Observations on the Migration and Differentiation of Neural Crest Cells in Somite Extirpated Salamander Larvae*

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Summary. In order to research the influences of the somites or their derivatives on the migration and differentiation of neural crest cells, unilateral extirpation of somites was performed in the larvae of the salamander (Hynobius lichenatus Boulenger). Two types of neural crest cells appeared in the somite-free environment. They could already be distinguished from each other shortly after beginning of migration. One type of the cells was distributed in the expanded space between the epidermis and the neural tube, with melanocytes differentiated from them. The other type of the cells migrated closely to the lateral wall of the neural tube. They aggregated as a cord on the ventrolateral side of the neural tube and gave rise either to neurons or to satellite cells of spinal ganglia. The sequence of cytological events along the development of the cells and the time required for each event were essentially identical with those of the melanocytes and spinal ganglion cells developing on the control side. The present results suggest not only that migration and differentiation of neural crest cells are independent of the possible regulation of the somites or their derivatives but also that the fate of neural crest cells is determined before the onset of migration.

It has been said that the migration as well as the differentiation of neural crest cells are greatly affected by the environment along their migratory pathways (LeDouarin and Teillet, 1974; Pratt et al., 1975; Weston et al., 1977, 1978; Derby, 1978; Greenberg et al., 1981; Loring et al., 1982; Newgreen and Thiery, 1980; Newgreen et al., 1982; Nakamura and LeLievre, 1982; Pintar 1978; Rovasio et al., 1983; Thiery et al., 1982; Tucker and Erickson, 1984; Boucaut et al., 1984; Lofberg and Ahlfors, 1978; Lofberg et al., 1985). The environment involves tissues or cells and extracellular matrices which may be synthesized by the cells (Greenberg and Pratt, 1977; Newgreen and Thiery, 1980; Pintar, 1978). However, exactly which tissues or cells affect the migration and differentiation of the neural crest cells has remained unclear. For a solution to this problem, a precise in vivo analysis of the roles of individual tissues forming the microenvironment for migrating neural crest cells was deemed necessary.

The present experiment was designed to elucidate the influences of the somites on the migration and differentiation of neural crest cells. The somites represent one of the essential materials comprising the microenvironment surrounding the migration

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pathway of the trunk neural crest cells from which spinal ganglion cells and melanocytes are derived. After the somites were unilaterally removed, it was observed as to whether or not the neural crest cells could migrate and give rise to spinal ganglion cells and melanocytes in a somite-free environment.

Experiments similar to the present study have already been done by Lehmann (1927) and Detwiler (1927, 1932, 1934). They excised somites and investigated the participation of mesodermal tissues in the metamerism of spinal ganglia and the development of neurons in them. They concluded that the metamerism required the normal arrangement of somites or their derivatives, and the normal connection between neurons and mesodermal tissues or epidermis was necessary for the development of neurons. However, the interaction of somites to the neural crest cell migration and differentiation at early stages was not explicated.

MATERIALS AND METHODS

The larvae of the salamander (Hynobius lichenatus Boulenger) were used. They were staged according to the criteria by Sawano (1947).

When a larva reached the tail bud stage (stages 22 to 24), the somites were extirpated unilaterally from the 4th to the still non-segmented part of the mesoderm, together with the intermediate mesoderm, upper part of the lateral mesoderm, epidermis covering them and endoderm beneath them, using a steel needle (Fig. 1). Neural crest cell migration did not yet occur at this stage. The actual numbers of the extirpated somites could be counted by comparison with the opposing side of somites after they had developed. Eventually, eight to twelve or more somites were removed. After the operation, the larvae were grown in Holtfreter’s solution at room temperature, and every 24 hr were fixed with 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.4). After the external gill stage, they were fixed every 3 or 7 days, with final fixation (stage 60) done 40 days after the operation.

For light microscopic observation, the fixed larvae were dehydrated through a graded series of ethanol, embedded in Epon 812 and cut into 2 or 4 μm thick serial sections. After removal of Epon 812 by Lane and Europa (1965), the sections were stained with hematoxylin and eosin.

For electron microscopic observations, the larvae were fixed with 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.4), then post-fixed with 1% osmic acid in the same buffer, dehydrated and embedded in Epon 812.
RESULTS

After the operation, the external appearance of the larvae showed normal development except for the exposed endoderm through the window made in the epidermis. On the operation side of the larvae, the epidermis covered the lateral surface of the neural tube; its ventral margin reached the level of the notochord and stopped at the exposed endoderm. The epidermis never spread any further ventrally to cover the denuded

Fig. 2. A photomicrograph showing neural crest cells just after the onset of migration in the somite free space (upper side). On the control side (lower), migrating neural crest cells, somite cells and epidermis can not be distinguished. Horizontal section, stage 30. EP epidermis, NC neural crest cells, NT neural tube. ×210

Fig. 3. Migrating neural crest cells in the somite free space showing different distribution pattern. The medial (neural tube side) cells are linked together close to the neural tube and the lateral (epidermal side) cells are loosely distributed. Horizontal section. EP epidermis, NC neural crest cells, NT neural tube. ×210
endoderm, though the window tended to become smaller after the digestive canal
developed. The dorsal fin and apical edge of the somites located on the non-operation
side shifted to the operation side passing the median plane. The histological and cyto-
logical development in the non-operation side showed processes identical with those in
normal larvae. Thus the non-operation side could be used as the control.

When the larvae reached stage 30, neural crest cells appeared in the newly formed
narrow space between the neural tube and the epidermis on the operation side (Fig. 2).
They soon became polygonal in shape, linking together to form a one or two-cell-thick
layer (Fig. 3). Careful observation revealed that the distribution of lateral cells was
looser than the medial cells. This differentiation in the distribution pattern of cells
became more prominent with the progression of development. The space between
the neural tube and the epidermis expanded gradually until finally reaching such a
size as to be able to accommodate a somite in a cross section (Fig. 4). Cells situated
laterally were scattered in this space and extended cytoplasmic processes in all direc-
tions as if to support this space (Fig. 4, 5). Melanocytes differentiated at stage 40 on
both sides of the larva (Fig. 6). On the control side, they were distributed beneath the
basal surface of the epidermis (Fig. 6a, 8a), but on the non-operation side, they occurred
everywhere in the space between the epidermis and the neural tube (Fig. 6a 7a).

The neural crest cells distributed medially formed a cord extended along the
ventrolateral wall of the neural tube throughout the operation area at the level of the

![Fig. 4.](image) The cell cord (arrow) formed in the wide space between the neural tube and epidermis. On
the control side a large spinal ganglion (SPG) can be seen. The difference between the two
is only the size in the cross-section. Neural crest cells aggregate at the same level of the
neural tube (NT) as the normal ganglion. The arrowhead indicates the incomplete dorsal
fin. Cross section, stage 37. C notochord, E endoderm, S somite. ×190
spinal ganglia which were formed on the control side (Fig. 4, 5). At first, the cells forming the cord had a soma of a craniocaudally elongated spindle shape containing an oval nucleus, a large amount of pigment granules and yolk platelets (Fig. 5b). After the disappearance of the yolk platelets and pigment granules, the cord became a compact strand with prominent nuclei (Fig. 6b). This process also took place in the spinal ganglia on the control side (Fig. 5c, 6c). Later, on both the operation and control sides, large cells having ample cytoplasm and a large round nucleus appeared in the cord and spinal ganglia (Fig. 7).

On the control side, the large cells in the spinal ganglia became still larger, while the other cells in it became smaller; the former cells being neurons and the latter satellite cells. They rapidly showed definite morphological characters, respectively (Fig. 8b). Finally all cells in the spinal ganglia became either neurons or satellite cells, and spindle shaped cells could no longer be observed. On the other hand, on the opera-

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**Fig. 5.** Horizontal plane of a larva at the same stage of Figure 4. a. The craniocaudal elongated cord (arrow head) close to the lateral wall of the neural tube (NT). On the other side (control side) four spinal ganglia (arrows) showing metamerism can be seen on the belly of each somite (S). The lateral cell distribution becomes more disperse. b. and c. The encircled parts in a. The cord cells (b) and apical ganglion cells (c) show the same features. EP epidermis. a: x 110, b, c: x 430
tion side, neurons also developed in the cord, but were always smaller in size and number than on the control side (Fig. 8c). Most of the other cells in the cord remained with a small amount of cytoplasm (compare Fig. 6b, 7a, 8c). By electron microscopic observation, they were distinguished into two types of cells. One type, probably neurons, had a spherical surface, whereas the other type invaded their cytoplasmic processes among the neurons and invested them with those processes showing a satellite cell nature (Fig. 9).

Subsequently the neurons in the cord decreased in number, though occasionally small sized neurons and satellite cells were observed (Fig. 10). Most of the other places at which the cell cord had existed were occupied by fibrocyte-like cells forming a thick membrane, which can be already seen in Figure 8d.

Observations suggestive of the fragmentation of the cell cord corresponding to the somites or myotomes were never obtained.

DISCUSSION

Lehmann (1927) and Detwiler (1927, 1932, 1934) suggested that the metamerism of spinal ganglia was controlled by somites or their derivatives and that the normal growth of neurons in the spinal ganglia was facilitated by environmental factors. The present experiment supports their conclusions.

Lehmann (1927) reported that neural crest cells did not transform into spinal ganglion cells in an environment completely lacking somites, and inferred that the differentiation of the spinal ganglion cells was induced by the medial surface of the somite or the axial cartilage. On the other hand, Detwiler (1932) recognized the
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Development of spinal ganglia and the differentiation of neurons in them in spite of the absence of muscles and axial cartilages and suggested a self-differentiation capability for neural crest cells. However, he did not mention the differentiation of satellite cells in the irregular-developed spinal ganglia. In the present experiment, the differentiation of neurons and satellite cells was demonstrated in the cord-like spinal ganglia which developed in the somite-free environment. Neurons, satellite cells and melanocytes did differentiate from the trunk neural crest cells in the somite-free microenvironment. Furthermore, morphological changes in each cell on the operation side proceeded parallel to those on the control side. These results indicate that the somites and their derivatives have no influence on the differentiation of neural crest cells.

It is also clear that the somites have no relationship to the aggregation of neural crest cells to form spinal ganglia, because in the present study, the neural crest cells aggregated and formed a compact cord in the somite-free environment. THIERY et al. (1982) and VINCENT and THIERY (1984) claimed that the migration of neural crest cells located in the space between the neural tube and the bulk of a somite was blocked towards the further ventral by the sclerotome. They therefore aggregated at that site and differentiated to the spinal ganglion cells. PRATT et al. (1975) suggested that the accumulation of hyaluronic acid expanded the space through which neural crest cells migrated, and WESTON et al. (1977, 1978) and DERBY (1978) suggested that the decrease in the concentration of hyaluronic acid narrowed this space and induced the aggregation of neural crest cells to form spinal ganglia. Under the present experimental conditions, there was no structure to block the migration of neural crest cells to the

![Fig. 7. Transverse plane of a larva at shortly after a stage from Figure 6.](image)

**Fig. 7.** Transverse plane of a larva at shortly after a stage from Figure 6. **a.** Cell cord, **b.** spinal ganglion. Developing neurons (arrows) can be seen in both of them. Other cells are in shape of spindle. Arrowheads: melanocytes, NT neural tube, M myotome. a, b: ×380
Fig. 8. a. Low power view of the horizontal plane of larva at stage 45. Arrows indicate the thin cytoplasmic processes of melanocytes widespread beneath the basal surface of the epidermis. Inset is the enlargement of the left one. b-d. The encircled parts in a. b. Developing spinal ganglion on the control side. Large neurons and small mantel cells can be distinguished. c. Most of cells consisting the cell cord show the same features from Figures 6 and 7. Difference between neurons and mantel cells is indistinct except one small sized neuron (arrow). d. The membrane like structure is consisted fibrocyte-like cells. NT neural tube, EP epidermis, M myotome, C notochord. a: × 40, inset: × 170, b-d: × 350
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further ventral, and even in the widely expanded space, most of the neural crest cells migrating along the lateral wall of the neural tube stopped and formed the cord on the ventrolateral side of the neural tube. This evidence indicates that neural crest cells do not aggregate passively by diminution of space or interruption of their migration.

It has been claimed that scaffoldings for the migration of neural crest cells might be the basal lamina of the neural tube and the epidermis and fibronectin on it (Newgreen and Thiery, 1980; Löfberg et al., 1980, 1985; Newgreen et al., 1982; Thiery et al., 1982). In the somite-free environment, scaffolding for the neural crest cells forming the cell cord was only on the basal lamina of the neural tube, so that they are associated with the lateral surface of the neural tube more closely than on the control side. However, it has been previously established that neural crest cells have a close association to the neural tube from the onset of migration (Takahashi and Yamadori, 1979; Löfberg et al., 1980; Hirano and Shirai, 1984, 1986), and in this experiment, neural crest cells forming the cell cord moved close to the lateral surface of the neural tube from the beginning of the migration and aggregated at the level of the developing spinal ganglia. Therefore, the neural crest cells migrating in the space between the neural tube and the somites may depend on the neural tube primarily, and the position of the spinal ganglia is not decided by the somites but presumably by the interaction with the

Fig. 9. An electron micrograph showing the development of neurons (N) and mantel cells (M) in the cell cord at the same stage of Figure 8. A neurons having a spherical surface is invested by thin cytoplasmic processes (arrows) of two mantel cells. Other cells are not identified in this photograph. ×9,000
neural tube or fibronectin on it. The basal lamina of the epidermis must have existed in the present experiment, but for unknown reasons, no relationship was recognized between the lateral migration of neural crest cells and epidermis.

It was clear that, morphologically, two types of cells grew in the primary culture of the neural crest cells (Cohen and Konigsberg, 1975; Sieber-Blum and Cohen, 1980; Ziller and LeDouarin, 1983). Ziller et al. (1983), Sieber-Blum and Sieber (1984) and Ito and Takeuchi (1984) cytochemically demonstrated the two types of cells in the cultured neural crest cells and claimed that the neural crest was a heterogeneous population. Further in vivo observations have suggested that the fate of neural crest cells is determined before the onset of their migration (Cochard and Coltey, 1983; Epperlein and Löffberg, 1984; Payette et al., 1984). The present experiment in the salamander larva indicated that two types of migrating neural crest cells in the somite-free environment could be observed shortly after their migration, and that they advanced their normal differentiation in the somite extirpated environment. This evidence supports the view that at least the segregation between neuronal cells and non-neuronal cells may occur in the neural crest.

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REFERENCES


