DEVELOPMENTAL BIOLOGY 141, 243-253 (1990)

The Origins of Neural Crest Cells in the Axolotl

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Accepted May 29, 1990

We address the question of whether neural crest cells originate from the neural plate, from the epidermis, or from both of these tissues. Our past studies revealed that a neural fold and neural crest cells could arise at any boundary created between epidermis and neural plate. To examine further the formation of neural crest cells at newly created boundaries in embryos of a urodele (*Ambystoma mexicanum*), we replace a portion of the neural folds of an albino host with either epidermis or neural plate from a normally pigmented donor. We then look for cells that contain pigment granules in the neural crest and its derivatives in intact and sectioned host embryos. By tracing cells in this manner, we find that cells from neural plate transplants give rise to melanocytes and (in one case) become part of a spinal ganglion, and we find that epidermal transplants contribute cells to the spinal and cranial ganglia. Thus neural crest cells arise from both the neural plate and the epidermis. These results also indicate that neural crest induction is (at least partially) governed by local reciprocal interactions between epidermis and neural plate at their common boundary.

INTRODUCTION

During neurulation, as neural folds rise and move together to form a neural tube covered by a layer of epidermis, some epithelial cells move out of the neural folds and take up residence temporarily between the epidermis and the neural tube where they form the neural crest. Soon cells leave the neural crest and migrate throughout the body of the embryo where they contribute to many tissues. In another migration that occurs slightly later, some cells from the epidermal placodes of the cranial region move out of the epithelium and contribute to the cranial ganglia.

While much research has focused on the migration and differentiation of neural crest cells, and, more recently, on the mechanisms by which neural crest cells segregate from the epithelium (Duband and Thiery, 1987; Martins-Green and Erickson, 1987), relatively little attention has been given to the origin(s) of neural crest cells. Investigators who have attempted to determine whether neural crest cells originate in the neural plate, in the epidermis, or in both of these tissues have arrived at several conflicting conclusions (for general reviews see Hörstadius, 1950; Weston, 1970; Le Douarin, 1982; Hall and Hörstadius, 1988).

Landacre (1921) concluded that the neural crest arose from different tissues in different species. His study did not directly address the origin of neural crest cells, however, but assumed that the position and appearance of the neural crest reflected the origin of its cells—from the neural plate, from the epidermis, or from neither tissue. In other studies, Raven (1931) and Baker and Graves (1939) used cellular morphology and cell marking to identify presumptive neural crest cells in the folds and to trace them through development. Both studies claimed that neural crest cells in *Ambystoma* arose in both the neural plate and the epidermis. However, when Fautrez (1942) repeated these studies—using a vital dye to mark tissues in *Ambystoma*—he concluded that the neural plate was the only source of neural crest cells. Through histological examination of mouse embryos, Verwoerd and van Oostrom (1979) concluded that neural crest cells arise solely from the epidermis. Using a histological technique that is purported to stain neural crest cells prior to emergence, Nichols (1981, 1987) reported that neural crest cells in the mouse arise initially from the epidermis, but that later neural crest cells form exclusively from the neural plate. To identify the origin of neural crest cells in the urodele *Hynobius*, Hirano and Shirai (1984) examined cell ultrastructure and traced cell fate by injecting Nile blue or India ink into the neural fold. They concluded that the neural crest originates only from the lateral edge of the neural plate. Brun (1985) transplanted neural plate and epidermis from normally pigmented *Ambystoma* embryos into albino hosts and was able to trace the pigmented cells through development. Melanocytes appeared only in hosts that received neural plate transplants, and he concluded that neural crest arises solely from the neural plate.

Some of these studies identify presumptive neural crest cells using characteristics that are not obvious, are not unique to neural crest cells, and/or are not expressed in the presumptive neural crest cells before they leave the epithelium. The subtle morphological characteristics that some investigators use to identify pre-

0012-1606/90 $3.00

sumptive neural crest cells in the neural folds (Landacre, 1921; Baker and Graves, 1939) require a somewhat subjective assessment of cells that exhibit "intermediate" characteristics. Some histochemical techniques (Nichols, 1981, 1987; Cochard and Coltey, 1983) and antibodies recognize neural crest cells (Barald, 1982; Ciment and Weston, 1982; Tucker *et al.*, 1984; Vincent *et al.*, 1983; Vincent and Thiery, 1984; Bronner-Fraser, 1986), but they may also recognize other cell types. Furthermore, antibodies often recognize only the cells that have begun to migrate away from the neural crest.

Cell marking techniques can be used to follow the development of neural crest cells. However, vital stains or particles that are applied to the cell surface (Stone, 1932; Fautrez, 1942; Hirano and Shirai, 1984) usually mark several adjacent cells, and the dye may diffuse from one cell to another after marking. Another technique replaces tissues in an unmarked host with a transplant from a donor whose cells have "marks" that do not move from cell to cell. "Marked" cells in such transplants either exhibit natural differences in cytological properties (Le Douarin and Teillet, 1974; Brun, 1985) or have been internally labeled prior to transplantation (Johnston, 1966; Weston, 1967).

Cell marking techniques that are used to determine the origin of neural crest cells should allow the boundary between marked and unmarked tissue to exactly match the boundary between the epidermis and the neural plate. Separating the epidermis from the neural plate within the fold can damage the potential neural crest cells. Therefore, most neural crest transplants or explants contain the entire neural fold (including part of the epidermis and neural plate). Though these transplants are useful in tracing the migration and fate of neural crest cells, they cannot ascertain the origin of neural crest cells because both potential sources of neural crest cells are marked.

Our recent work (Moury and Jacobson, 1989) indicates that neural folds will form at new, ectopic boundaries between neural plate and epidermis and that neural crest derivatives (melanocytes and cranial ganglia) form from cells that originated in these newly created folds. In this study, we examine whether neural crest cells originate from the epidermis, from the neural plate, or from both of these tissues. We create new boundaries by transplanting pieces of either neural plate or ventral epidermis taken from normally pigmented donor embryos into the neural fold region of albino host embryos. We allow these embryos to develop and then examine them for the presence of pigmented cells in the neural crest and its derivatives.

MATERIALS AND METHODS

Embryos of the Mexican axolotl, *Ambystoma mexicanum* (Shaw), were obtained from the Indiana University Axolotl Colony and kept in 10% Holtfreter's solution (Holtfreter, 1931) prior to the experiments. Holtfreter's solution was prepared according to the formula given by Jacobson (1967). Embryos between stages 14 and 16 (Schreckenberg and Jacobson, 1975) were dejellied manually, and their vitelline membranes were removed with sharpened forceps. Operations were performed in Syracuse watch glasses lined with a mixture of Permaplast (American Art Clay Co.) and paraffin and filled with 100% Holtfreter's solution. When the transplant had healed in place, the embryos were transferred to 50% Holtfreter's solution in plastic tissue culture dishes lined with 1% agar and maintained at 17°C. The other methods followed in preparing and maintaining embryos have also been described in detail by Jacobson (1967).

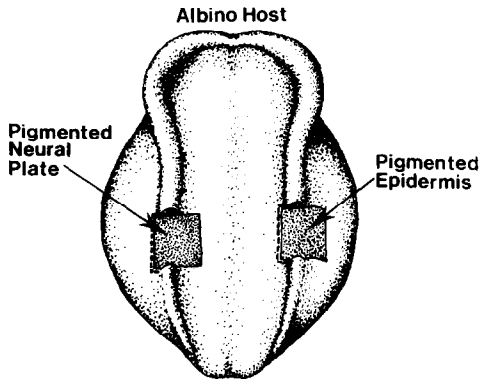

FIG. 1. Diagram of experiments in which the neural fold of an albino host embryo is replaced with a piece of either neural plate or ventral epidermis from a pigmented donor. (The operations depicted here would not be performed simultaneously on a single embryo.) Newly created boundaries between the epidermis and the neural plate are indicated by a dashed line.

Using a sharpened tungsten needle, a strip containing neural plate, neural fold, and epidermis was removed from an albino host embryo and replaced with either neural plate or epidermis from a normally pigmented donor embryo (Fig. 1) so that pigmented cells from the graft could be traced as the host embryo developed. Neural crest cells may require exposure to some "factor(s)" in the environment of their normal migratory pathways in order to differentiate (see Le Douarin and Teillet, 1974; Vincent and Thiery, 1984; Bronner-Fraser, 1986, 1987). We removed as little epidermis as possible from any fold that was to be replaced with neural plate, and we removed as little neural plate as possible from any fold that was to be replaced with epidermis. This should allow the new folds to form at nearly the position they would occupy in a normal embryo and thereby place any neural crest cells that form at the new boundaries in a more "normal" environment.

We excluded the neural folds from all donor grafts to guard against transplanting any pigmented neural crest cells into the albino host. Epidermal grafts came from the ventral regions of the donor embryos—well away from the neural folds. The lateral edge of each neural plate transplant was medial to the neural fold. Furthermore, since the notoplate (the "neural" epithelium that lies above the notochord) is tightly bound to the underlying notochord and appears to have properties very different from the rest of the neural plate (Jacobson *et al.*, 1986), we also avoided including this region in the neural plate transplants. (The parameters of such transplants were more fully illustrated in Moury and Jacobson, 1989.) Finally, since the derivatives produced by the cephalic neural crest differ from those produced by the trunk neural crest (Le Douarin and Teillet, 1974; Le Douarin *et al.*, 1977; Le Douarin, 1982), folds from the cranial, neck, and spinal regions were replaced with epidermis or neural plate grafts from corresponding regions in the donor.

Following *in vivo* observation, experimental embryos were fixed in Kahle's fluid (Jones, 1966), dehydrated, cleared through an ethanol/*n*-butanol series, embedded in Paraplast-plus, and sectioned at 6 μm. Sections were stained with 0.2% neutral red (Jones, 1966) and examined histologically by light microscopy. When illuminated with red light to reduce the contrast between the section and its background, pigmented cells transplanted into an albino host could be identified in paraffin sections by the presence of pigment granules (Moury and Jacobson, 1989). Pigment granules are small and widely scattered in the cells of early embryos, but they are clearly visible under the microscope (though they tend to be obscured in photographs, see Fig. 2). In older embryos, pigment granules appeared to aggregate within the cells and thus they become quite prominent.

We attempted to remove all mesodermal cells that adhered to the epithelial transplants. Any pigmented mesodermal cells that we overlooked could give false positive results if they became located in the neural crest pathway or in the mesenchyme; therefore, when examining sectioned material, we ignored occasional pigmented cells that were not located in the neural crest proper (i.e., the "nonmigrating" neural crest, Fig. 2) or in a neural crest derivative.

RESULTS

As indicated by our previous study (Moury and Jacobson, 1989), folds form at the new boundaries between neural plate and epidermis whether the donor tissue is epidermis or neural plate. These folds closely resemble their natural counterparts and appear to participate in neurulation in the normal manner (Figs. 3a–3f). Shortly after the operation, the graft heals into place (Figs. 3a, 3d). At this time, all pigmented cells are on the surface

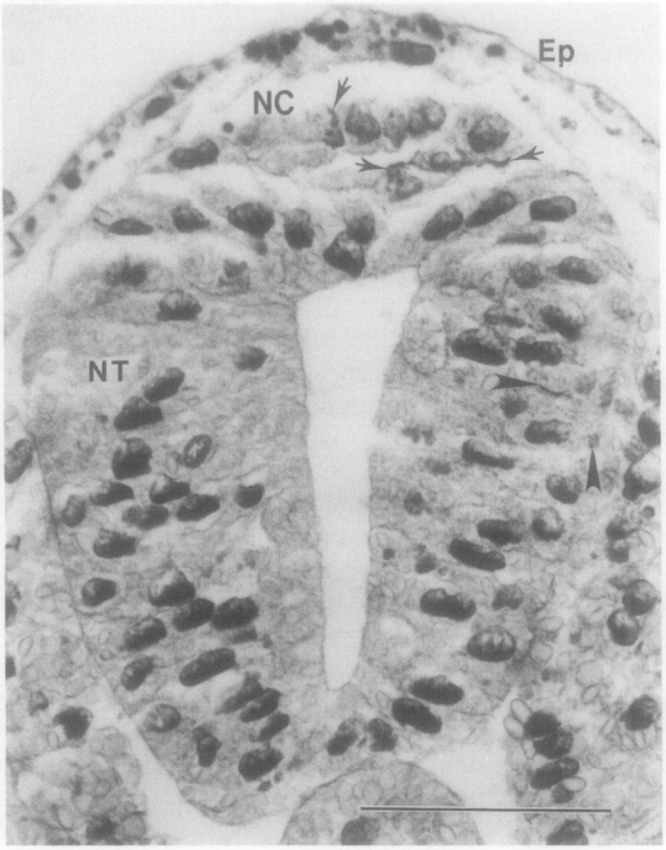

FIG. 2. Paraffin cross section (normal illumination) through the neck region of an experimental embryo. The neural tube (NT), the epidermis (Ep), and the nonmigrating neural crest or neural crest proper (NC) are clearly visible, but pigment granules are obscured by other darkly staining cytoplasmic inclusions (such as yolk platelets) in photographs of early embryos (even when photographed using red light). This embryo received a pigmented neural plate transplant, and a few of the pigment granules are labeled (with arrowheads in the neural tube, and with arrows in the neural crest proper); no cells with pigment granules are found in the epidermis. Time elapsed since operation: 40 hr. Line scale = 100 μm.

of the embryo, and they are extremely dark compared to adjacent albino tissues. As neurulation proceeds, a fold that incorporates tissues from both the pigmented graft and the albino host forms at the newly created boundary and then rolls toward the midline where it meets its natural counterpart (Figs. 3b, 3e). Though the wound healing process delays movement of the new fold toward the midline, there seem to be no major differences in the newly created folds and their natural counterparts. During fold formation, any pigmented tissue that remains on the surface continues to appear dark, but pigmented tissue that becomes covered by colorless albino epidermis appears gray. When they meet at the midline, the folds fuse and tissues from the left and the right sides join. Neural plate tissue transplanted from a pigmented donor becomes a part of the neural tube and is visible as a gray area beneath the colorless epidermis of

the albino host (Fig. 3c). Most of the epidermal tissue that has been transplanted from a pigmented donor remains on the surface and integrates with the epidermis of the albino host (Fig. 3f).

Folds Replaced with Neural Plate

We replaced the neural folds of 15 albino embryos with pieces of neural plate from pigmented donors. We fixed and sectioned three of these 3–5 days after the neural folds had closed at the midline. In two of these specimens, some cells in the neural crest proper (see Fig. 2) contained pigment granules.

We allowed the remaining 12 embryos to develop for 10–18 days. In some of these, superficial examination *in vivo* revealed that darkly pigmented cells migrated from the graft into the surrounding tissues. In three specimens, some of these cells eventually migrated into the skin and developed the dendritic morphology that is characteristic of melanocytes (Figs. 4a, 4b). These melanocytes developed in two specimens that had received a transplant in the cranial region. The other specimen in which melanocytes developed had received a transplant in the trunk region, and cells in one of its spinal ganglia also contained pigment granules.

In one transplant made in the cranial region, some pigmented cells migrated into the deep tissues of the head (Fig. 5a). Pigmented cells that became part of the neural tube remained on the same side as the original implant, but pigmented cells were present in the eye that was contralateral to the original implant (Figs. 5a–5c). In the eyes of normal embryos, both the pigmented retinal epithelium and the choroid contain pigmented cells. The pigmented retinal epithelium arises directly from neural plate tissue, while the choroid is invaded by migrating neural crest cells that differentiate into melanocytes (Ris, 1941). In our specimen, the pigmented cells in the eye had obviously migrated across the midline, and furthermore, the "pigmented" retinal epithelium (which is not pigmented in albino embryos) was visible as a layer that lay more proximal to the lens than the donor-specific melanocytes (Figs. 5b, 5c). Therefore, these were neural crest-derived melanocytes in the choroid.

We found no cells from the neural plate transplants in the epidermis, epidermal placodes, or epidermal derivatives of the host embryos at any stage of development.

Folds Replaced with Epidermis

We transplanted pigmented epidermis into the neural fold of 19 albino embryos. In two of the three specimens that we fixed and sectioned 3–5 days after the folds had closed at the midline, some cells in the neural crest proper contained pigment granules.

We had replaced the neural folds in the cranial and neck regions with pieces of ventral epidermis in eight of the remaining specimens. When fixed and sectioned (after 10–18 days), five of these embryos revealed cells with pigment granules in one of the cranial ganglia (either V, IX, or X, depending on the level of the graft; Figs. 6a, 6b). Pigmented cells were also found in some epidermal placodes and their derivatives (such as the otic vesicles).

In the other eight specimens, we had transplanted epidermis into the neural folds in the trunk region of the embryo. In three of these embryos, we found that some cells in one of the spinal ganglia contained pigment granules (Figs. 7a, 7b).

After transplanting pigmented epidermis into the neural fold of an albino host, we found no melanocytes and no cells containing pigment granules in the neural plate or in any of its derivatives.

DISCUSSION

Where Do Neural Crest Cells Originate?

Both the epidermis and the neural plate contribute cells to the neural crest. When we replaced a portion of a host embryo's neural fold with a piece of tissue taken from a donor embryo's neural plate or epidermis, donor-specific cells became part of the neural crest proper. (Some donor-specific cells also appeared in the neural crest pathways and in the mesenchyme, but we ignored these because they could have been mesodermal cells that adhered to the epithelial transplant.) As these embryos developed, pigmented cells appeared in some neural crest derivatives. Melanocytes and (in one case) a spinal ganglion formed when pigmented neural plate replaced the neural fold in an albino host, and some cranial or spinal ganglia contained donor-specific cells when pigmented epidermis or neural plate replaced the neural fold in an albino host.

Though melanocytes are usually considered to be neural crest derivatives (DuShane, 1935, 1938; Raven, 1936; Hörstadius, 1950; Le Douarin, 1982; Hall and Hörstadius, 1988), they may also arise directly from the neural plate. Niu (1954) observed an overabundance of host-specific cranial and caudal melanocytes (in addition to neural crest defects) after he removed the neural folds (including the presumptive neural crest) and either replaced them with flank epidermis from a donor of a different species or allowed the epidermis and neural plate to rejoin. Brun (1985) noted the production of melanocytes after transplanting a piece of pigmented neural plate into the neural plate of an albino host. Matsuda (1983) demonstrated that explanted neural plate cells could become melanocytes when grown in culture, perhaps in response to some "factor" in fetal calf serum.

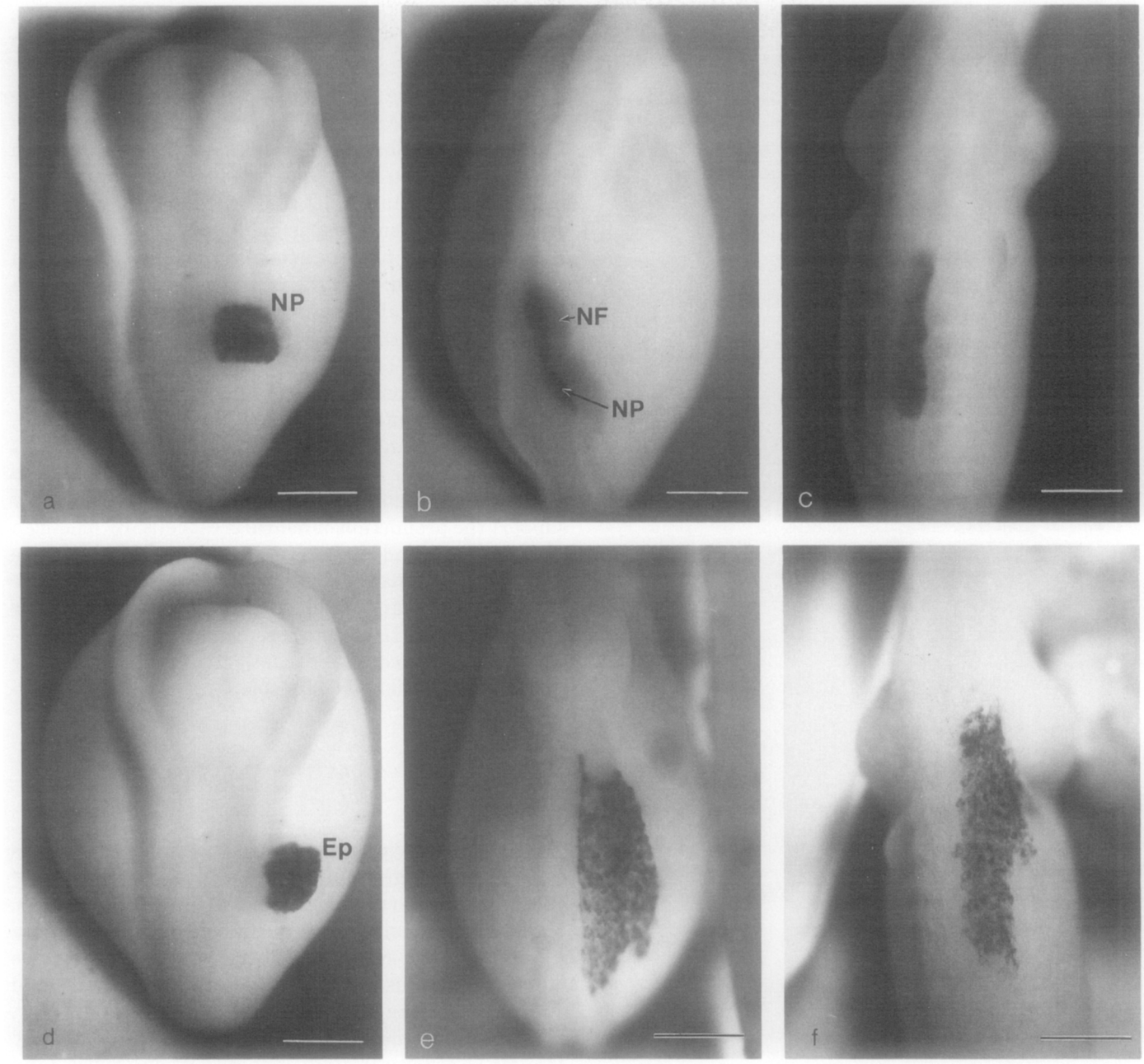

FIG. 3. New neural folds form when the natural neural folds of an albino host are replaced with pigmented neural plate or pigmented epidermis. Line scales = 500 μm. (a) Pigmented neural plate (NP) transplanted into an albino host. Time elapsed since operation: 1.5 hr. (b) A new neural fold (NF) has formed at the boundary between the pigmented neural plate and the albino epidermis. Time elapsed since operation: 13 hr. (c) The pigmented neural plate tissue has become incorporated into the host's neural tube and is visible through the host's colorless epidermis. Time elapsed since operation: 2.5 days. (d) Pigmented epidermis (Ep) transplanted into an albino host. Time elapsed since operation: 1.5 hr. (e) Though the neural folds are touching, the pigmented and the albino epidermis have not yet fused at the midline. Time elapsed since operation: 18 hr. (f) Fusion of the epidermis is complete—pigmented and albino epidermal cells integrate at the midline. Time elapsed since operation: 2.5 days.

Many cells in the ganglia of the peripheral nervous system originate in the neural crest, but epidermal placodes also contribute cells to the cranial ganglia (Yntema, 1937, 1943; Narayanan and Narayanan, 1980). In the cranial region, pigmented cells from the epidermal transplant appeared in epidermal placodes and their derivatives and in cranial ganglia. Therefore, cells from a cranial transplant may have become part of the cranial ganglia via the epidermal placodes rather than via the neural crest. In the trunk, however, no epidermal placodes develop, and the peripheral nervous system, including the spinal ganglia, is believed to arise exclu-

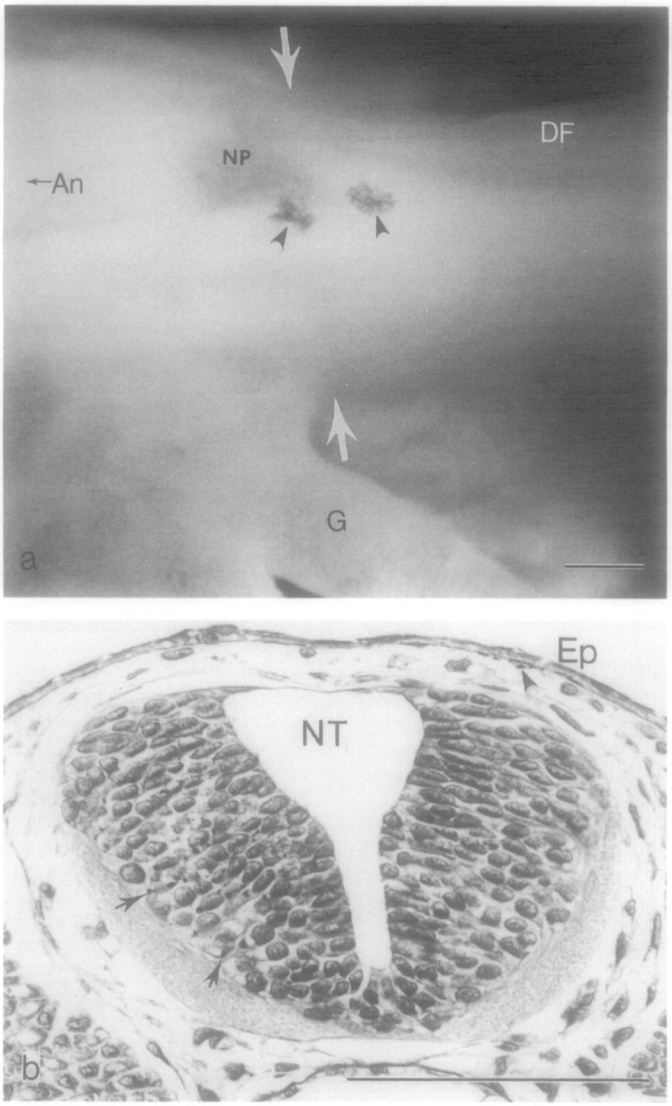

FIG. 4. Melanocytes originating in the donor neural plate appear in the skin. Time elapsed since operation: 11 days. Line scales = 200 μm. (a) External view of larva showing the pigmented neural plate transplant (NP) and two melanocytes (arrowheads). White arrows indicate the approximate plane of section for b. The dorsal fin (DF), the gills (G), and the anterior direction (An) are labeled to aid in orientation. (b) Cross section of the same larva (normal illumination) showing pigmented cells (arrows) in the neural tube (NT) and a melanocyte (arrowhead) beneath the epidermis (Ep).

sively from the neural crest (Detwiler and Van Dyke, 1934; Hörstadius, 1950; Le Douarin, 1982; Hall and Hörstadius, 1988). Consequently, the cells from the pigmented epidermal transplant that appeared in spinal ganglia (Figs. 7a, 7b), must have once been part of the neural crest.

Competence to become neural crest cells is not limited to cells in the natural neural fold. In contrast to proposals that limit the presumptive neural crest cells to an "anlagen" that lies in the neural fold between the neural plate and the epidermis (Raven, 1931, 1936; Detwiler, 1937), our experiments indicate that "nonfold" cells (in both tissues) are competent to become neural crest cells. Extirpation of the neural folds reduces or eliminates the formation of neural crest derivatives (Detwiler and Van Dyke, 1934; DuShane, 1935, 1938; Niu, 1954; Jacobson and Jacobson, 1973) and clearly demonstrates that the neural folds contain cells that will become the neural crest. However, such experiments do not demonstrate that competence to form neural crest cells is restricted to the neural fold. Removal of the neural fold creates a large wound that separates the epidermis and the neural plate and that could reduce the number of neural crest derivatives by delaying the necessary interactions between these tissues.

In our experiments (see also Moury and Jacobson, 1989), a transplant could give rise to neural crest cells even if neither its original position in the donor embryo nor its new position in the host embryo would normally support the production of neural crest cells. By placing a graft in the wound left by removal of the neural fold, we minimized the spatial separation between neural plate and epidermis and reduced the time necessary for wound healing. In some cases, this treatment apparently allowed adequate contact between the tissues for some neural crest cells to develop.

How Might Interactions between Tissues Affect Neural Crest Cells?

Different cell types at a boundary between two tissues can induce changes in one another's commitment and/or they can interact mechanically to generate forces. Induction is a cumulative phenomenon rather than a simple "on/off" switch, and a responding cell can be induced to various levels before it reaches the threshold at which a change is noted (see Jacobson and Sater, 1988). Many tissues eventually respond to induction by changing (differentiating) into new tissue types (and hence creating new boundaries with neighboring tissue types). These new boundaries, in turn, may become the sites of new interactions. Mechanical interactions at the boundary drive morphogenetic movements that can bring new tissues into contact, again creating possible sites of new interactions. Therefore, a sequential series of several inductive and/or mechanical interactions may affect an epithelial cell as it becomes a neural crest derivative. Note that our experiments juxtaposed epidermis and neural plate from neurulae. Similar experiments performed at earlier stages may elicit the formation of a different (or more complete) range of neural crest derivatives, since cellular responses to these interactions and the interactions themselves can change

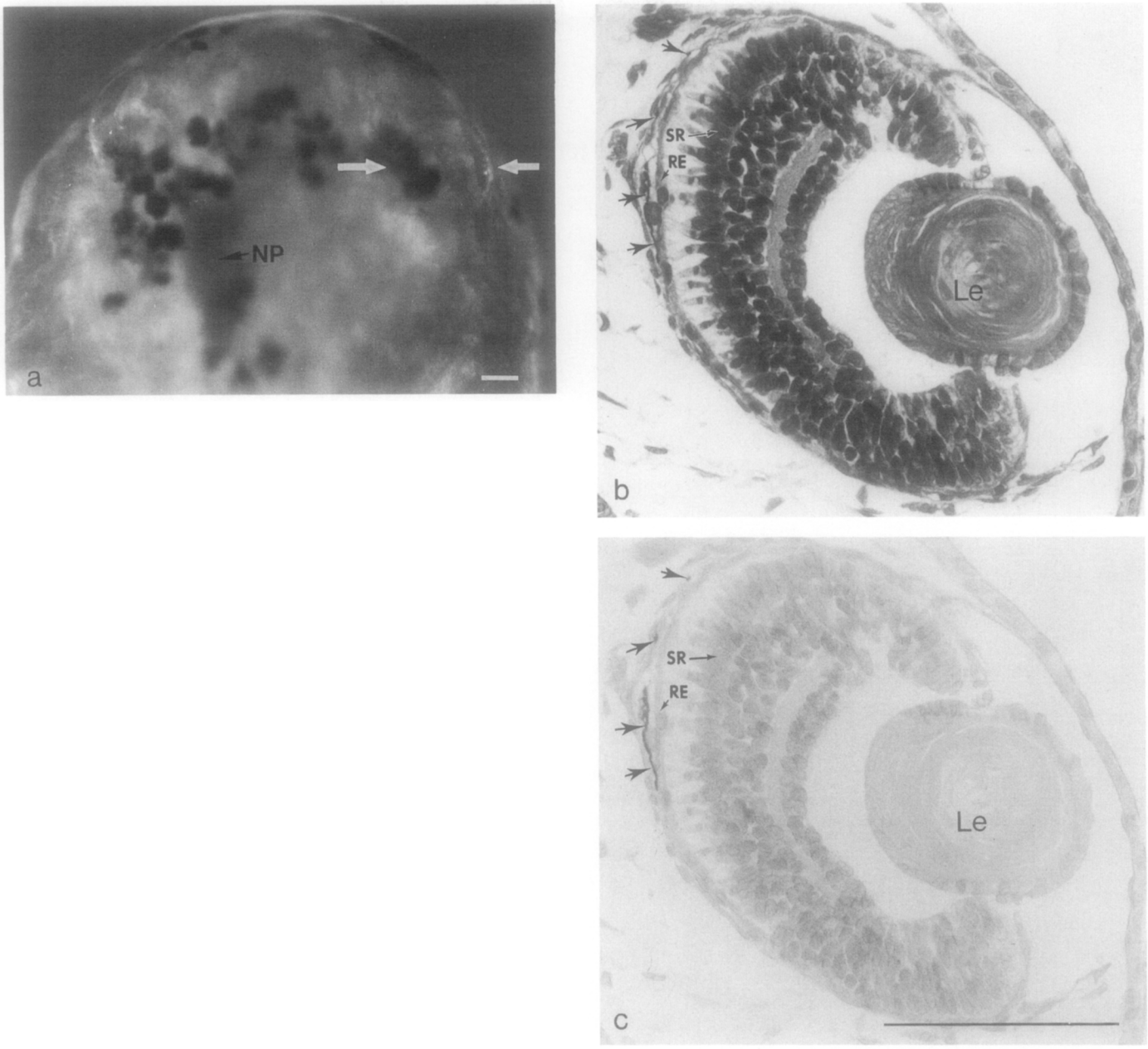

FIG. 5. Melanocytes originating in the donor neural plate appear around the eye. Time elapsed since operation: 18 days. Line scales = 200 μm. (a) External view of larva showing the neural plate transplant (NP) on the left side. White arrows indicate the approximate plane of section for b and c. (b) Cross section of the right eye showing the sensory retina (SR), the retinal epithelium (RE), and the melanocytes in the choriod layer (arrows). The lens (Le) is labeled to aid in orientation. Section is viewed with normal illumination. (c) Section in b is viewed with red light to accentuate pigment granules.

through time. We summarize some of the major interactions that seem to occur during the formation of neural crest cells in Figs. 8a-8c.

Neural induction causes ectodermal cells to become neural plate cells, thus setting the neural plate apart from the epidermis as a separate tissue (Figs. 8a, 8b). At the edge of the neural morphogenetic field, where the neural folds and neural crest cells will form, the influence of neural induction may be very low (Nieuwkoop *et al.*, 1952; Nieuwkoop, 1985) or "neuralizing" and "epidermalizing" influences may overlap (Rollhäuser-ter Horst, 1977, 1980). Cells may be affected by their distances from the "inducer(s)," and/or by temporal changes in the inductive signal(s), in the competence of the tissues to respond to an inductive signal or in the local environment. Thus, neural induction(s) could create several different subpopulations of cells within the neural plate (different levels of induction above the

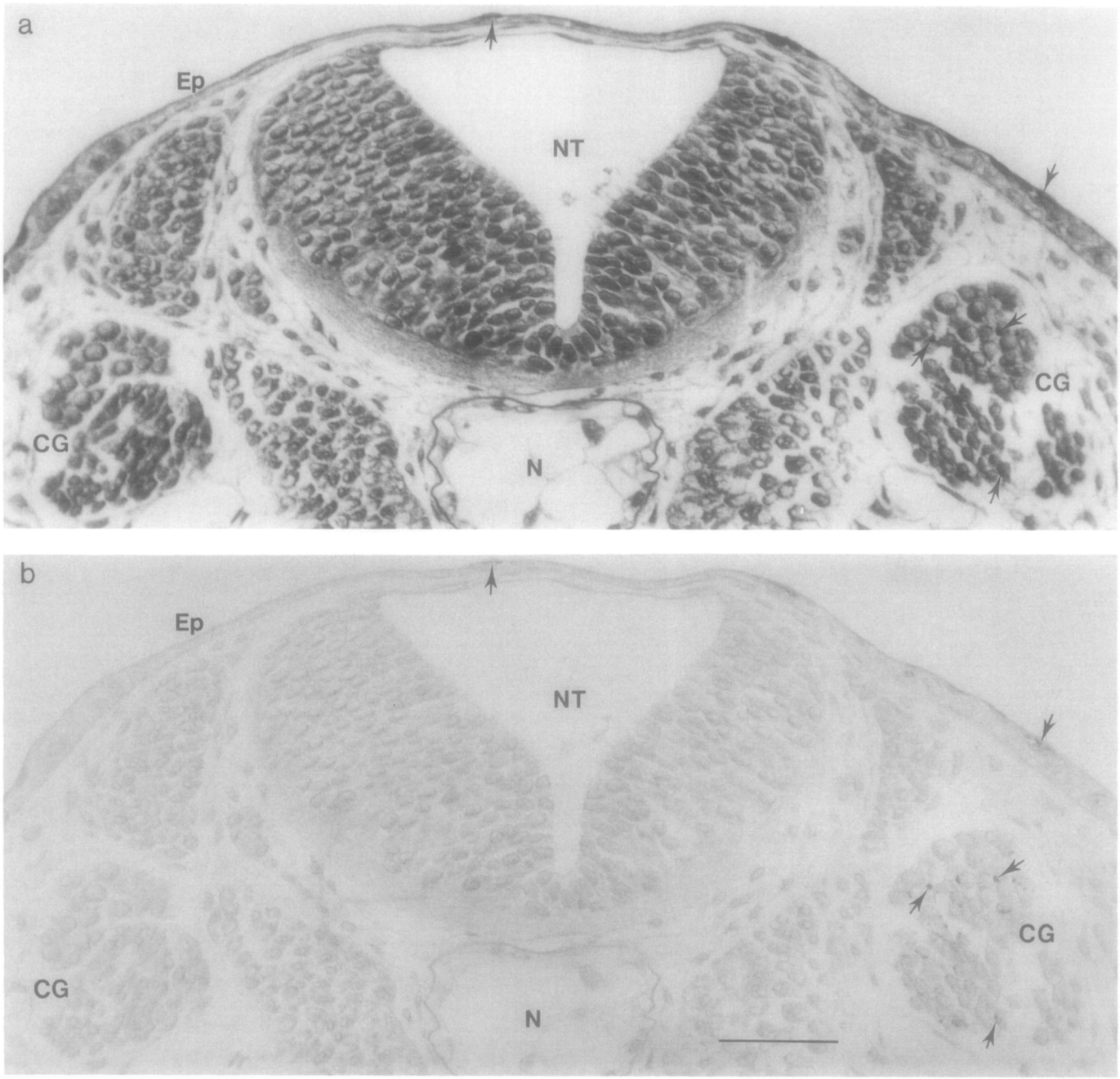

FIG. 6. Cross section through the myelencephalic region of an albino embryo whose right cranial neural fold has been replaced with pigmented epidermis. On the right side, cells containing pigment granules (arrows) appear in the cranial ganglion (CG) and in epidermis (Ep) that came from the graft. No pigmented cells appear in the neural tube (NT), the notochord (N), the nongraft epidermis, or the cranial ganglion on the left side. Time elapsed since operation: 8 days. Line scale = 100 μm. (a) Section viewed with normal illumination. (b) Section viewed with red light to accentuate pigment granules.

neural theshold) and/or within the epidermis (different levels of induction below the neural threshold).

Local interactions between cells in the neural plate and epidermis cause neural crest cells to form and help them to leave the epithelium (Figs. 8b, 8c). Neural folds and some types of neural crest derivatives formed when nonfold neural plate and epidermis (products of neural induction) were juxtaposed experimentally. However, because our experiments were performed after the neural plate had differentiated, and we did not observe neural plate contributing to epidermis or vice versa, the formation of neural folds and neural crest cells appears to result from local interactions at the boundary, rather than from neural induction. These local interactions

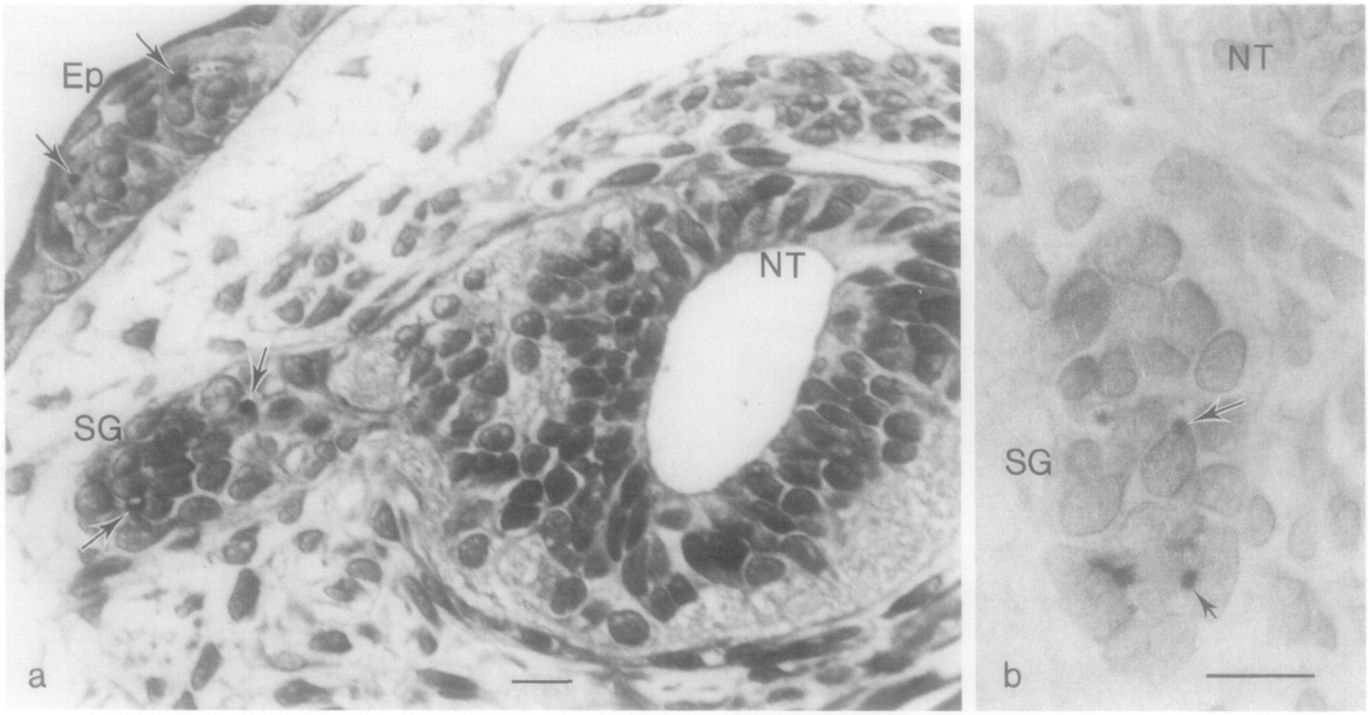

FIG. 7. Cross sections through the spinal cord of an albino embryo whose trunk neural fold has been replaced with pigmented epidermis. Cells containing pigment granules (arrows) appear in the spinal ganglion (SG), and in epidermis (Ep) that came from the graft, but not in the neural tube (NT). Time elapsed since operation: 15 days. Line scales = 25 μm. (a) Low magnification view (normal illumination) of the spinal ganglion and surrounding tissues. (b) When viewed in red light, a higher magnification of another section through the spinal ganglion in Fig. 6a clearly shows several cells that contain pigment granules.

most likely involve reciprocal inductions in which epidermis induces competent cells from the neural plate to become neural crest cells, and neural plate induces competent cells from the epidermis to become neural crest cells (Fig. 8b). Initially, these local interactions would only affect cells that are near the boundary; then, as the rising neural fold brings more of the epidermis and neural plate into contact, cells in regions that are farther from the original boundary could interact.

Local interactions also generate forces that help to raise the neural folds (Moury and Jacobson, 1989) and that may help to free cells from the neural plate and epidermis as neural crest cells (Fig. 8c). For example, as the neural folds fuse at the midline, a "wedge" of epithelial cells (probably most are epidermal cells) appears to become isolated between the epidermis and the neural tube. These cells are in the future neural crest area, and it is possible that they become part of the neural crest.

There must also be a mechanism by which individual cells can actively crawl out of the epithelium because, in some animals, neural crest cells in some regions migrate out of the epithelium before the neural folds fuse (Le Douarin, 1982; Hall and Hörstadius, 1988). Future neural crest cells may pull free of the epithelium when their apical surfaces become reduced to such a small area that internally produced forces are sufficient to break the apical junctional complexes that bind them to one another (Jacobson, 1987). As the rising neural folds bring more neural plate and epidermal cells into contact, cells in areas farther from the boundary could be released from the epithelium. The sequence of events just described is consistent with changes in cellular morphology that Nichols (1981, 1987) observed during the segregation of neural crest cells in mice. Furthermore, the morphology of the long, flask-shaped cells in the neural plate (especially along its border with the epidermis) and the elongated cells in epidermal "tabs" that extend beneath the neural plate at the base of the neural folds (Moury and Jacobson, 1989) is consistent with the expected morphology of the cells that are losing their attachments as they crawl (or are pulled) out of the epithelium.

Some, but perhaps not all, neural crest cells are multipotent when they leave the epithelium. Several studies have found individual neural crest cells that are bipotent for melanin and neurotransmitters (Sieber-Blum and Cohen, 1980; Loring *et al.*, 1982; Satoh and Ide, 1987; Bronner-Fraser and Fraser, 1988), and, in one of our transplants, cells from a neural plate graft became melanocytes and part of a spinal ganglion. However, in most of our experiments, neural crest cells that originated in the nonfold neural plate became melanocytes,

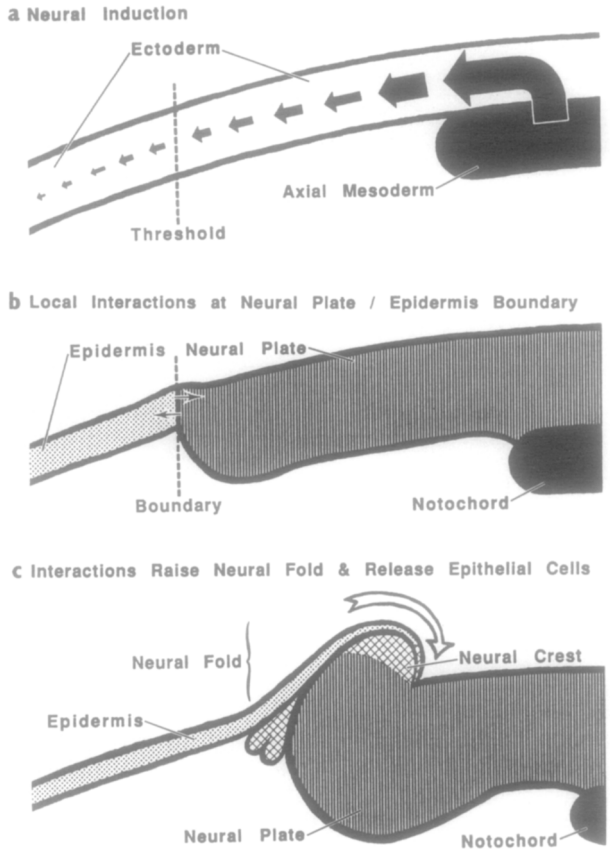

FIG. 8. Diagrams illustrating the probable sequence of interactions that results in the release of neural crest cells from the epithelium. (a) During gastrulation, the neural inductive signal (solid arrows) originates in the axial mesoderm (black) and travels through the ectoderm (white), diminishing with distance from its source. The threshold defines the future boundary between neural plate and epidermis. (b) Ectoderm induced to levels above the threshold becomes neural plate (hatch), and ectoderm induced to levels below the threshold becomes epidermis (stipple). Local interactions, perhaps including inductions (solid arrows), then take place across the boundary between these tissue types. (c) During neurulation, forces generated by the local interactions at the boundary help the neural fold to rise and roll toward the midline (open arrow) and help cells from both neural plate and epidermis to crawl out of the epithelium (crosshatch). These released cells can then contribute to the neural crest. (The "loose" cells represent cells that are leaving the epithelium to become part of the neural crest; they do not indicate that neural crest cells migrate into the tissues prior to fusion of the neural folds in axolotls.)

while most neural crest cells that originated in the nonfold epidermis became cells in ganglia. We speculate that cells originating nearer to the boundary may be competent to form a wider range of neural crest derivatives than cells originating farther from the boundary and that by removing the tissues near the boundary (i.e., the natural neural folds) we (and Niu, 1954) may have eliminated several subpopulations of multipotent cells (and their derivatives). More data are needed, however, to test these speculations.

This study is part of a Ph.D. dissertation by J. D. Moury at The University of Texas at Austin. We are grateful to the Indiana University Axolotl Colony for providing the genetic strains of axolotl embryos. This work was supported by NIH Grant HD 25902, and by a B.R.S.G. grant through the University Research Institute of The University of Texas at Austin.

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