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Neural Crest Replaced by Gastrula Ectoderm in Amphibia

Effect on Neurulation, CNS, Gills and Limbs

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Summary. Early Axolotl gastrula ectoderm was grafted into early Triturus neural stages in place of excised neural folds at the gill and anterior trunk level.

Macroscopically the young graft behaves like normal neural fold material: it follows the closing host neural plate to the dorsal midline, folds into the host's interior and, especially in the gill region, moves ventrad beneath the host's epidermis. These movements cannot be interpreted as active migration. They are the result of passive displacements by morphogenetic forces inside the embryo.

Histologically the graft differentiates into neural and neural crest tissue, the quantitative relation depending on the host's region. At the gill level the graft forms mesenchyme and other neural crest elements and hardly any neural structures. In the trunk about one half of the graft forms a secondary, surplus CNS. Problems of induction, differences between gill and trunk region and between graft and normal fold behaviour are discussed.

Limbs develop normally. The dorsal layer of the blastema is furnished by graft cells. Host and graft tissue can stay separate or form a combined blastema.

Key words: Heteroplastic heterochronic grafting – Morphogenetic movements – Induction – Differentiation.

Introduction

In an earlier report (Rollhäuser-ter Horst 1975) we described the behaviour of Amphibian neural crest cells in the neck and anterior trunk region during early larval development, studied by means of isotopic exchange of Triturus and Ambystoma neural folds. Donors and hosts were of the same developmental stage (Harrison stage 14–15). In some unreported cases, however, Ambystoma material of younger stages (Harrison stage 13) was grafted into older hosts

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(stage 15). No significant difference was observed, the younger material behaved exactly as older fold material. We began to doubt whether under these circumstances "activities" of neural fold material such as closure of folds and the following ventrad migration of neural crest cells could be interpreted as active accomplishments of this material or whether they are passively moved by morphogenetic forces inside the host embryo.

Furthermore we wanted to test the inductive abilities of the mesoderm underneath the neural folds. As it constitutes the interphase between neuralizing archenteron roof and epidermalizing lateral mesoderm is should be a strong inducer of neural crest and its derivatives (Rollhäuser-ter Horst 1977, 1979).

We excised neural folds of Triturus neural stages and grafted instead strips of young reactive ectoderm from Ambystoma gastrulae.

Material and Methods[1]

Host Embryos. Triturus taeniatus, Triturus alpestris, neural stages with wide open neural plate (Harrison stage 14–15).

Donors. Ambystoma mexicanum gastrulae with beginning blastopore (Harrison stage $10^1/_4$).

Operation. Neural folds of hosts were excised without damage to underlying mesoderm and replaced by strips of Ambystoma ectoderm (Fig. 1). Varying length. Bilateral excision, bilateral or unilateral graft.

Culture. 7 to 9 days at 19° C.

Medium. $\frac{1}{2}$ Holtfreter solution (Holtfreter 1931) without Na H CO_3 plus 20,000 I. U. penicillin/1,000 ml.

Fixation. Bouin.

Staining. PAS.

Results

The young grafted Ambystoma ectoderm has considerably larger cells than the excised Triturus neural folds. Nevertheless it blends readily into the older host and does not interfere with neurulation. 24 hours after the operation the neural plate is closed and the grafted material has disappeared from the embryo's surface. From now on dark strands of Ambystoma cells move – or rather are moved – ventrad beneath the light Triturus epidermis (Figs. 2, 3). This stream of graft material is most prominent in the gill region but thin strands and/or single cells can also be observed in the ear and anterior trunk region. The picture resembles the streams of dark cells observed in Triturus embryos whose neural fold has been replaced by an Ambystoma neural fold (Rollhäuser-ter Horst 1975, Stone 1922 a.o., for older literature see Hörstadius 1950). Larvae with unilateral graft show dark strands of graft tissue on the opposite side also.

Further development is not quite as normal as in our previous series with exchanged neural folds. Rhombencephalon and neural tube in the operated

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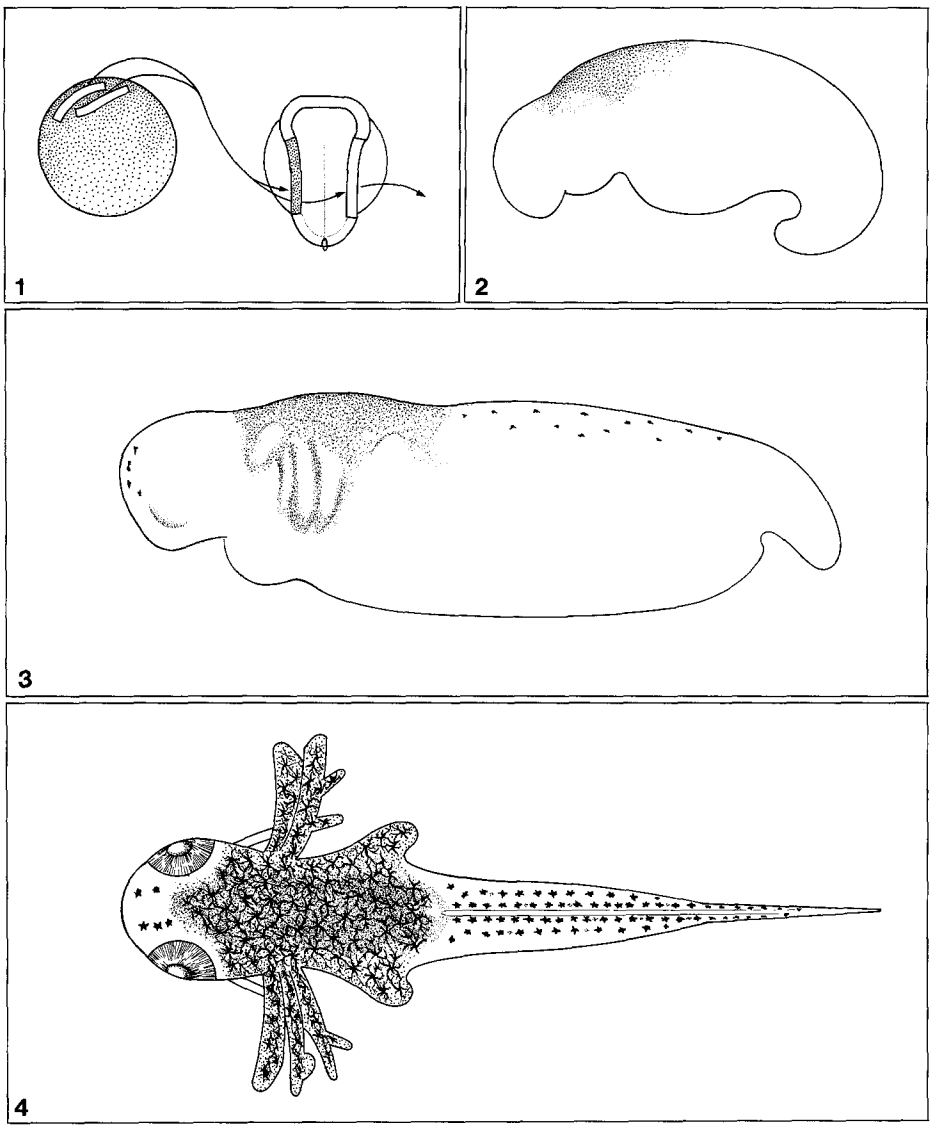


Fig. 1. Diagram of operation. Excision of both *Triturus* neural folds, replacement by one or two Ambystoma gastrula ectoderm grafts

Fig. 2. *Triturus taeniatus* host, 2 days after operation. Dark grafted ectoderm has rolled into embryo during neural fold closure. Remains visible through light Triturus epidermis. Beginning ventral movement of dark tongues in gill region

Fig. 3. *Triturus taeniatus* host, 3 days after operation. Dark grafted tissue forms solid stripes underneath thin gill epidermis, thin flow of graft material around ear and pronephric (limb bud) region

Fig. 4. *Triturus alpestris* host, 8 days after operation. Gills completely filled with graft cells. Posterior head, gills, anterior trunk and limb buds Ambystoma pigmentation. Operated trunk area without dorsal fin, broader back on account of surplus CNS. Anterior head, posterior trunk and tail host pigmentation (band pattern)

area are doubled (unilateral graft) or tripled (bilateral graft), the dorsal fin is missing or rudimentary, gills can be short and stubby. Pigmentation, however, is again donor specific, though slightly delayed. Limbs develop normally (Fig. 4).

33 embryos were raised for 7 to 9 days and prepared for histological examination.

Most grafted Ambystoma cells can be identified microscopically, especially where they lie close together (central nervous system = CNS, masses of loose or condensed mesenchyme). They are larger in size than Triturus cells, contain more embryonic cell pigment and often show a spot of heteropigment near the nucleus. Melanophores are larger than those of host origin, they have longer processes extending in all directions and are more loosely connected with the epidermis.

The grafted ectoderm develops into two general directions: neural tissue and neural crest derivatives. The quantitative relation between these tissues varies in different regions of the host.

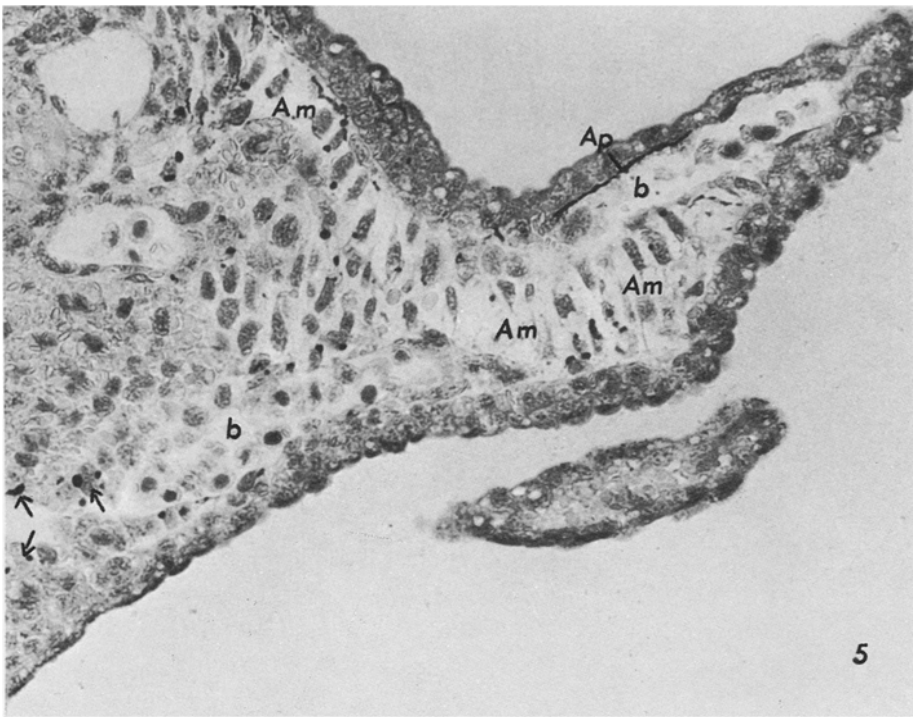
In the gill region, where most of the graft material leaves the dorsal midline soon after fold closure, almost 100% develops into neural crest elements (Fig. 5). Gills are completely filled by Ambystoma mesenchyme and melanophores. They can be of surprising perfection considering the unripe stage of the graft at the time of their outgrowth. Proximally the head shows large amounts of Ambystoma mesenchyme and strands of graft cells extend far into the mandibula (chondrocytes? tooth papillae?). In cases of unilateral grafting a considerable number of Ambystoma cells cross the dorsal midline and can be found in the opposite gills.

At more anterior and posterior levels of the embryo, where initial ventrad shifting of graft material is less conspicuous, the relation neural tissue/neural crest derivatives is higher, in the trunk approximately 1:1. Here neuralized Ambystoma material forms one (unilateral graft) or two (bilateral graft) surplus CNS, closely apposed to the host CNS and showing the same regional characteristics (Fig. 6). They accompany the host CNS as individual structures, fusion as with implanted fold material (Rollhäuser-ter Horst 1975) does not occur. Ganglia and nerves can consist of and/or be accompanied by graft cells.

Ambystoma neural crest cells reach all sites normally occupied by neural crest elements in spite of their comparative youth. Our special interest concentrated on the anterior trunk and limb bud region. Here Ambystoma neural crest cells underline the lateral Triturus epidermis, as mesenchyme and corium cells they migrate ventrad down to the host's belly midline. Ambystoma melano-

Fig. 5. 7 days after operation, unilateral graft, cross section gill level, 1:225. Short *Triturus* gill filled with *Ambystoma* graft mesenchyme (*Am*), young *Ambystoma* melanophores (*Ap*) and wide blood vessels (*b*). Graft cells fill proximal part of head and extend into mandibula, crossing over to other side of head (*arrows*). No graft CNS

Fig. 6. 8 days after operation, unilateral graft, anterior trunk level, 1:208. *Triturus* host notochord (*Tn*) and neural tube (*Tt*). *Ambystoma* graft forms surplus neural tube (*At*). *Ambystoma* melanophores line dorsolateral epidermis (*Ap*), no band pattern. *Ambystoma* melanophores and mesenchyme furnish dorsal layer of limb bud (*arrows*)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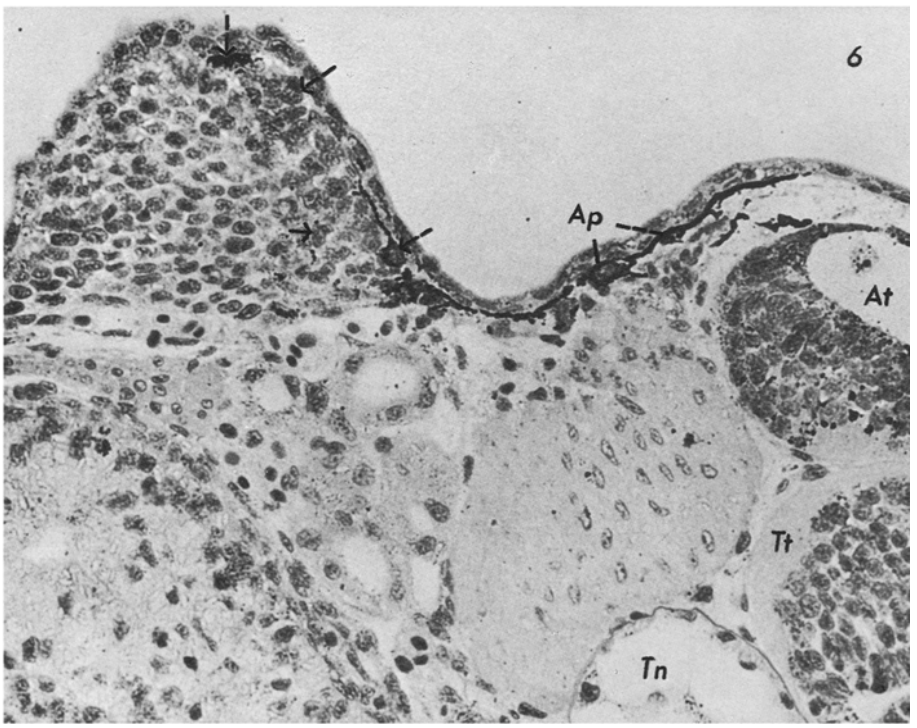
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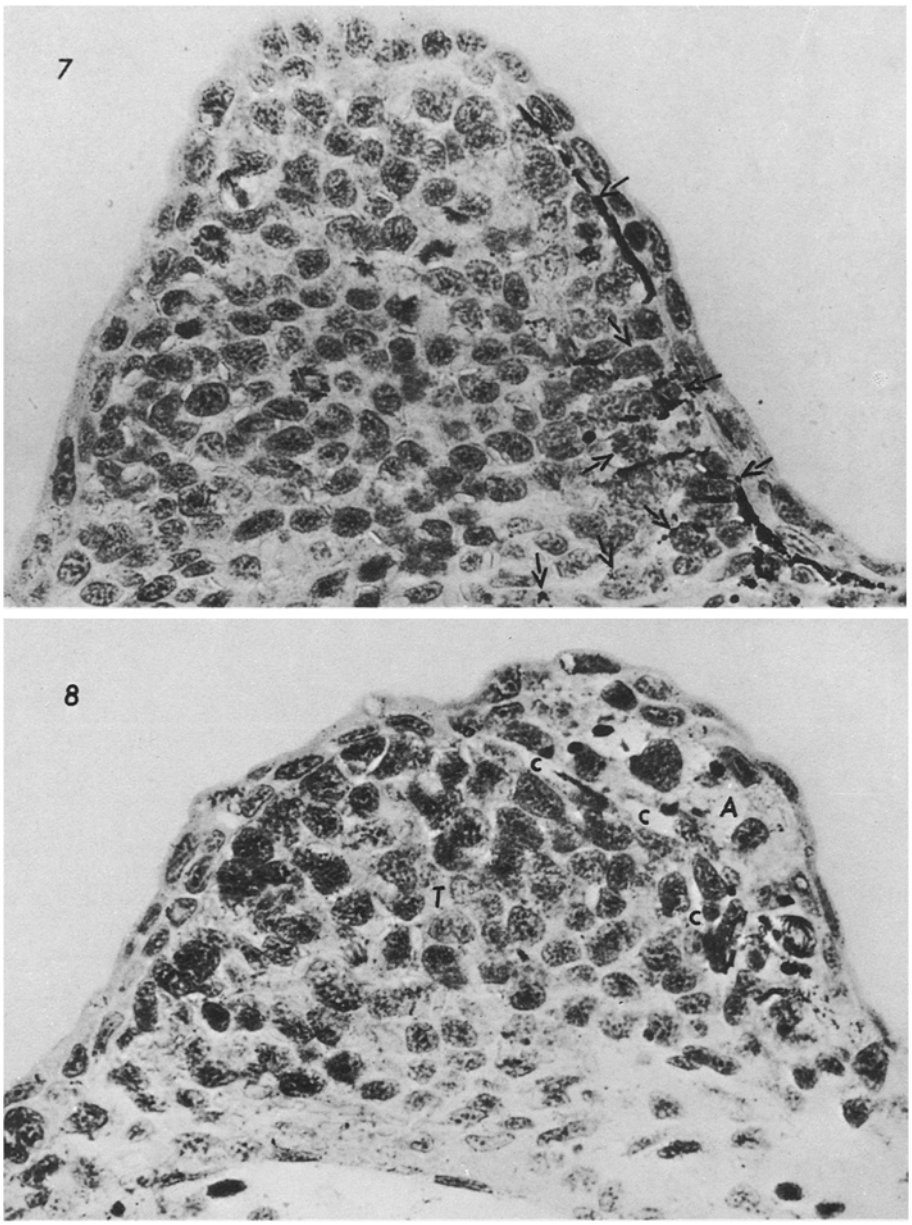

Fig. 7. 8 days after operation, limb bud, 1:360. Grafted *Ambystoma* cells (*arrows*) closely interwoven into dorsal part of *Triturus* limb blastema

Fig. 8. 8 days after operation, limb bud, 1:400. *Ambystoma* graft cells (*A*) furnish dorsal cap on *Triturus* limb blastema (*T*). Tissues separated from each other by narrow cleft (*c*)

phores abound between dorsal midline and flanks. They do not follow the host's band pattern but they look larger and healthier in the host's pigment zones. Limb buds show large amounts of graft cells. They either form the dorsal part of a combined blastema (Fig. 7) or are slightly separated from the host mesoblast (Fig. 8), forming an Ambystoma cap or wedge on the dorsal side of the Triturus blastema. Mesenchyme, melanophores and corium cells contribute to this Ambystoma layer underneath the Triturus epidermis.

Discussion

1. Morphogenetic Events

Early gastrula ectoderm grafted in place of an older host's neural folds undergoes almost the same spectacular movements and shiftings as normal neural fold material. We therefore doubt that these movements – closure of neural folds and the following ventrad shifting – are active achievements of these tissues. Closure of neural folds is a consequence of contraction and closure of the neural plate. The young graft is passively pulled toward the dorsal midline and into the embryo in spite of its youth and undetermined stage. The same applies to the ventrad shifting after neural plate closure, especially prominent in the gill region. This shifting is generally considered to be an active migration of neural crest cells toward their final destination (Stone 1922, Raven 1936, Hörstadius 1950, a.o.). In the present experiments the shifting cells ontogenetically are pre-neurular. They are not yet determined to move in a special direction in their new surroundings. The unripe tissue is pulled or pushed to the proper sites by morphogenetic forces inside the embryo.

Hörstadius and Sellmann (1946) have already raised the question "which tissues call or guide the ectomesenchyme along its pathway" (Hörstadius 1950). The present unripe material needs more than a guidance. It is passively displaced regardless of its youth and lack of proper determination.

Dorsal crossing of neural crest material to the embryo's opposite side is also independent of the tissue's ontogenetic stage. The same amount of unripe ectoderm cells is dispatched to the other side as of ripe neural fold grafts (Rollhäuser-ter Horst 1975). The crossing thus is not controlled by the graft – nor by normal neural crest – but by factors inside the host.

We do not question the strong migratory tendency of ripe neural crest cells. The early ventrad shifting of neural fold tissue in the gill region, however, seems to be a different process, independent of the material involved. Similar passive cell transport may – to a lesser extent – occur all along the embryo's axis. In later stages neural crest cells ripen and migrate actively. Both phenomena – passive transport and active migration – interact in normal development.

2. Induction and Differentiation

The inductive influences upon the grafted material are neural and ectomesenchymal as expected. Neural influence emanates from the adjacent neural plate

as well as from the edge of the archenteron roof (Mangold 1933, ter Horst 1948, a.o.). Exact evaluation of inductive processes in these complex surroundings, however, is hardly possible. The grafted material is in constant motion and during its journey comes in contact with many unknown stimulants (Weston 1970, Le Douarin et al. 1977). Three aspects will be discussed.

a) Different Amounts of Induced Neural Tissue Along the host's Axis. The strips of grafted ectoderm were of the same width throughout their length. All along their medial edge they have direct contact with adjacent neural plate and underlying archenteron roof. Thus the initial neural stimulus should be alike at all levels during neural plate closure. After closure, however, events differ along the host's axis. In the gill region most of the grafted material is swept ventrad, away from the CNS' neuralizing influence. Here only little neural material develops but great amounts of ectomesenchyme. Three factors may be responsible and/or interact:

– initial neural stimulus in the gill region is weaker and/or ectomesenchymal stimulus stronger than in the trunk;

– neural stimulus is shorter as plate closure starts in the neck and gill region;

– after displacement away from the CNS the weakly neuralized graft cells experience a deneuralization and redetermination.

b) Morphology of the Induced Trunk CNS. In the anterior trunk ventrad shifting is less pronounced and approximately $\frac{1}{2}$ of the graft tissue develops into a surplus CNS, directly apposed to the host CNS. Both systems remain individual, separate structures. They do not fuse as in the case of fold exchange (Rollhäuser-ter Horst 1975), when graft material furnishes the most dorsal part of a combined, normal CNS. At the time of operation the host neural plate is definitely determined and in the process of contraction and closure. It takes no notice of the adjacent unripe graft but behaves as in case of fold excision. It overcomes the apparent lack of fold material by regulative abilities and forms a smaller but complete CNS of its own. The neuralized graft has no choice but to form a secondary CNS. Later fusion is inhibited by meninges.

c) Comparison with Normal Neural Folds. At the time of operation normal neural folds are solidly determined to form neural tissue and ectomesenchyme at the gill as well as at the trunk level as shown by many explantation, implantation and transplantation studies. The neural part of fold tissue adheres closely to the adjacent neural plate. It resists ventrad displacement in the gill region as well as isolation in the trunk region and instead furnishes the most dorsal part of the CNS. Normal, complete development of dorsal fin, meninges, ganglia and other structures depend on this fold part of the CNS.

3. Limb Buds

Limb buds show a high amount of graft cells: melanophores, corium cells and dense mesenchyme. This corresponds with the results of fold exchange

(Rollhäuser-ter Horst 1975), when neural crest elements underline the epidermis of the future limb bud before a blastema develops and additional neural crest cells continue to immigrate into the developing blastema from dorsal directions. In the present study fusion of host and donor tissue does occur but can also be disturbed. Differences in age and ripeness still seem to represent an obstacle. This obstacle, however, is not as serious as in the case of the CNS, as combined blastemas can develop. Limbs are younger structures than the CNS and by the time of their budding the difference in age between host and graft seems to be almost overcome. Furthermore a limb bud is a more "open system" than the rigidly closed and meninge protected CNS. Later fusion of originally separated host and graft tissue seems possible.

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