INTRODUCTION

The central nervous system of adult mammals is known to lack regenerative capacity when a lesion is inflicted upon it. This phenomenon seems to have directed the interest and determined the perspective of researchers in the past, who employed the technique of transplantation of nervous tissue like spinal ganglia or of segments of peripheral nerves in the brains of adult mammals. Findings in these studies, in almost all cases, have been viewed in relation to the questions whether the transplanted nerve cells show any regenerative growth of their processes and whether the transplanted nerve grafts have any 'neurotropic' influences upon the host tissue. Ranson, Saltykow and Altobelli were the first investigators to study the fate of transplanted cortical tissue in the cerebral cortex of adult mammals. More recently Wenzel et al. have found that in adult mice transplanted slabs of cortex in the brains of host animals show various phases of differentiation of the nerve cells and sprouting of their processes. Ranson, Dunn and LeGros Clark, in their studies on the survival of transplanted spinal ganglia and fetal nervous tissue into the brains of young animals emphasized that the employment of young animals, whose brains are still growing and differentiating, assures the differentiation of transplanted neuronal elements.

All these studies have used standard histological stains, including silver stains, and possibly because of this the findings of these studies have been treated with some degree of skepticism. Furthermore, with these staining techniques the investigators could not detect active migration of the transplanted neuronal elements, even when it might have been present, and their incorporation as normal cells into the brains of host animals. In our studies, which are more directed towards the problems of migration of the transplanted precursors of nerve cells into the host brain, and their proliferation and differentiation in the new environment, we have employed young...
animals as the recipients in whose brains various structures like cerebellum, hippocampus and olfactory bulbs show extensive postnatal neurogenesis\textsuperscript{2,4}. In order to distinguish the host cerebellar cells from the donor cells, we labelled the precursors of neuronal elements in the donor animals before transplantation and studied their fate in the brains of the host animals autoradiographically.

**MATERIAL AND METHODS**

Laboratory-bred Long-Evans hooded rats were used as donors as well as recipients; they were 7 days old when surgery for transplantation was performed. Eight animals from one litter, that served as donors, were injected with \(^{3}\text{H}\)thymidine intraperitoneally (dose, 10 \(\mu\text{Ci/g body weight; specific activity: 6.7 Ci/mM, 1 mCi of the radiochemical dissolved in 1 ml of isotonic saline. After 1 h, these animals were anesthetized and surgically prepared for removal of slabs of the cerebellum for transplantation. These slabs were washed thoroughly twice in Ringer’s solution before transplanting them in the cerebellum of the host animals to insure that they did not contain any unutilized radiochemical. The recipients were anesthetized with Metofane (methoxyflurane) for the surgery. In each case the cerebellum was exposed and a longitudinal cut was made in the vermis, and then the transplant was gently pushed inside the slit. The cerebellum was covered, the skin sutured and the incision coated with 3% celloidin. When the animals had recovered from anesthesia, they were placed back with their respective mothers. On the average one donor provided cerebellar tissue for transplantation for 2–3 recipients. A total of 16 recipient animals were operated on for transplantations.

After 3 h, 1, 2, 4, 6, 10 and 16 days following surgery, two or more animals at each
survival stage were sacrificed and perfused with 10% neutral formalin. The brains obtained from these animals were kept in the fixative for 2 weeks before they were cut in coronal blocks and embedded in Paraplast. The blocks containing the cerebellum were cut serially at 8-10 μm thickness. The sections were deparaffinized, coated with Kodak NTB-3 nuclear emulsion and sealed in light-proof boxes containing Drierite as the dessicant for exposure at 5°C. After 10 weeks the slides were developed and stained with cresyl-echt violet.

RESULTS

Nature of the transplants

In the brains of animals that survived for 3 h following surgery, the transplants could be identified by the presence of intensely labelled cells (Fig. 1). In many fragments of the transplants the cytological organization of cerebellum of a 7-day-old rat was recognizable. Although cytological characteristics of the intensely labelled cells in the external and internal granular layers could not be determined, almost all the unlabelled cells in these regions appeared normal. This suggested that cells in the proliferative and migratory zones of the external granular layer, and the mitotically active cells and undifferentiated granule cells in the internal granular layer of the transplants could survive and remain viable. Pyknotic cells, if any, were found mainly along the borders of fragments of the transplants.
Fig. 3. Low magnification view of the transplant. Within this, regions showing pathological involvement (path), islands of labelled cells (isl), normal cytoarchitecture (norm) and streams of migrating cells (migr) can be identified. 4 days survival. × 8.

In one animal, in addition to the fragments of the transplant, few intensely labelled cells were found attached to the lesioned surface of the cerebellum, and they were regarded as the dissociated labelled cells from the transplant (Fig. 2). However, within the host cerebellum no labelled cells were found, which indicated that free $[^3H]$thymidine in the transplant was not present at the time of transplantation of the slabs.

**Pattern of changes in the transplants**

In the animals that survived for 2–4 days postoperatively, the transplants were still present. During this period some small fragments of the transplants appeared degenerated and showed the presence of phagocytic activity. Few intensely labelled cells, a large number of leukocytes, involvement of pial cells and infiltration of capillaries characterized these degenerated fragments.

Very close to the lesioned regions in the host cerebellum large portions of transplants showed the following changes (Fig. 3). These changes may be described in 3 categories.
Fig. 4. A degenerated fragment of the transplant with an island of normal looking cells, some of which appear intensely labelled (arrow a). Another island (arrow b) appears to be detached from the degenerating portion of the transplant, but it is completely surrounded by the pia membrane. Note pathological involvement of tissue surrounding these islands. 2 days survival. × 15.

Fig. 5. An island of normal looking cells (arrows) surrounded by the degenerating portions of the transplant. Cells in this island appear round and compactly organized reflecting the cytoarchitecture of the proliferative zone of the external granular layer. 2 days survival. × 40.

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Firstly, there were diffuse and uncircumscribed regions showing degenerative changes, and these were characterized by the absence of normal looking cerebellar cells, and the presence of pyknotic cells, necrosis, a large number of leukocytes and extensive capillary elaboration. Within these regions of degenerative activity highly enclosed islands of labelled as well as normal looking unlabelled cells were found (Fig. 4). Cytologically these islands of cells appeared normal and viable. In these islands of cells very few lightly labelled cells were observed, which suggested that although these islands were cytologically normal there was very little proliferative activity taking place in them (Fig. 5). To what extent these islands of normal cerebellar cells could survive over a protracted period of time and contribute to the transplantation of their component cells into the host cerebellum could not be determined.

Secondly, there were portions in the transplants which were characterized by aggregates of normal looking cerebellar cells maintaining the cytological organization similar to that of the external granular layer of a normal cerebellum (Fig. 6). In these aggregates very few intensely labelled cells, many unlabelled cells and few mitotic cells were observed. This suggested the possibility of continuance of proliferative activity in these cells. The cells in these localized aggregates did not appear elongated and spindle-shaped, and it indicated that they were not in a migratory state. The im-

![Fig. 6. A normal appearing region of the transplant. Its cytological organization is very similar to that of the proliferative zone of the external granular layer. Few intensely labelled and lightly labelled cells (arrows) and a mitotic figure (arrow m) may be noted. In the lower left region a stream of migrating cells emerging from this portion of the transplant is seen, and within this stream two labelled cells may be identified (arrows). 4 days survival. × 32.](Brain Research, 38 (1972) 233-249)
Fig. 7. Stream of migrating cells emerging from the transplant. It appears to branch at two points, and at these points clusters of intensely labelled cells are seen (arrows). Few lightly labelled cells within the core of the stream may be noted. Notice that within the stream no pathological reaction is present. 4 days survival. × 32.

Fig. 8. Stream of migrating cells in its mid-course. The spindle shape of the migrating elements is evident. A number of intensely and lightly labelled cells (arrows) and one mitotic figure (arrow m) can be identified. At the bottom, infiltration of capillaries along the borders of the stream is seen and the migrating cells appear to glide past these capillaries. 4 days survival. × 32.
Important observation made on these aggregates of normal cells was that they were not tightly circumscribed by pia membrane and instead were continuous with masses of cells which were migratory elements.

Thirdly, continuous with the aggregates of normal cells in the transplants, described above, were the large numbers of cells organized in thick streams of mi-

Fig. 9. The labelled cells of the transplant (arrows) attached to the medullary layer of the host cerebellum, which serves as one of the paths of migration. Such transplanted elements after their migration appear to settle in the basal layers of the internal granular layer of the host cerebellum. egl, external granular layer; igl, internal granular layer; mdl, medullary layer; ml, molecular layer. 2 days survival. \( \times 32 \).

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Fig. 10. A stream of migrating cells containing few labelled and many unlabelled cells in the medullary layer of the host cerebellum. Due to crowding of the migrating cells medullary layer is not distinct, but it can be identified by the presence of internal granular layer flanking on both the sides. Some of the intensely and lightly labelled cells seem to have settled in the basal aspects of the internal granular layer. egl, external granular layer; igl, internal granular layer; ml, molecular layer. 2 days survival. × 32.

migrating cells (Figs. 7, 8). Evaluation of serial sections revealed that in all likelihood the origins of these streams of migrating cells were the aggregates of normal looking proliferating cells. Within these streams of migrating elements a small number of cells was found intensely labelled and the rest appeared unlabelled; frequently mitotic cells were found in their course. Judging from the over-all cytological organization of the transplants, all the component cells of the streams of migrating cells, labelled as well as unlabelled, were considered as the cells arising from the transplanted precursors of nerve cells. The streams of migrating cells appeared to follow tortuous courses, extend outside the transplants and penetrate into the host cerebellum at various points of contact between the transplant and the host tissue. However, at those regions of contact, where the pia membrane had intervened between the transplant and the host tissue, the migrating cells did not appear to penetrate into the host cerebellum.

*Paths of migration of the donor cells into the host cerebellum*

Generally speaking, sites of penetration of the streams of migrating cells from the transplant were the medullary, external granular and internal granular layers of the host cerebellum, and these determined the paths of migration of the donor cells.
The most commonly observed path of migration for the donor cells was the medullary layer of the host cerebellum (Figs. 9, 10). From a comprehensive evaluation of animals with 2–4 days and 10–16 days of survival after surgery, it was found that the migrating cells following the medullary layer of the host cerebellum tended to settle in the internal granular layer from its basal aspect, and remained relatively intensely labelled. The latter observation suggested that the donor cells, in this case, probably did not proliferate after their migration into the host cerebellum. The external granular layer as a path of migration by the donor cells appeared to be utilized by the migrating elements only in a few instances. In such cases the donor cells appeared to penetrate actively into the external granular layer of the host cerebellum and were incorporated as the integral components of it (Fig. 11a, b, c). In the external granular layer of the animals that survived for 6 days postoperatively a large number of lightly labelled cells were detected. There were only one or two instances where the migrating cells failed to penetrate into the external granular layer of the host cerebellum, and instead accumulated along the external surface of this region. In these cases pia membrane was found involved in intervening between the migrating cells and the host.
Fig. 13. Intensely labelled transplanted cells (arrows) in the internal granular layer of the host cerebellum. This region is close to the hemispheres, far away from the site of transplantation. 10 days survival. × 32.

Fig. 14. Few intensely labelled (arrows) and many lightly labelled transplanted cells in the internal granular layer of the host cerebellum. Cytology of the lightly labelled cells can be readily established, and they appear fully differentiated granule cells. This region of the internal granular layer is close to the lesioned surface of the host cerebellum. 16 days survival. × 32.
tissue, and donor cells appeared in a degenerative state (Fig. 12). This suggested that the donor cells after their migration and incorporation into the external granular layer of the host cerebellum were involved in the proliferative activity just like the other cells in this layer.

In addition to the above two paths of migration which provided for active migratory behavior of the donor cells, in some instances it was observed that the labelled donor cells were attached to the internal granular layer of the host cerebellum at its lesioned surface and that they were incorporated into it. There was no clear cut evidence that these labelled donor cells actively migrated into the internal granular layer of the host cerebellum. It is possible that by virtue of the fact that the donor cells did survive the trauma of surgery and were able to attach themselves to the lesioned surfaces of the internal granular layer of the host cerebellum, the combination of neurogenetic and reparative processes taking place in the host cerebellum facilitated their incorporation into the host cerebellar tissue.

Ultimate fate of the donor cells migrating into the host cerebellum

The animals that survived for 10–16 days after the surgery provided clear cut evidence on the incorporation and differentiation of the donor cells transplanted into
the host cerebellum. The lesioned vermis of the host cerebellum appeared to have undergone various reparative morphogenetic changes, and despite these changes the 3-layered architecture of the cerebellum was maintained. It is in these regions of the vermis that labelled cells in the internal granular layer and molecular layer were found. On the average, 5–10 intensely labelled cells in the internal granular layer and 4–7 intensely labelled cells in the molecular layer per section were present. In the medullary layer no labelled cells were seen. The cytological characteristics of these intensely labelled cells could not be determined. However, in these very regions of the host cerebellum many lightly labelled cells in the internal granular layer were seen, and they could be readily identified as the granule cells (Figs. 13, 14). Similarly, a sizeable number of lightly labelled basket and stellate cells, and a few glial cells in the molecular layer were identified (Fig. 15).

**DISCUSSION**

The findings reported here have shown that successful transplantation of precursors of granule, basket and stellate cells of the mammalian cerebellum and their subsequent growth and differentiation is possible. The success in this study was largely due to our employing the precursors of nerve cells and undifferentiated pre-migratory cells rather than fully differentiated neurons for transplantation, and young animals as recipients. During normal postnatal neurogenesis of the cerebellum in mammals, the precursors of neurons show active migratory behavior from the external granular layer into the molecular and internal granular layers. This tendency on the part of precursors of neurons proved very valuable in yielding streams of migrating cells from the transplant which penetrated into the cerebellum of the host. But for the active migratory behavior of the precursors of neurons, these cells too would have been involved in the degenerative processes taking place in many regions of the transplanted slabs of the cerebellum. In contrast to the precursors of neurons and the pre-migratory elements of the transplant, Purkinje cells, Golgi cells and possibly also the differentiated granule cells of the transplant did not migrate into the host cerebellum, possibly because they could not remain viable for a long duration. What exactly are the factors that determine viability of one group of cells as against the other, is not fully known.

Although viability of the transplanted cells and their migratory tendency are necessary requirements for the successful transplantation of cells, the expression of these characteristics appeared to be influenced by the presence of and interaction with other non-neural tissue, like the pia membrane. The degenerative processes observed in the transplants involved excessive proliferation of the pial cells, in addition to an extensive growth of the capillaries, and presence of leukocytes and necrosis. Whenever a viable-looking portion of the transplant appeared surrounded by the pia membrane, its component cells did not show any migratory behavior. It appeared as if the pia membrane had restricted their migratory activity. On the other hand, it was the absence of pia membrane in and around the streams of migrating cells which was correlated with the active migratory behavior of the precursors of the nerve cells.
cells from the transplants. Beyond these correlative observations the exact nature of influence of the pia membrane on the transplant as a whole and on the migratory cells could not be determined.

Complementary to the viability and migratory behavior of the transplanted precursors of neuronal elements is the milieu in which they find themselves after their migration. The growing cerebellum of young recipient rats, which were of the same age as the donors, seems to have provided the ideal histogenetic environment in which the migrating cellular elements could proliferate, grow and differentiate. In the light of recent studies by Zalewski\textsuperscript{29,30}, it is conceivable that the differentiating Purkinje cells and other neuronal elements in the cerebellum of host animals had trophic influence upon the transplanted cells and hence they could survive and differentiate. If these cells were transplanted into the cerebellum of adult rats, it is possible that they would not have been incorporated at all. But this remains to be determined experimentally.

Although transplantation of slabs of cerebellar tissue may not appear as a desirable technique due to extensive pathological involvement of the transplant as well as the host tissue, in fact it is the most suitable method to resolve the problems of viability of transplanted cells, of migration of cells from the transplant to the host tissue, and of interaction between the transplanted tissue and other non-nervous tissue. The most interesting observation emerging from this study is that despite severe pathological conditions produced by the experimental surgery, the host cerebellum appeared normal in its final picture. Why did these pathological conditions not produce chronic cerebellar pathology? How could the host cerebellum, which was lesioned for the purpose of transplantation, achieve normal cytoarchitectural organization? Questions of this nature, though difficult to answer at this stage, provide some thoughts on postnatal neurogenesis of the cerebellum. It seems that postnatal neurogenesis in the cerebellum, and possibly in the hippocampus and olfactory bulbs too, may be a self-regulating process whereby despite structural damage to the tissue following the surgical lesion it somehow achieves as far a normal looking cerebellum as possible.

This approach, it is hoped, will make possible the investigation of some fundamental problems of neuroembryogenesis in mammals. Since the homogeneous appearing cells of the external granular layer, during normal postnatal neurogenesis, sequentially give rise to the basket and stellate cells of the molecular layer, and to the granule cells of the internal granular layer it is generally considered that the cells of the external granular layer are 'indifferent'\textsuperscript{21}, implying that they are pluripotent. To what extent the cells of the external granular layer, or to that extent the germinal cells of the neuroepithelium in embryonic stages\textsuperscript{24,25}, are pluripotent can be experimentally tested by varying the ages of donor and host animals for transplantation of the precursors of nerve cells from different sources into the cerebellum, or even into the hippocampus and olfactory bulbs. Studies along these lines, which are in progress, will provide a better understanding of the factors determining specificity and differentiation in the developing nervous system.
SUMMARY

In the cerebellum of 7-day-old rats slabs of cerebellum from donor animals of the same age were transplanted. One hour prior to the surgery the donor animals were injected with [3H]thymidine intraperitoneally in order to label the mitotically active cells of the external granular layer. Following transplantation, the host animals were sacrificed at different intervals. The brains were processed for autoradiography and the fate of the transplanted precursors of nerve cells was evaluated. The short-survival animals revealed the presence of pathological changes as well as survival and proliferation of certain aggregates of cells in the transplants. These cell-aggregates represented the viable portions of the external granular layer of the transplanted cerebellar slabs. From these cell-aggregates in the transplants streams of migrating cells penetrating into the host cerebellum were observed. In the long-survival animals (16 days postoperative) labelled granule cells in the internal granular layer, and labelled basket and stellate cells in the molecular layer were seen. These findings suggested that transplantation of the precursors of nerve cells in the developing mammalian brain is possible, and that the transplanted neuronal elements may differentiate into cytologically normal nerve cells.

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REFERENCES

5 Altobelli, R., Inneesti cerebrali, Gazz. int. med. Chir., 17 (1914) 25-34.

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13 LEGROS CLARK, W. E., The problem of neuronal regeneration in the central nervous system. II. The insertion of peripheral nerve stumps into the brain, J. Anat. (Lond.), 77 (1943) 251–260.
17 RANSON, S. W., On the medullated nerve fibers crossing the site of lesions in the brains of the white rat, J. comp. Neurol., 13 (1903) 185–207.
18 RANSON, S. W., Transplantation of the spinal ganglion, with observations on the significance of the complex types of spinal ganglion cells, J. comp. Neurol., 24 (1914) 547–558.