


Fig. 2. Morphological changes in graft and in recipient's brain tissue after transplantation of brain areas. a) Area of necrosis in graft on 3rd day after operation. Hematoxylin-eosin, 100×; b) cellular barrier consisting mainly of glial cells together with a few young connective-tissue cells on the boundary between the graft and recipient's brain, 5th day after operation. Nissl's method, 180×; c) glial-fibrous capsule on boundary between graft and recipient's brain 15 days after operation. Hematoxylin-eosin, 180×; d) general view of transplanted tissue on 11th day after operation. Nissl's method. 180×; e) amitotic division of neuron. Nissl's stain, immersion; f) hypertrophy and hyperchromia of neuron of recipient's brain close to site of grafting. Nissl's stain, immersion; g) neuron of ordinary size in recipient's brain tissue some distance from site of grafting. Nissl's stain, immersion.

Macroscopically, until 7 days after the operation the graft was separated from the recipient's brain by a groove. By the 11th day and later the groove was no longer visible, but the grafted area could be deduced by the irregular shape of the prominences in the temporo-parietal region of the right hemisphere, surrounded by an oval or round depression of brain tissue. Because of this the brain was asymmetrical in shape, and this persisted to some degree until 45 days.

The morphological and regenerative changes in the graft and in the recipient's brain tissue were equally distinct in animals of the two groups.

On the first day after the operation changes of a dystrophic character took place in the graft and in the recipient's brain: segmental lysis of the basophilic substance, vacuolation of the cytoplasm, and swelling of the neurons. These changes were most marked near the line separating the graft from the recipient's brain and were less marked in the cerebral hemisphere to which the graft was applied. Besides dystrophic changes, necro-



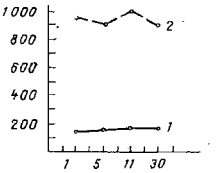


Fig. 3 Fig. 4

Fig. 3. General view of graft on 25th day after operation. Hematoxylin-eosin.

Fig. 4. Time course of change in number of binucleolar neurons in newborn rabbits after operation. 1) Control, 2) experiment. Abscissa, time after operation (in days); ordinate, number of binucleolar neurons.

biotic changes also were observed, as shown by the presence of areas of brain tissue with pale, swollen neurons, with indistinct outlines, without basophilic substance, and with vacuolated nuclei and cytoplasm, as well as of foci of necrosis (Fig. 2a), which were present both in the graft and in the recipient's brain. Nuclear debris could be observed in the foci of necrosis and the glial cells there were often preserved. Dystrophic changes were present in the brain for a long time but the necrobiotic changes mainly ended by the 7th day after the operation. Many free-lying erythrocytes could be observed between the graft and recipient's brain.

On the 3rd, 5th, and 7th days after the operation of a concentration of macrophages appeared between the graft and the recipient brain, moderate at first but later of considerable magnitude (Fig. 2b).

Starting with the first day after the operation proliferative activity of gliocytes was observed in the brain tissue and the graft, and took place by both mitotic and amitotic cell division. A clear decrease in the number of neurons due to death took place in the graft on the 3rd day and later, but the remaining neurons were structurally viable (Fig. 2d). On the 3rd day after the operation changes of edematous type, or to some extent of ischemic type began to predominate in the brain tissue: rims of pale spaces appeared around the cells, hyperchromasia of the neurons was detected, and changes in shape of the nuclei were observed in some cells, with translucency of the cytoplasm. On the 5th-7th day after the operation, on the boundary between the graft and the recipient's brain, besides macrogliocytes it was also possible to see a few neutrophilic leukocytes and cells of lymphoid type. On the 7th day elongated connective-tissue cells (fibroblasts and fibrocytes) also were present in this situation.

On the 11th and 15th days after the operation, processes of formation of a glial-fibrous capsule, which separated the graft from the recipient's brain, assumed predominance (Fig. 2c). Until the end of the experiment viable neurons could be seen in the graft, but their total number was reduced. The general appearance of the graft on the 25th day after the operation is shown in Fig. 3. By the end of the experiment the graft was completely separated from the recipient's brain by a surrounding glial-fibrous capsule.

Regenerative changes were minimal in the nerve tissue. No reliable patterns of mitosis were observed in the neurons. Occasional figures of amitosis was revealed in neurons by the presence of an incomplete con-

striction band in their nuclei (Fig. 2e). Cytotomy of the neurons during amitotic division was never once observed. Frequently in the histological sections neurons lying very close together could be seen. In some cases this picture could simulate one of amitosis, as was confirmed by a careful study of these cells.

Previously [2] the writer showed that after mild brain trauma in newborn rabbits an increase in size of the neurons in the injured hemisphere could not be detected visually. After transplantation of areas of the brain hypertrophy of some neurons was found visually both in the graft and in the recipients's brain (Fig. 2f, g). This process was particularly clearly defined close to the line separating the graft from the recipient's brain. Hypertrophy of these neurons occurred soon after the operation (1st and 3rd days) and attained its highest degree of development on the 11th and 15th days.

Some workers regard an increase in the number of binucleolar neurons as a sign of compensatory and adaptive changes in the brain [4, 5]. In our material an increase in the number of binucleolar neurons was observed during the first days after the operation, and persisted until the end of the experiments. The time course of the number of binucleolar neurons in newborn rabbits after transplantation of areas of the brain, compared with the normal situation, is illustrated in Fig. 4.

In the course of these experiments evidence was thus obtained that in rabbits aged 2-4 days transplantation of quite considerable areas of the brain is possible. Under these circumstances some neurons in the graft died, but some survived this situation and remained viable until the end of the experiments. Regeneration of brain tissue does not take place at the cellular level. Amitotic division of neurons can evidently take place in rare cases; mitosis was never found in neurons in our material. Marked hypertrophy of the neurons occurred. This indicates that regeneration of nerve tissue during postnatal ontogeny takes place entirely at the intracellular level.

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