ACTA NEUROBIOL. EXP. 1990, 50: 397-403

Symposium "Recovery from brain damage: behavioral and neurochemical approaches"
4-7 July, 1989, Warsaw, Poland

DEVELOPMENT OF VESSELS IN THE FOETAL CORTICAL TRANSPLANT DEPENDING ON THE PLACE OF GRAFTING IN THE RAT BRAIN

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Key words: foetal cortical transplants, ventricular grafts, callosal grafts, striatal grafts, development of vessels, rat

Abstract. Formation of new blood vessels within the graft is crucial for the survival of brain grafts. Moreover, it must occur rapidly to prevent ischaemic changes in the grafted neurons. A study was made of the development of the vascular system in the foetal cortical grafts depending on the place of grafting in the rat brain. Pieces of neocortical tissue from an 18-day old rat foetus were transplanted into the lateral ventricle, the striatum or the corpus callosum of 2 months old Wistar rats. The vascular system of the graft was visualized from coronal sections of the brain by means of Pickworth's technique 3, 7, 14 and 28 days after transplantation. After 3 days the vessels in the graft were absent. After 7 days the vessel pattern was poor and very simple and after 14 days the vessels formed large number of branches in the graft. After 28 days the pattern of the vascular network in the graft was similar to that of the vessels of the host brain. The size and branching of the vessels showed considerable variations depending on the localization of the graft.

INTRODUCTION

The formation of blood vessels and the integration of the transplanted tissue with the vascular system of the host brain is an essential factor in the survival of brain grafts. Moreover, this must occur rapidly in order to prevent ischemic changes in the grafted neurons (7, 10, 14). Apart from the nutritional function, the vascular system of the graft plays an important role in transmitting from the graft to the host's brain some substances produced and released by the graft. This is particularly important when the purpose of the transplantation is to supply the host's brain with neurotransmitters, neuromodulators or neurohormones and when the direct axonal connectivity from graft to host is poor or completely lacking.

No systematic evaluations of the graft vascular system development are available. Therefore we have undertaken investigations into the development of the vessels in a neocortical graft implanted into the brain of adult rats of the same strain depending on the localization of the graft in the brain.

The study is a continuation of investigations of the development of the vascular system in cortical grafts in rats with experimentally induced micrencephaly (5, 6, 18).

MATERIAL AND METHODS

The material consisted of 102 male rats of the Albino Wistar strain aged 2-3 months. They were divided into three groups according to the intended site of transplantation. In group I the transplantation was done into the striatum, in group II into the white matter and in group III ino the lateral ventricle.

Each group of animals was divided into four subgroups according to the survival time, which was 3, 7, 14 or 28 days.

For the implantation the parietal cortex of a foetus on the 18th day of gestation was used. After a cesarean section fragments of the foetal brain cortex were taken, their total volume being about 2 mm^3, and introduced in a stereotaxic apparatus of the David Kopf type into the paper brain structure of an adult rat.

Before decapitation the animals were placed for 60 s in a chamber filled with carbon dioxide to obtain a dilatation of the vessels and fill them better with blood. After fixation in 4% neutralized formalin the brains were cut serially into 150 μm sections on the freezing microtome. The vascular system of the transplant was visualized by Pickworth's method (13).

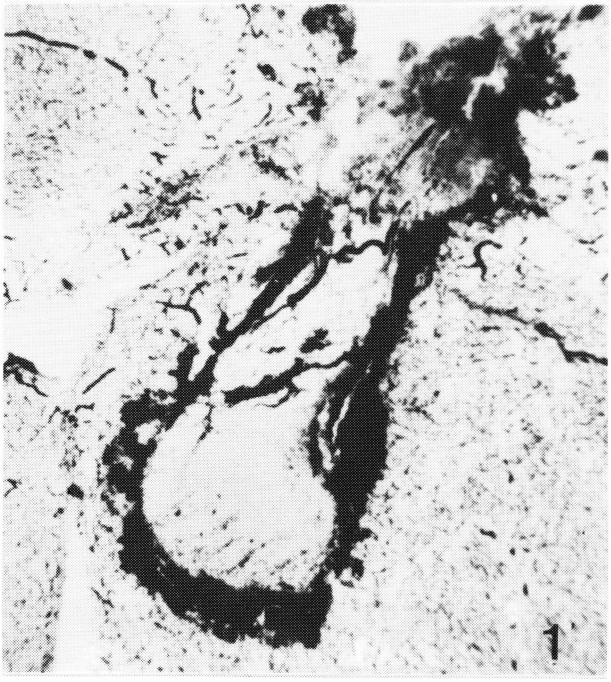

Fig. 1. Transplant in the striatum on the 3rd day after implantation deprived of blood vessels and surrounded by a wall of red blood cells, × 25.

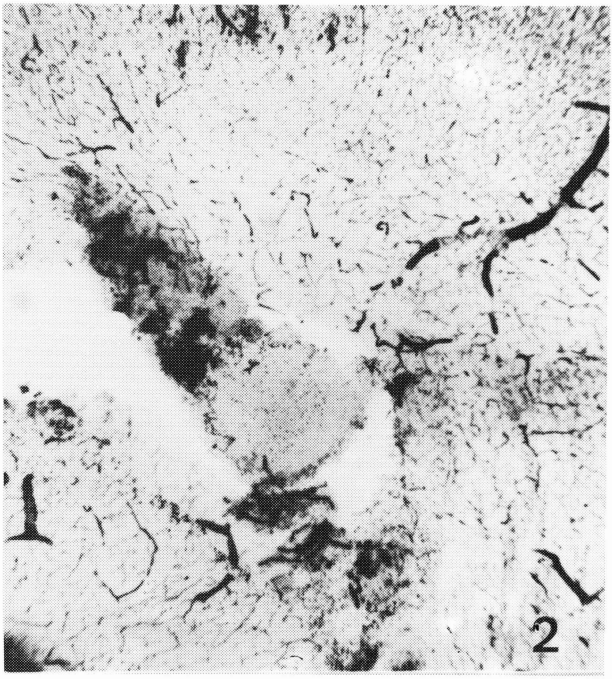

Fig. 2. Transplant in the striatum on the 3rd day after implantation. In the surrounding tissue distended blood vessels directed towards the graft, ×25.

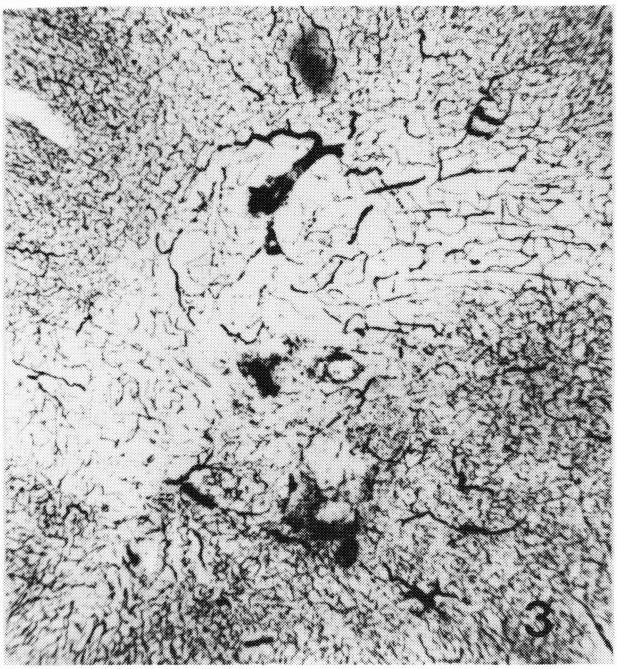

Fig. 3. Transplant in the corpus callosum on the 7th day after implantation with a developing vascular network within it. Some of vessels with a wide lumen surround the graft or penetrate towards its centre, × 25.

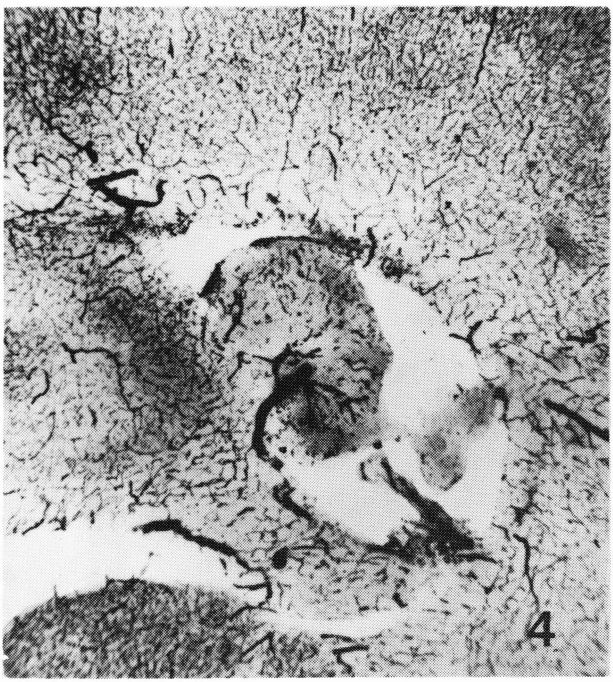

Fig. 4. Transplant in a lateral cerebral ventricle on the 7th day after implantation; a large blood vessel connects it with the host's brain, × 25.

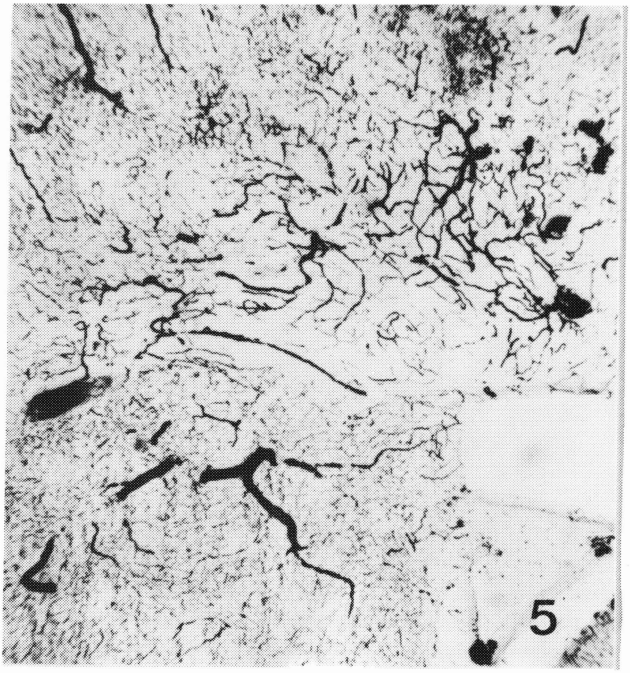

Fig. 5. Transplant in the corpus callosum on the 7th day after implantation. A chaotic unordered character of the vascular network in the transplant, × 25.

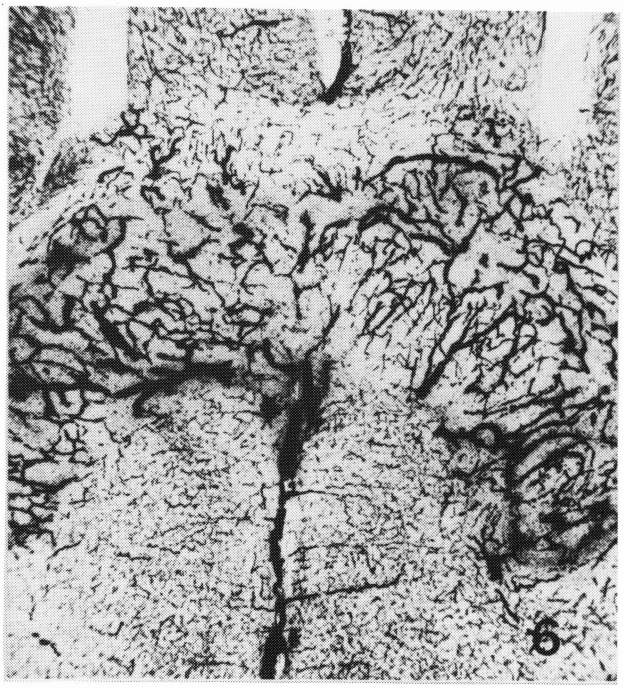

Fig. 6. Transplant on the 14th day after implantation localized in the corpus callosum, characterized by large dimensions and a dense network of large blood vessels, × 25.

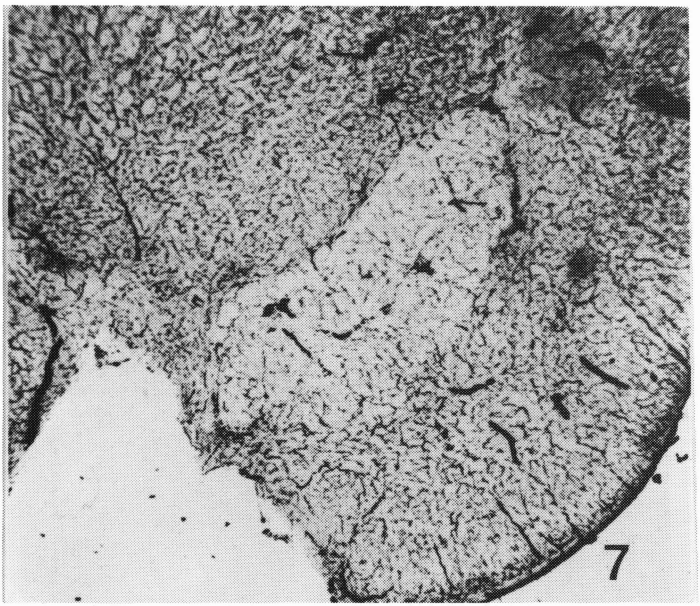

Fig. 7. Transplant on the 14th day after implantation situated in the ventral part of the striatum shows a minute dense mesh of vessels, × 25.

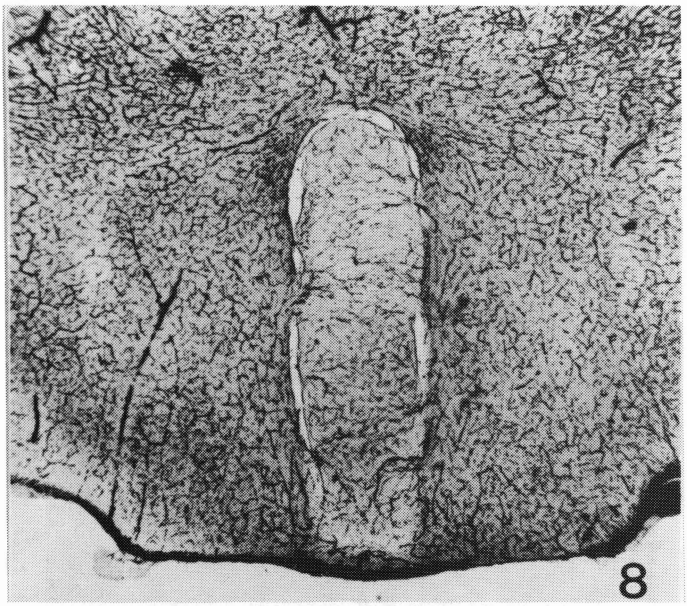

Fig. 8. Transplant on the 14th day after implantation localized in the ventricle III lumen, characterized by a dense vascular network. The arrangement of the vessels in the transplant is in general transverse, in some places integration of those vessels with the host vascular system is visible, × 25.

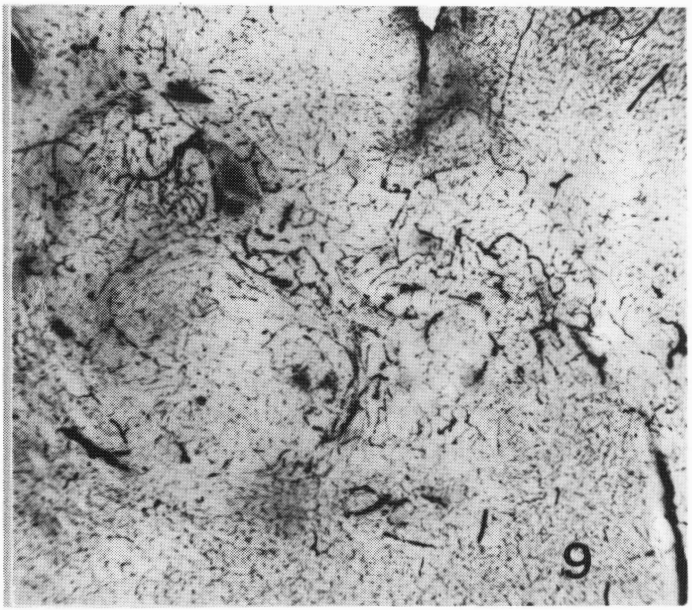

Fig. 9. Transplant on the 28th day after implantation localized in the striatum, comprising the white matter and the cortex is characterized by an irregular network of fairly large blood vessels, × 25.

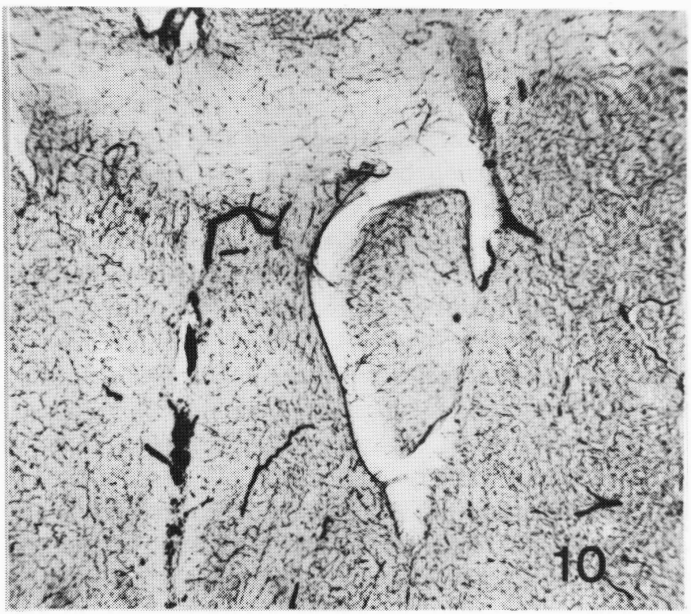

Fig. 10. Transplant on the 28th day after implantation, placed in the lateral cerebral ventricle exhibits a minute vascular network of density similar to that in the host brain, × 25.

When the implant had extensive dimensions, the greater part of the brain with the transplant was cut, frozen and stained by Pickworth's method, the remainder was embedded in paraffin and stained by the HE and the Klüver-Barrera methods.

RESULTS

Transplants from animals decapitated three days after implantation were almost devoid of blood vessels. Around them a wall of red blood cells could be seen as a consequence of the operation procedure. Especially within the striatum the extravasated red blood cells around the transplant separated it sometimes from the surrounding tissue (Fig. 1). In some places large vessels of the host could be seen around the transplant, directed towards it (Fig. 2).

Transplants surviving for 7 days exhibited symptoms of a vascular network developing within them and on their edge. On the periphery of a transplant there appeared large vessels surrounding it (Fig. 3) or constituting a link between the transplant and the host's brain (Fig. 4). The course of the vessels in the transplant was disorderly and chaotic (Fig. 5). In general, the vessels within the transplant were of a larger calibre than those in the surrounding tissue (Fig. 5), but they had few branchings. Owing to this difference in the structure and architecture of the vessels the transplant was in most cases distinctly separated from the surrounding tissue.

After 14 days of survival in some transplants, especially in the white matter (corpus callosum), there developed dense network of large blood vessels with numerous branchings (Fig. 6). Transplants in this localization were sometimes of large size. Those situated in the striatum exhibited a delicate network of vessels with diameter similar to or smaller than that of vessels in the surrounding tissue (Fig. 7). This angioarchitectonic difference made it possible also in this case to distinguish clearly the boundaries of the transplant. Similar features of the delicate mesh of small vessels, sometimes arranged transversely, was observed in transplants placed in the ventricle III (Fig. 8). It should be mentioned that placing the transplants in ventricle III had not been planned. The foetal tissue had been implanted into the lateral ventricle. Its translocation probably occurred because of its flowing with the cerebrospinal fluid.

Twenty-eight days after transplantation the transplants in the striatum were of large dimensions, occupying almost the whole hemisphere. The vascular network within them continued to be chaotic and showed wide angioarchitectonic differences as compared with the surrounding tissue (Fig. 9). It was built of vessels of various diameters, similar to or larger than the vessels of the host's tissues.

On the other hand, transplants in the lateral ventricle were never large, probably because their growth was limited by the ventricle walls. Their vascular network was minute and delicate, similar in density to that of the host's vessels. Those transplants adhered to the ventricle walls or were attached to them. Some few vessels connected the transplants with the surrounding tissue (Fig. 10).

DISCUSSION

During foetal life the first brain vessels appear in the rat on the 12th day (3). In the present investigations the embryonal cortex was taken for transplantation from the brains of 18-day foetuses. Therefore, it may be assumed that the transferred tissue already contained a vascular system, but it was not fully developed yet. Comparatively little is known about the factors controlling the growth and development of the vascular network in the transplant. It is not clear what mechanism initiated the vascularization process, what was contributed by the host's vascular system and by the transplant itself or what factors determine the final pattern of that system in the grafted tissue fragment (14, 15).

The origin of the vessels in the transplant, especially the question whether they penetrate from the host's brain or are derived from the tissue of the donors may be determined by the use of monoclonal antibodies as marker of the cells of the vascular mesothelium. Baker et al. (1) applied this method for finding the origin of the transplant vessels in cross-species grafting. They demonstrated that integration of the host's vascular system with that of the transplant begins around the 10th day after grafting. Full integration takes place about the 30th day. Baker et al. (1) also demonstrated that the presence of the donor's vessels in the implant can still be detected as late as four months after transplantation. The authors never noted the transplant vessels growing into the host's brain.

The use of immunochemical methods revealing the components of the basement membrane of the vessels (laminin or type IV collagen) confirm that in the early phase the graft has its own vascular system, which at first shows no links with the host's system. At a later period some vessels of te donor tissue undergo necrosis and a new vascular network forms within the transplant. According to Raisman et al. (14), the origin of the cell from which the newly forming vessels arise — whether from the donor or the recipient tissue — cannot be ascertained.

Some light may be thrown on this problem by the use of [^{3}H]thymidine labelling donor tissue (14). The method of labelling allows us to visualize that the mesothelium cells of large marginal vessels of the

transplant are derived from the grafted tissue and so is the mesothelium of the newly formed arterioles. On the other hand, the cell nuclei of the smooth muscle of the arterioles did not show any labelling with the marker. In this connection Raisman et al. (14) suggest that the vascular system of the graft is "a chimera of donor and recipient vessels since its arterioles show the presence of unlabelled smooth muscle cells originating from the host's brain and of labelled mesothelial ones derived from the implanted donor tissue."

A good method of visualizing cerebral capillaries is the histoenzymatic reaction for alkaline phosphatase. The site of the activity of the enzyme is the plasmatic membrane (plasmalemma) of the mesothelium of the capillary vessels (16). Unfortunately this technique is limited to the visualization of the capillaries and does not show larger vessel.

The Pickworth technique (13) is somewhat superior to the one described above. The red blood cells, present in all vessels, react with benzidine and sodium nitroprusside, changing their colour to black under the influence of perhydrol. They are a good marker of the whole vascular system. The continuity of the vessel picture, however, depends on the degree of their being filled with blood. Placing the animals before decapitation in a carbon dioxide atmosphere causes hyperemia and improves the filling of the vascular system. On comparing the advantages of both these techniques of vessels visualization it was decided to apply the Pickworth technique.

There is evidence that changes in the vessels of the nervous system occur continuously throughout the whole life (17). The transplant is a peculiar form of nervous tissue which has its own program of development and ageing. The growth of the graft and the development of its vascular network is probably the resultant of the development program of the implanted neurons, the host neurons and other factors connected with the transplantation procedure. Those factors include anoxia resulting from the interruption of the continuity of the vessels while collecting the material (11), the impairment of the blood-brain barrier (4, 8), the penetration of mitogenic substances from the lesioned neurons and axons (4) and the appearance of neuron-survival-promoting factors (12). Angiogenetic factors probably also play a certain role in the reconstruction of the vascular network (2, 9).

As regards the development of the vascular network in the graft and its localization, the transplants develop unhindered in the white matter and the striatum, reaching large dimensions. Their vascular system consists of large vessels chaotically dispersed. Frequent large marginal vessels develop on the periphery, surrounding the transplant and giving numerous branchings or linking it with the surrounding tissue.

On the other hand, the grafts growing in the lateral ventricle or ventricle III do not attain a large size owing to the limiting ventricle walls. The vascular network within them is delicate but fairly dense. In arrangement and density it is similar to the surrounding tissue.

To sum up, it may be said that on the third day after grafting the embryonal cerebral cortex into the brain of an adult rat only a few blood vessels could be detected in the graft. The development of the vessels appears on the 7th day after implantation. The vascular network, however, is still scarce and chaotic and the vessels show few branchings. It is only as late as 28 days after implantation that the pattern of the vascular network becomes in some grafts similar to that in the host brain.

This investigation was supported by Project CPBP 06-02.II.3.3. of the Polish Academy of Sciences.

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