

Development of neural tube basal lamina during neurulation and neural crest cell emigration in the trunk of the mouse embryo

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SUMMARY

In the trunk of higher vertebrates, the neural crest (NC) cells remain temporarily within the dorsal portion of the neural tube after fusion of the neural folds; shortly thereafter they emigrate, invading surrounding spaces and tissues. One of the factors postulated to be important in the initiation of migration of NC cells is the disruption of the basal lamina (BL) over the dorsal portion of the neural tube. It has been assumed by many that the BL must be discontinuous in order that the NC cells can leave the neural tube; and indeed, experiments performed in our laboratory, and by others, have shown that NC cells cannot penetrate an intact BL. Therefore, we have undertaken a systematic ultrastructural study to evaluate the condition of the BL during neural fold elevation and NC cell emigration. Our results show that: (i) BL surrounding the neural epithelium (NE) becomes progressively more extensive from neural fold to migratory stages. It first forms on the lateral portion of the neuroepithelium of the neural folds and then extends ventrally into the region adjacent to the notochord; (ii) BL becomes continuous beneath the epidermal ectoderm (EE) that overlies the NC cell region only during the terminal stages of NC cell emigration; (iii) BL does not form over the dorsal portion of the neural tube until NC emigration is terminated; and (iv) the morphology of the BL changes as development proceeds. We conclude that absence of a BL over the premigratory NC cell population in the trunk of mouse embryos is a necessary but not a sufficient condition for emigration to take place.

INTRODUCTION

The basal lamina (BL) is an extracellular matrix structure that surrounds parenchymal tissues and separates them from connective tissue. It is 40–120 nm thick and consists of a 'lamina rara' composed mostly of electron-transparent material that lies immediately adjacent to the plasma membrane and is overlain by a 'lamina densa' composed of electron-dense material (Farquhar, 1978; Kefalides, Alper & Clark, 1979; Bluemink, Faber & Lawson, 1984; Madri, Pratt, Yurchenco & Furthmayr, 1984; Laurie, 1985; Laurie & Leblond, 1985).

Basal laminae have been shown to be important in the maintenance of orderly tissue structure, in remodelling of tissues in response to injury, and in the control morphogenetic events (Bernfield, Cohn & Banerjee, 1973; Bernfield, Banerjee, Koda & Rapraeger, 1984; Vracko, 1974, 1982; Banerjee, Cohn & Bernfield, 1977;

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Bernfield & Banerjee, 1982; Martinez-Hernandez & Amenta, 1983; Ormerod, Warburton, Hughes & Rudland, 1983; Madri *et al.* 1984; Timpl, 1985). In addition, its modification or disruption has been postulated to be involved in the initiation of migration of neural crest (NC) cells (Tosney, 1978, 1982; Erickson & Weston, 1983; Erickson, 1986). Light microscope studies of the trunk region of chick and mouse embryos using specific stains (Erickson & Weston, 1983) and immunohistochemistry (Newgreen & Thiery, 1980; Sternberg & Kimber, 1986) have suggested that, prior to migration, the NC cells are constrained within the neural tube by a continuous basement membrane. (Basement membranes are composed of BL plus some associated extracellular matrix components, Bluemink *et al.* 1984; Laurie & Leblond, 1985; BL alone cannot be resolved by the light microscope.) However, electron microscope studies have shown that the BL is discontinuous just prior to NC cell emigration from the neural tube (Tosney, 1978, 1982; Newgreen & Gibbins, 1982; Erickson & Weston, 1983). Many workers have concluded, therefore, that the BL would have to be disrupted in order for the NC cells to leave the neural tube and that this disruption (or disintegration) might be an important factor in the initiation of migration. However, no systematic studies have determined if the BL is complete well before NC cell separation from the neural epithelium (NE) or if the BL over the presumptive NC cells has never developed. Consequently, we have undertaken a systematic ultrastructural study to determine the timing of deposition and disruption of BL during neurulation and early NC cell development in the trunk region of mouse embryos.

The neural crest arises in an anterior-to-posterior wave in avian (Tosney, 1978; Bancroft & Bellairs, 1976) and in murine (Erickson & Weston, 1983) embryos. Therefore the entire process of development can be followed by serial sectioning individual embryos. We have chosen 9.5-day-old mouse embryos because at this stage the complete spectrum of NC cell development can be studied. In addition, to confirm that the development of BL through time at a particular axial level of the embryo is equivalent to the development of BL along the axis of 9.5-day-old embryos, we have examined the process of deposition of BL at the 15th somite level (or equivalent) in 10-, 17- and 24-somite embryos. Our observations demonstrate that BL does not form over the portion of the neural tube containing NC cells until after all NC cells have emigrated.

METHODS

Balb/c mice were mated and day 0 was defined as the day that vaginal plugs were discovered. Embryos between 8.5–9.5 days of gestation were removed from the uteri and freed from the extraembryonic membranes with watchmaker's forceps, briefly washed in phosphate-buffered saline (PBS; mouse tonicity) and immediately fixed.

Processing for TEM

Embryos were fixed for 2 h at room temperature in a solution containing 2.5% glutaraldehyde and 1% paraformaldehyde in a 0.1 M-sodium cacodylate buffer, pH 7.4 or in a 3% glutaraldehyde solution in 0.05 M-phosphate buffer, pH 6.8. The embryos were washed in buffer, then postfixed for 1 h at room temperature in a 2% aqueous solution of osmium tetroxide and stained

in a saturated solution of uranyl acetate for 2 h at 4°C in the dark. This was followed by dehydration in an ascending acetone series and embedding in Epon-Araldite. Sections, 2 µm thick, were cut on a Reichert ultramicrotome and stained in 1% toluidine blue in sodium borate for light microscopic observations. Thin sections were cut with the same instrument and stained with a saturated solution of uranyl acetate in 70% ethanol followed by lead citrate (Reynolds, 1963). The ultrastructural observations were made at 80 kV on a JEM 200 transmission electron microscope.

RESULTS

We have serially sectioned three 9.5-day-old embryos (24–26 somites) and examined by transmission electron microscopy (TEM) the entire trunk from the posterior neuropore to the cervical region. From this spectrum, we have chosen six levels to illustrate the development of BL (Fig. 1A). At the most posterior levels, the future NC cells are found within the ridges of the elevating neural folds (neural fold stages, Fig. 2A–C); a short distance anteriorly, where the neural folds have already fused, they occupy the dorsal portion of the neural tube (pre migratory stage, Fig. 2D); a few somites farther anteriorly, the cells migrate away from the neural tube in large numbers (migratory stage, Fig. 2E); and finally, in the most anterior portion of the trunk, emigration is coming to an end (terminal migratory stage, Fig. 2F). We studied the process of BL deposition around the neural tube, over the presumptive NC cells, and under the epidermal ectoderm (EE) that overlies the neural epithelium.

At the axial level of the posterior neuropore (early neural fold stage), the NE is not well organized; cells are only slightly elongated and little demarcation is evident between the NE and adjacent mesenchyme (Fig. 2A). No BL is present in the region of close apposition of the EE and presumptive NC cells. Under the EE, no BL is present dorsal to area *a* in Fig. 2A, but pieces occur in more lateral areas (area *b* in Fig. 2A; data not shown). At an intermediate neural fold stage the NE has become increasingly well defined (Fig. 2B). In the region of close apposition of the EE and the NE, there is no BL over the region that presumably contains the NC cells, and the BL is patchy beneath the EE (area *a* in Fig. 2B; Fig. 3A). Ventral to area *b* in Fig. 2B, the BL underlying the EE becomes continuous (Fig. 3B, double arrowhead). BL deposition has begun on the NE; it appears as patches on what will become the lateral portions of the neural tube (area *c* in Fig. 2B; Fig. 3C). The observations of the late neural fold stage (Fig. 2C) are similar to the previous stage; no BL is present in areas *a* and *b*. However, the BL on the lateral portions of the neural tube (area *c*) is more extensive. On the ventral-most part of the neural tube, deposition of BL has just begun. At the pre-migratory stage (Fig. 2D), the BL remains absent over the NC cells (Fig. 4A,B) and is still patchy under the EE as far as the point indicated by the arrow in Fig. 2D. Lateral to the arrow in Fig. 2D, the EE is underlain by a continuous BL. Around the lateral and ventral portions of the neural tube, the BL is well developed, but points of discontinuity still can be seen (Fig. 4C). At migratory stages (Fig. 2E), the BL is nearly continuous under the EE, whereas it remains absent over the dorsal neural tube (areas *a* and *b*). By this stage, the BL around

the lateral and ventral portions of the neural tube is complete. Finally, when the last NC cells are leaving (Fig. 2F), the BL is continuous under the EE (Fig. 5D) and is being deposited over the dorsal-most part of the neural tube (Fig. 5A–C).

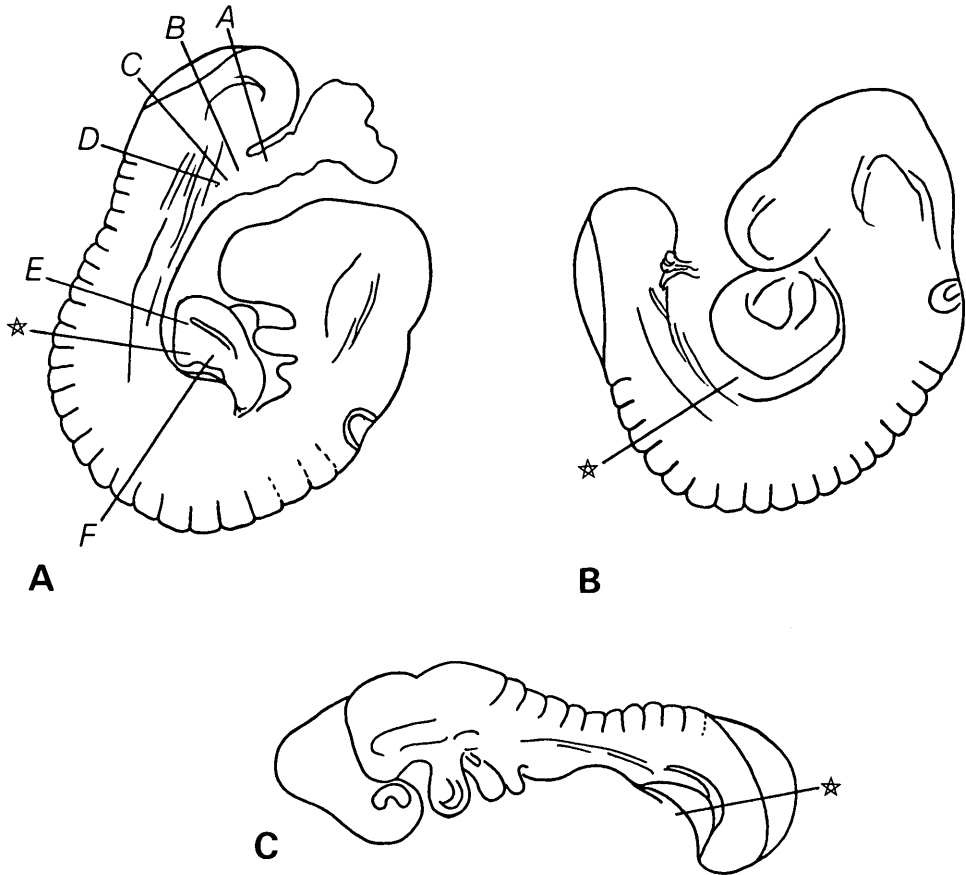
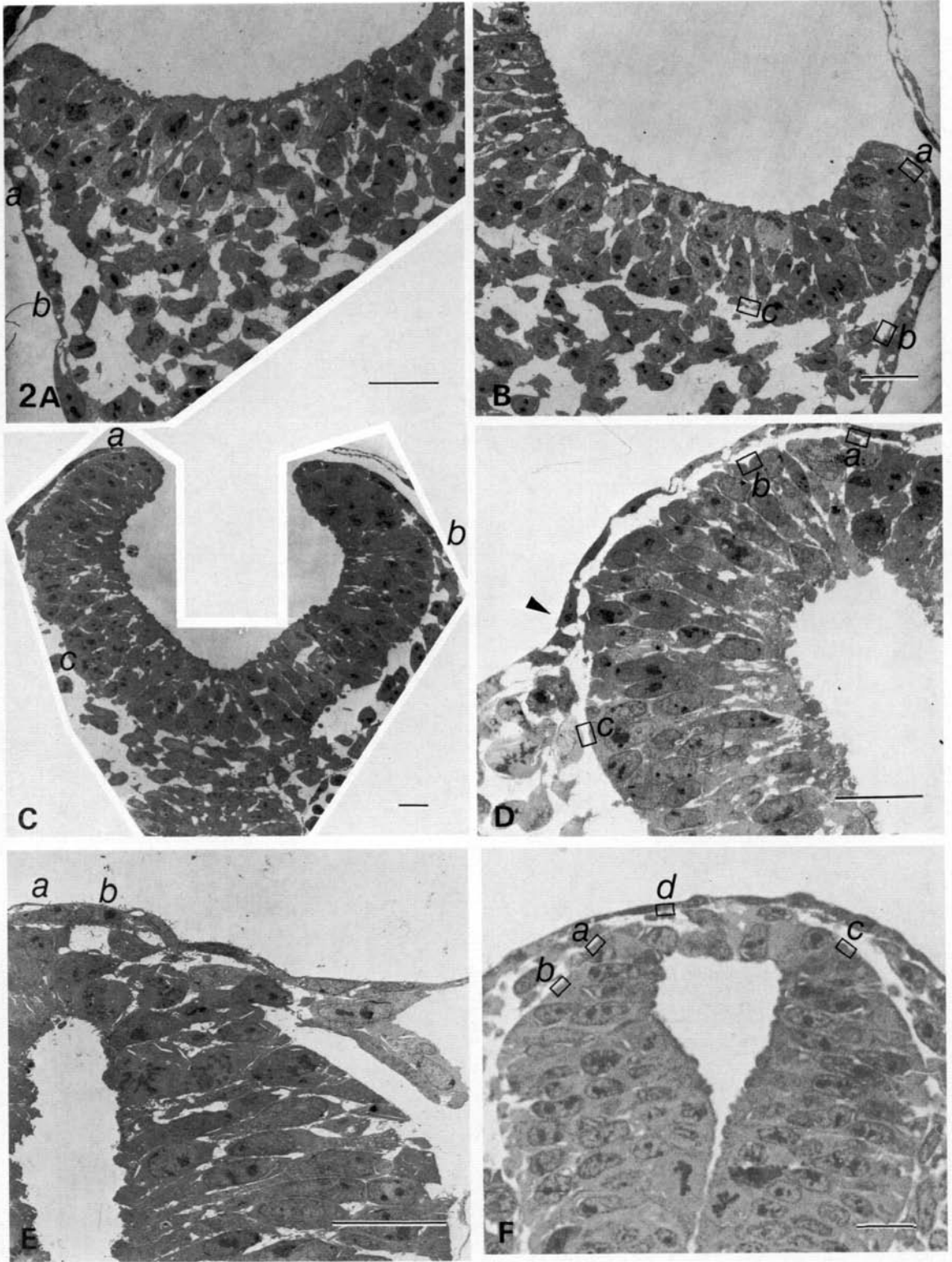


Fig. 1. *Camera-lucida* drawings of 8.5- to 9.5-day-old mouse embryos that were sectioned for this study. (A) 9.5-day embryo (24–26 somites). Cross sections showing progressive development of the basal lamina are taken from axial levels indicated by labelled lines A–F and are shown in detail in Figs 3–5. The 15th somite (star) is shown in Fig. 8 for comparison with the same level in younger embryos. (B) 8.5-day embryo (10 somites). Cross section through location of future 15th somite is marked and is shown in detail in Fig. 6. (C) 9.0-day embryo (17 somites). Cross section through 15th somite is marked and is shown in detail in Fig. 7.

Fig. 2. Cross sections of 9.5-day-old mouse embryos at the levels marked in Fig. 1A. (A–C) Neural fold stages with cross sections taken through the neuropore. The presumptive neural crest (NC) cells occupy the tips of the neural folds. Note that the neural epithelium becomes progressively more organized and well defined. (D) Late premigratory stage. The neural folds have fused to form the neural tube. The future NC cells occupy the dorsal-most part of the tube. (E) Migratory stage. This cross section shows NC cells migrating away from the neural tube. (F) Cross section of the neural tube showing the terminal stages of emigration of NC cells; most of the NC cells have separated from the neural tube. Lower case letters label the areas marked by boxes that correspond to the higher magnification shown in Figs 3–5. Scale bars, 20 μm .



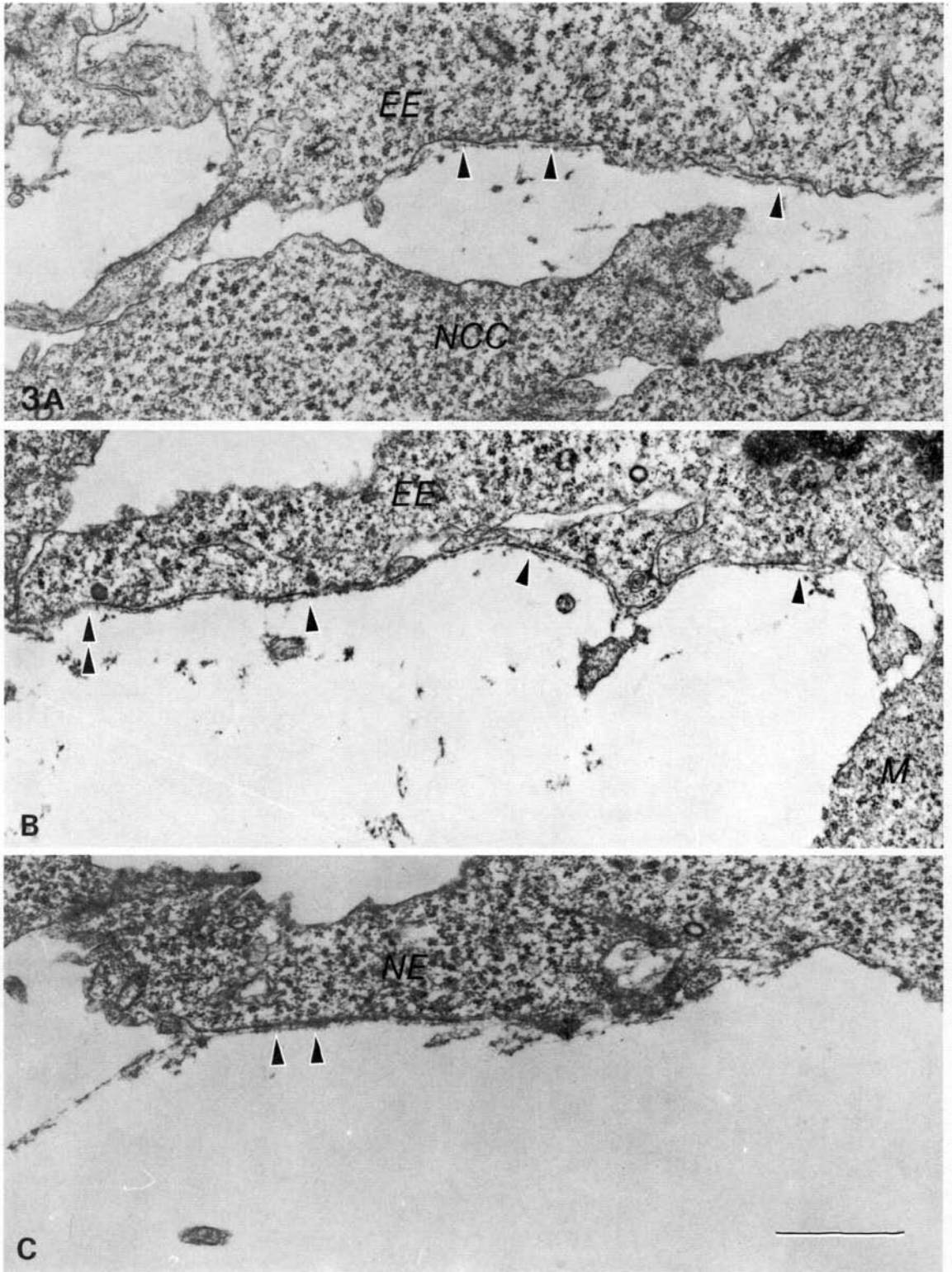


Fig. 3. For legend see p. 226

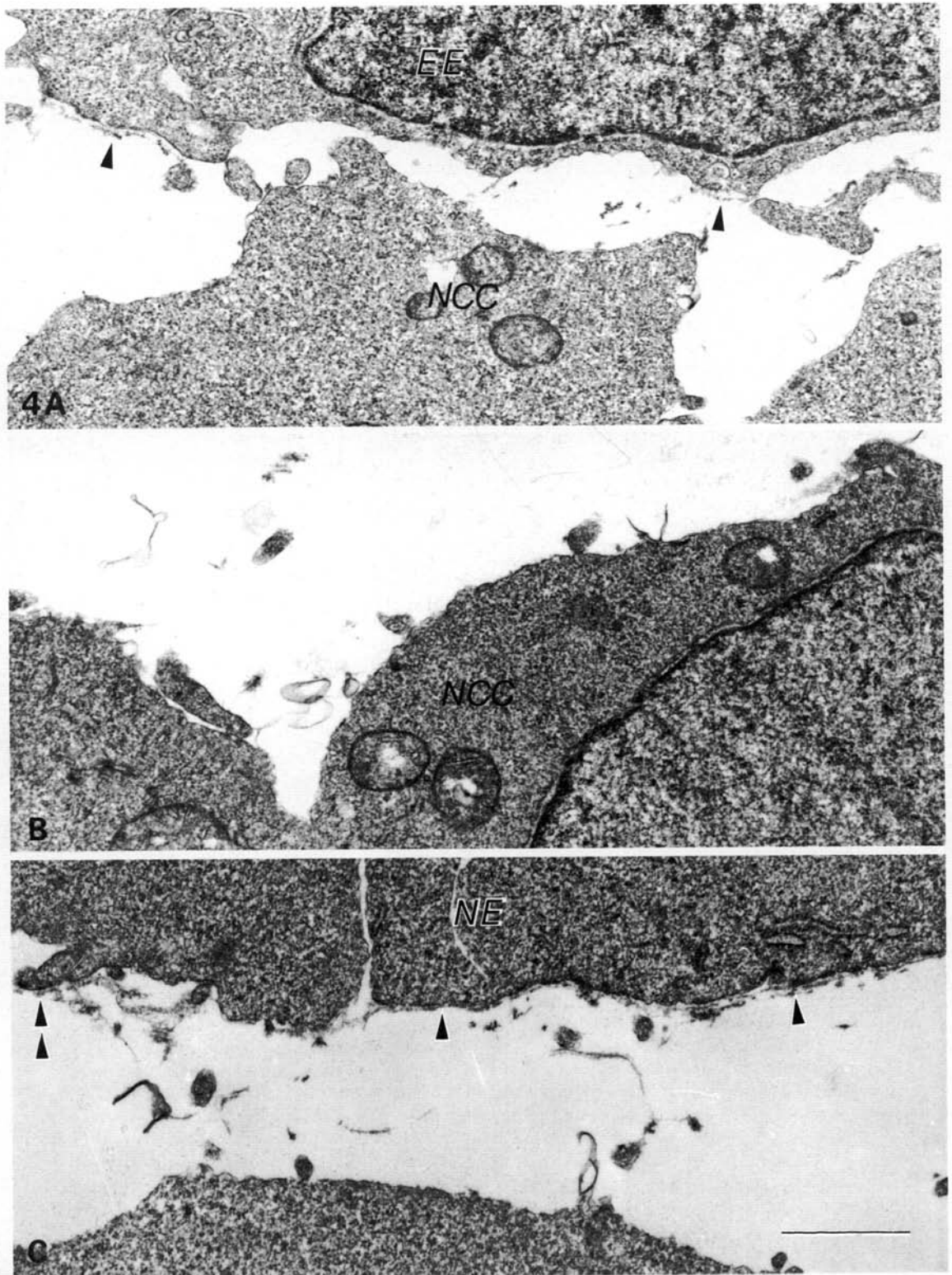


Fig. 4. For legend see p. 226

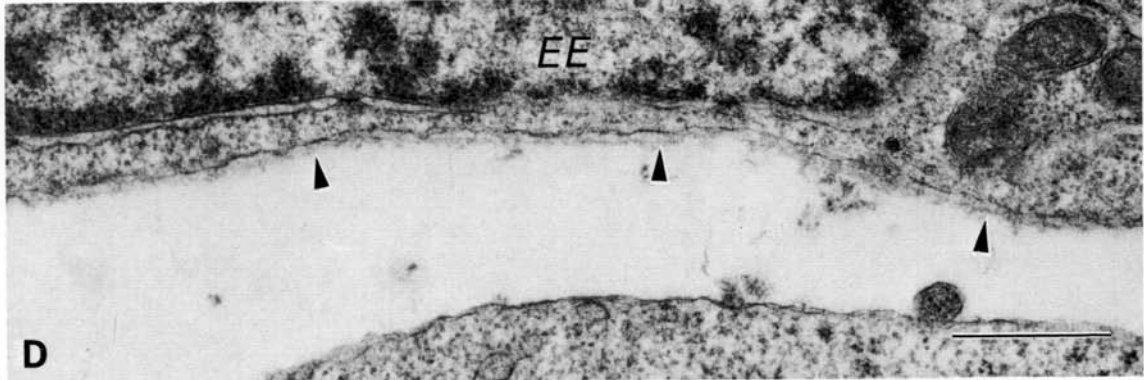
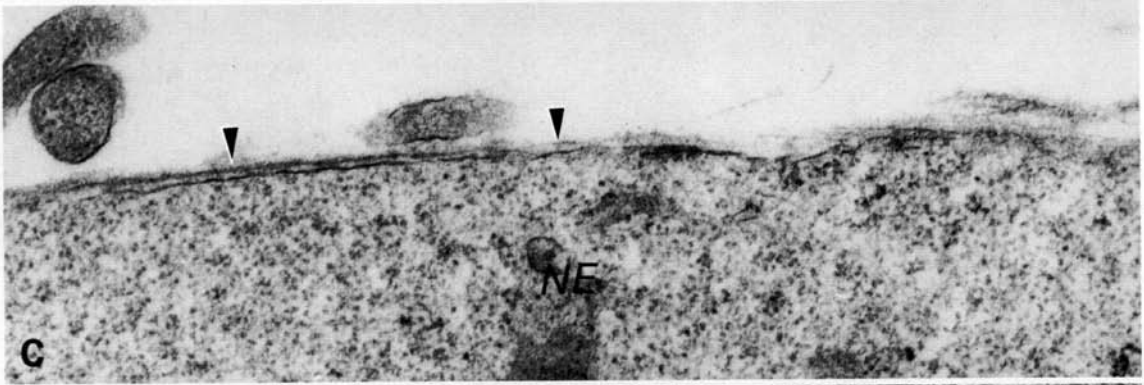
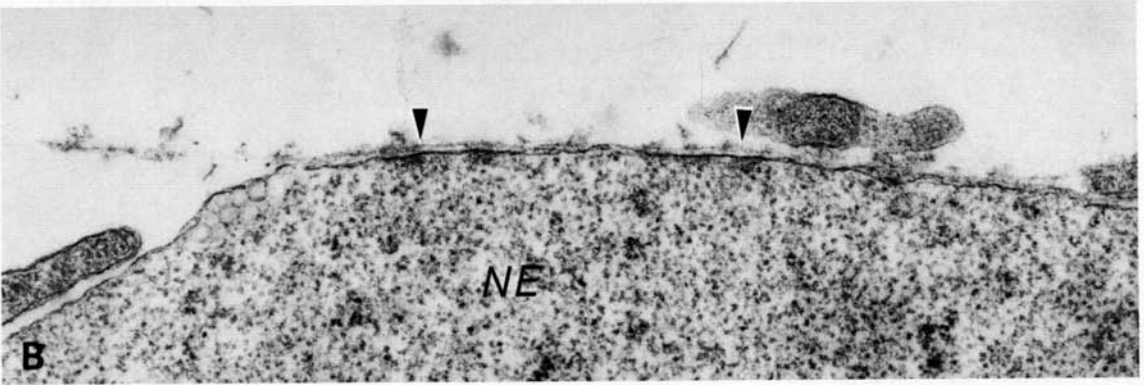
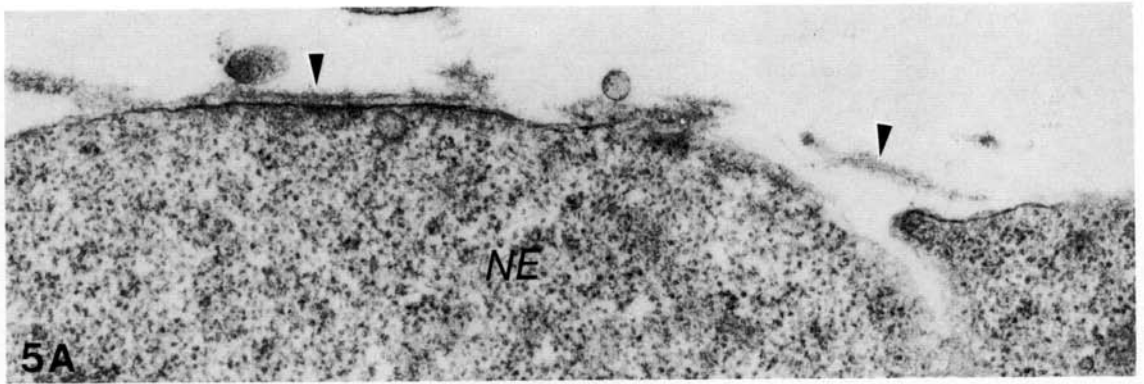
In order to confirm that progressive development of the BL from posterior to anterior levels of the trunk of single embryos is equivalent to a time sequence at a single somite level, we have examined the 15th somite or equivalent in 8.5-day (10 somite) embryos (Fig. 1B and Fig. 6) and in 9-day (17 somite) embryos (Fig. 1C and Fig. 7), in addition to the 15th somite in the 9.5-day (24–26 somite) embryos (Fig. 1A and Fig. 8). In the 10-somite embryo, the region where the 15th somite will eventually form is at the level of the posterior neuropore where the neural folds are widely opened (intermediate neural fold stage; Fig. 6A,B). Although BL is starting to be deposited laterally on the NE (Fig. 6E, arrows), there is no BL over the presumptive NC cells (Fig. 6C,D). In the 17-somite embryo, at the level of the 15th somite, the neural folds have fused to form the neural tube (Fig. 7A) but the NC cells occupying the dorsal portion of the tube show no signs of initial migration (pre migratory stage, Fig. 7B). A space between the presumptive NC cells and the EE has not yet opened; there are pieces of BL under the EE in this region, but no BL is seen on the future NC cells (Fig. 7C,D). However, some extracellular matrix components are found in this area. More laterally on the neural tube, BL is present (Fig. 7E). At the 15th somite level of 24–26 somite embryos, the NC cells are migrating abundantly from the neural tube (migratory stage; Fig. 8A,B). There is no BL over the NC cells that are still in the neural tube (Fig. 8C,D), nor is there any BL material on those that are actively migrating (not shown). However, the BL is starting to be deposited under the dorsoventral NE (Fig. 8E, arrowheads).

In addition to the progressive expansion of the regions covered by BL, the morphology of the BL itself also changes during development. At earlier stages, the BL is thin (40 nm) and poorly organized (Fig. 9A). It becomes thicker (60 nm) and more prominently layered at migratory stages (Fig. 9B), but does not become fully developed (80 nm) until postmigratory stages (Fig. 9C).

Fig. 3. Details from boxes in Fig. 2B (intermediate neural fold stage). (A) Region of apposition between the epidermal ectoderm (*EE*) and the presumptive NC cells (*NCC*). Small pieces of basal lamina can be seen on the *EE* (arrowheads), but are not present over the presumptive NC cells. (B) The BL (arrowheads) underlying the *EE* becomes continuous ventral to the area enlarged here. The point at which the BL becomes continuous is marked by a double arrowhead. *M*, mesenchyme. (C) Region of the neural epithelium (*NE*) where it is becoming well separated from the underlying mesoderm. BL deposition has begun locally (arrowheads), but pieces remain widely separated. Scale bar, 1 μ m.

Fig. 4. Details from boxes in Fig. 2D (late pre migratory stage). (A) Area in which the epidermal ectoderm is apposed to the NC cells (*NCC*). Pieces of BL underlie the *EE* (arrows), but no BL is observed on the NC cells. (B) Nearby area showing the total absence of BL over the NC cells. (C) Lateral region of the neural epithelium (*NE*). The BL underlying the *NE* (arrowheads) is continuous ventral to this point (double arrowhead). Scale bar, 1 μ m.

Fig. 5. Details from boxes in Fig. 2F (terminal stage of emigration). By this stage BL has been deposited over all but the dorsal-most portion of the neural tube. (A) Extensive pieces of BL are being deposited in this area. (B,C) Areas of the neural epithelium (*NE*) ventral to which a continuous BL (arrowheads) is present. (D) The BL (arrowheads) is now continuous under the *EE*. Scale bar, 1 μ m.



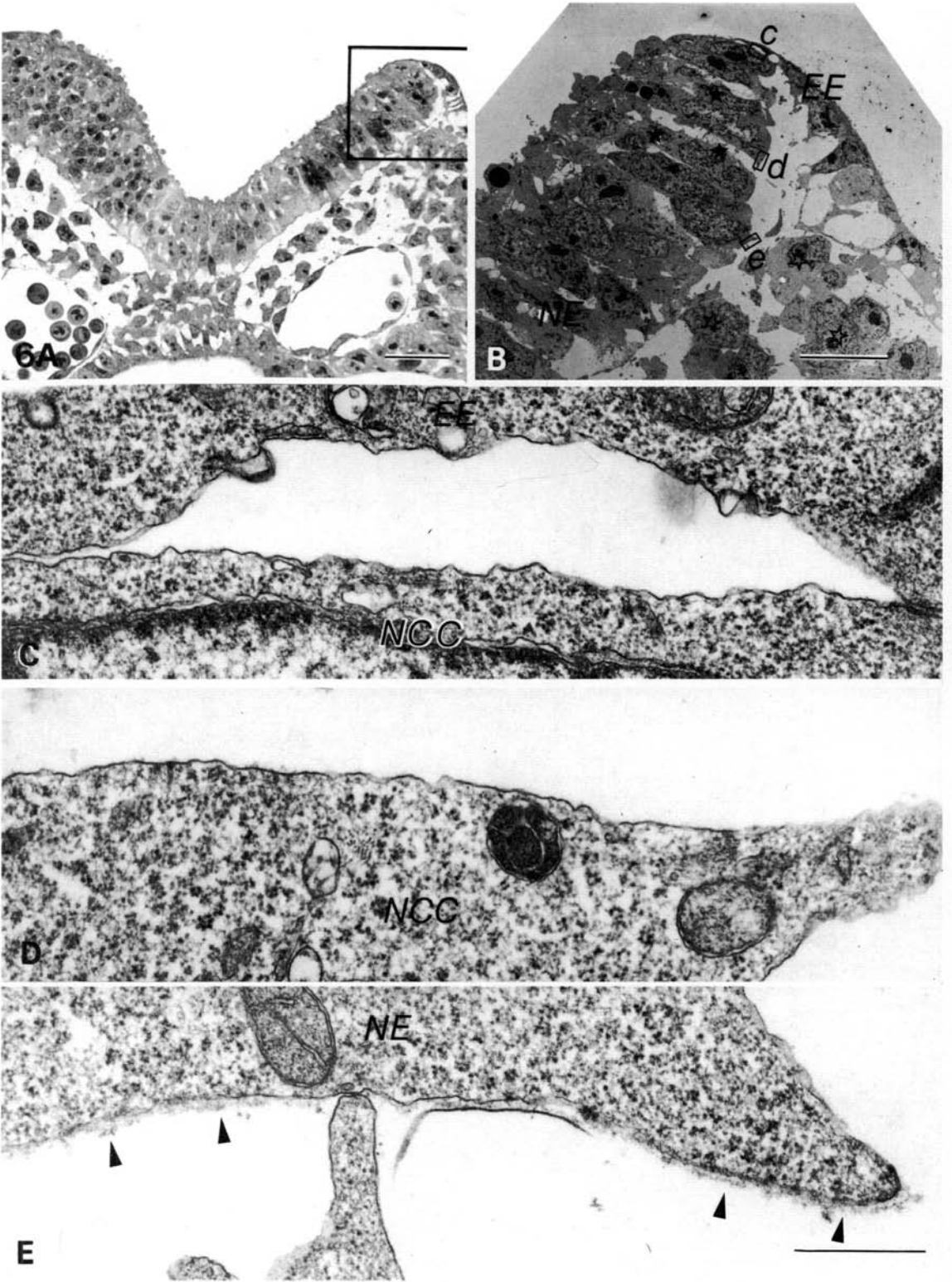


Fig. 6. For legend see p. 230

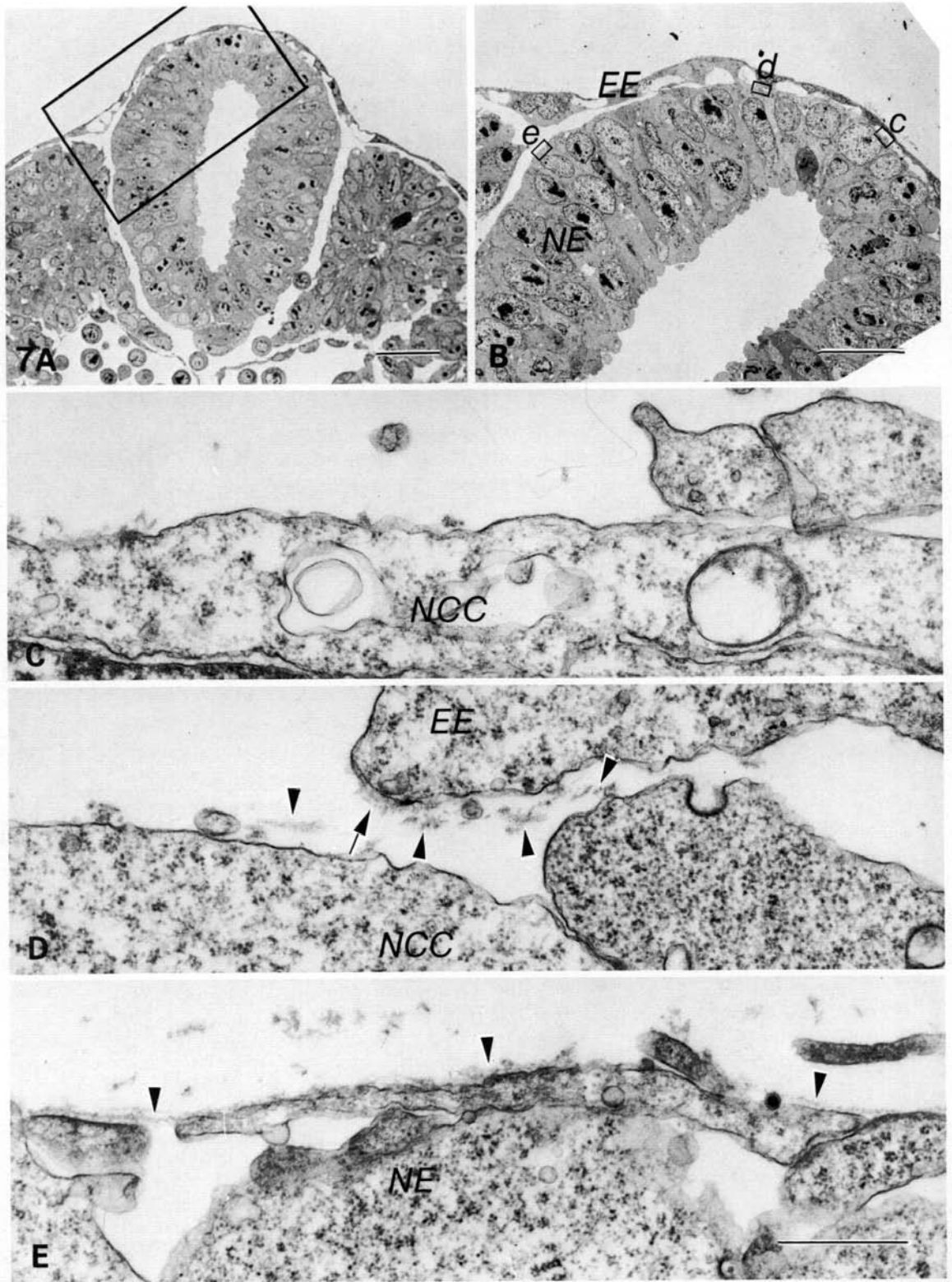


Fig. 7. For legend see p. 230

DISCUSSION

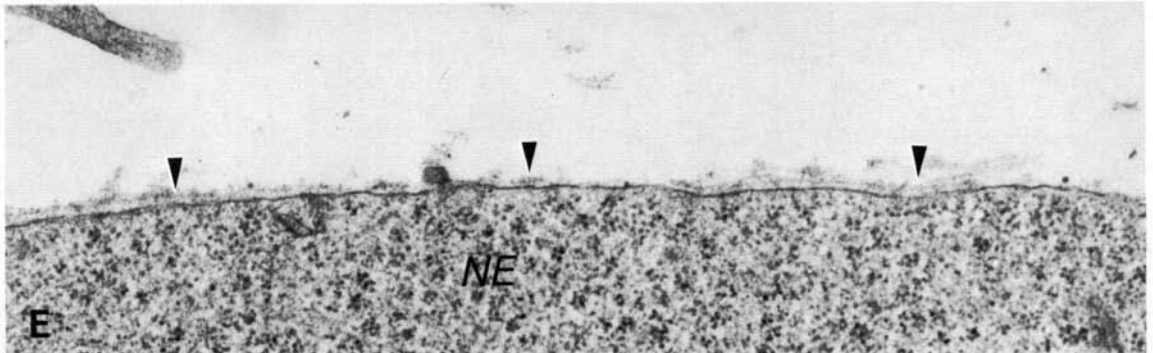
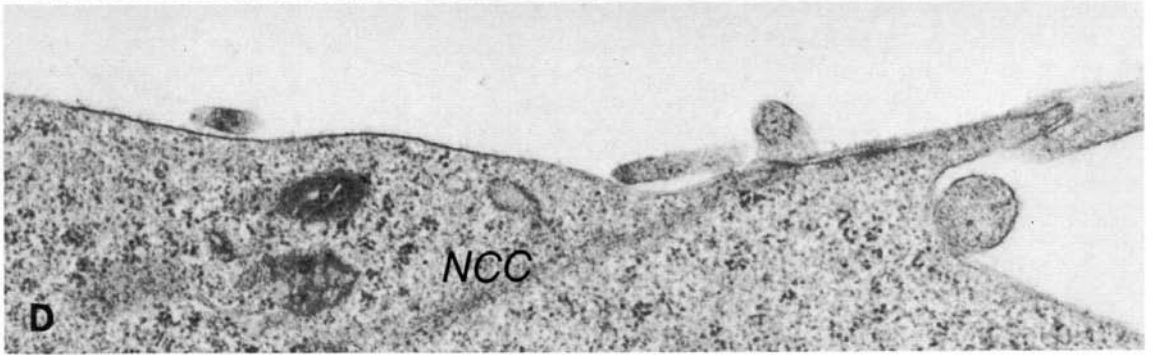
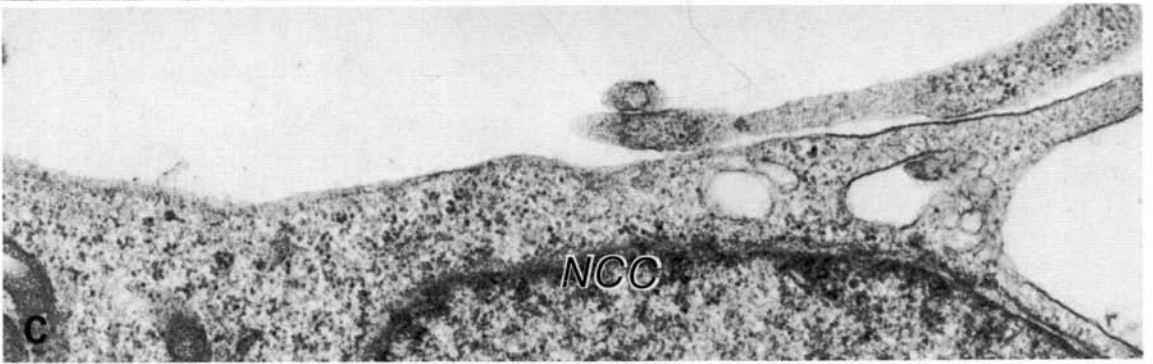
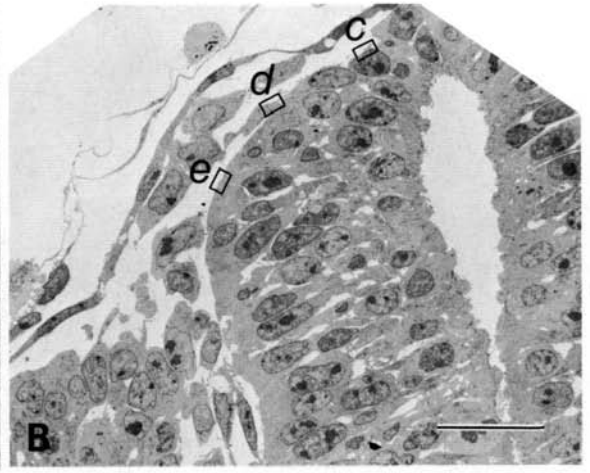
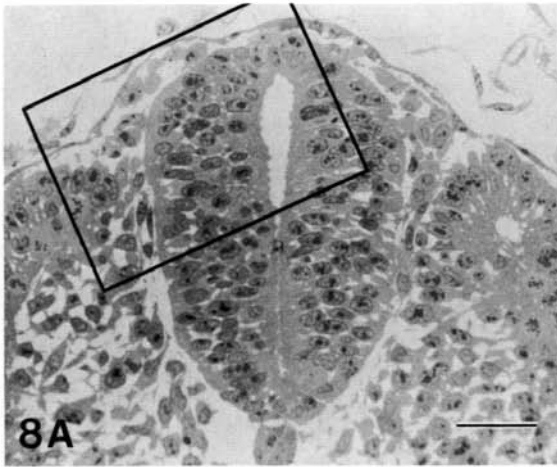
Our observations are summarized in Fig. 10. We have found that in the trunk of mouse embryos deposition of BL around and in the vicinity of the neural tube is a continuous process that can be summarized as follows: (i) BL investing the NE becomes progressively more extensive from neural fold to migratory stages. It first forms laterally and then extends ventrally into the region adjacent to the notochord; (ii) BL becomes continuous beneath the EE that overlies the NC cell region only during the terminal stages of NC cell emigration; and (iii) BL does not form over the dorsal portion of the neural tube until NC cell emigration has terminated. These conclusions apply whether one examines NC cell development in single 9.5-day-old embryos or whether one follows a particular level through development in embryos of different ages. Although the shape of the posterior neuropore changes with development (compare Fig. 2B with Fig. 6A), the pattern of deposition of BL remains the same. Extracellular matrix molecules were found in the narrow space between the premigratory NC cells and the overlying EE, as well as pieces of BL under the EE (Fig. 7D). It is quite possible that immunohistochemical techniques that identify components such as laminin, fibronectin and collagen IV could produce the appearance of a continuous basement membrane in this region when viewed with the optical microscope.

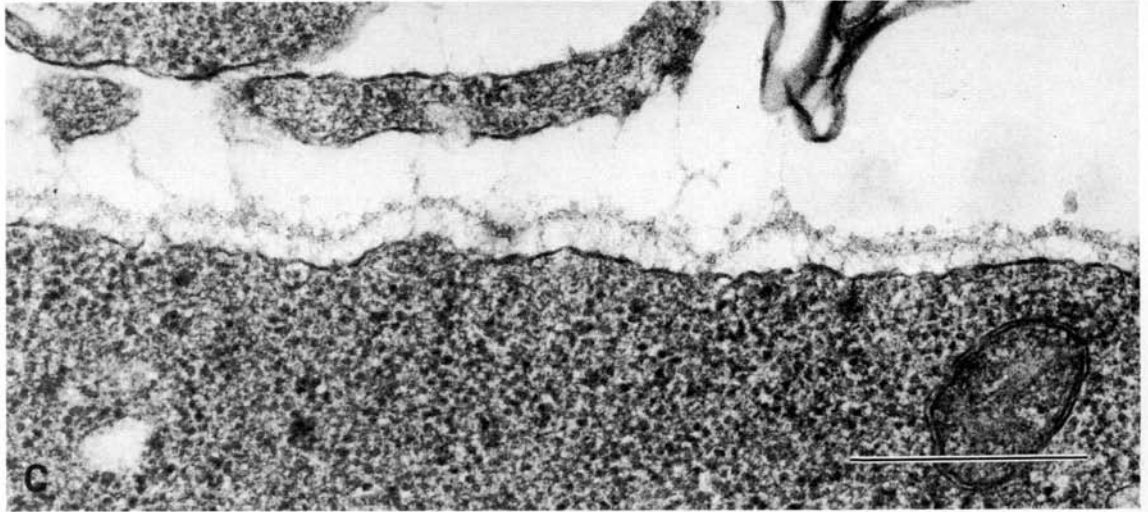
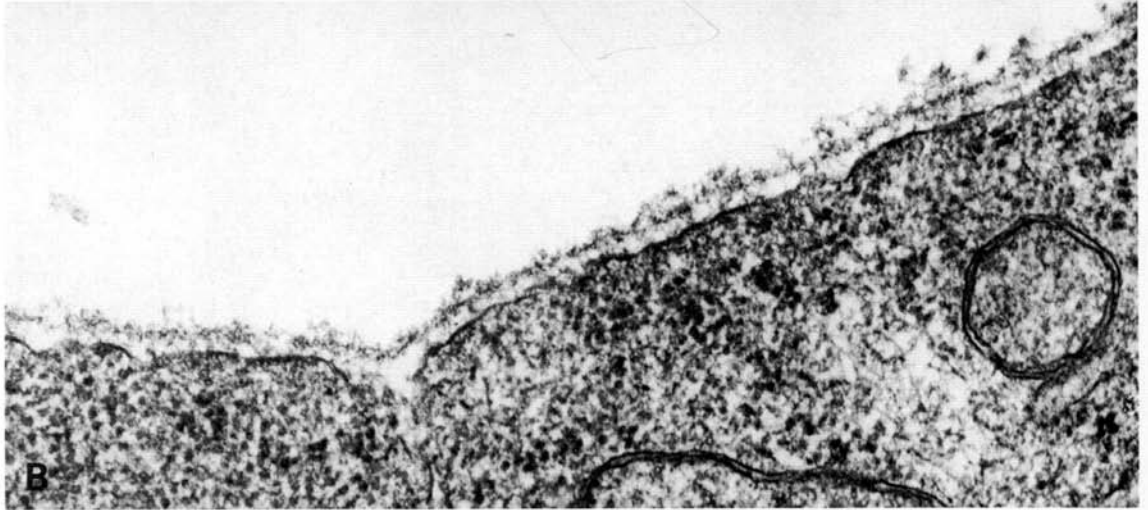
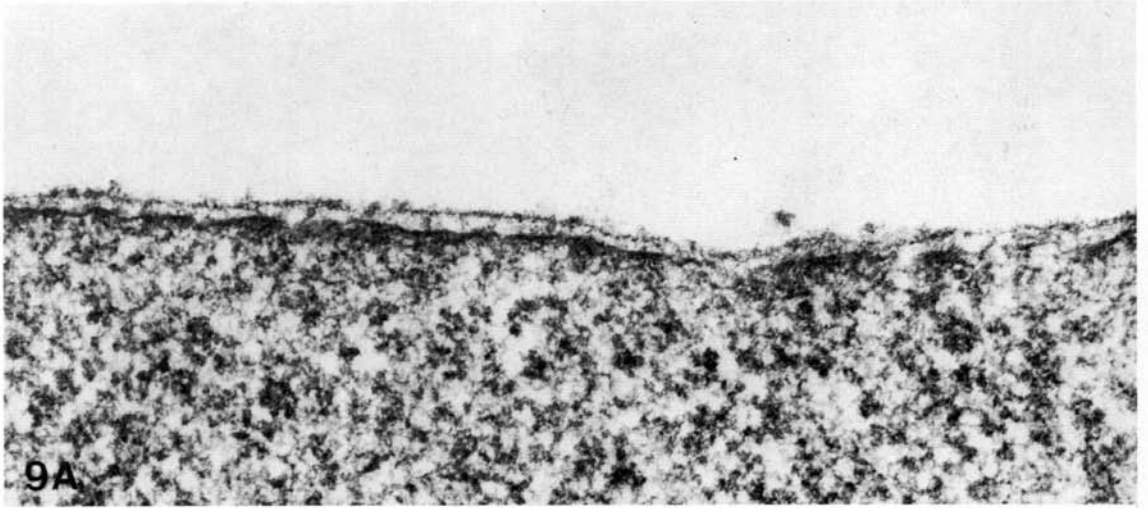
Because the BL is not complete over the dorsal portion of the neural tube until after emigration is complete, it follows that the trunk NC cells do not have to penetrate or degrade the BL in order to leave the neural tube. Moreover, our observations suggest that the future NC cells are distinct from the remaining neural epithelial cells very early in development because they lack a BL, whereas the neural epithelial cells around the lateral and ventral portions of the neural tube secrete and deposit a complete BL. These latter observations are consistent with

Fig. 6. (A) Cross section of an 8.5-day (10 somite) embryo at a level equivalent to the 15th somite (light micrograph). Scale bar, 40 μm . (B) TEM enlargement of box *a* in 6A; right neural fold showing the neural epithelium (NE) containing the presumptive NC cells (filled stars) at the tip of the fold, the epidermal ectoderm (EE) and mesoderm cells (open stars). Scale bar, 20 μm . (C,D) Details from boxes *c* and *d* in 6B; no BL is present under the EE nor over the presumptive NC cells. (E) Details of box *e* in 6B; BL (arrowheads) is starting to be deposited on the neural epithelium (NE). Scale bar, 1 μm .

Fig. 7. (A) Cross section of a 9-day-old (17 somite) embryo at the 15-somite level (light micrograph). Scale bar, 40 μm . (B) TEM enlargement of box *a* in 7A. Scale bar, 20 μm . (C,D) Details from boxes *c* and *d*; no BL is present over the presumptive NC cells but pieces of matrix (arrowheads) are found in the space between the NC cells and the epidermal ectoderm (EE). One small piece of BL has been deposited on the EE (arrow). (E) Detail from box *e* in 7B; BL (arrowheads) is being deposited on the lateral NE. Scale bar, 1 μm .

Fig. 8. (A) Cross section of a 9.5-day-old (24–26 somite) embryo at the 15-somite level (light micrograph). Scale bar, 40 μm . (B) TEM enlargement of box *a* in 8A. Boxes mark areas shown at higher magnification in Fig. 8C–E. Scale bar, 20 μm . (C,D) Details from boxes *c* and *d*; no BL is present on the stationary or migratory NC cells. (E) Detail of box *e* in 8B; BL (arrowheads) is being deposited on the dorsolateral NE. Scale bar, 1 μm .





the notion that the NC cells have begun the transition from epithelium to mesenchyme very early in neurulation.

Our results are in contrast to those of Nichols (1985) who reported that, in the head of 8-day-old mouse embryos, BL is first actively disrupted by NC cell processes and then deteriorates to allow departure of the remaining NC cells. This is perhaps another difference between NC cell development in the head and in the trunk (cf. Le Douarin, 1982 for review).

The deposition of BL also is delayed beneath the EE overlying the neural tube (similarly reported by Löffberg *et al.* 1985, for the axolotl). One possible explanation is that one or more components of BL may be in short supply in the ECM in this region, partially preventing BL deposition under the EE. A related possibility is that NC cells produce proteases that degrade certain BL components and thereby prevent assembly of the BL over the dorsal portion of the neural tube as well as on the overlying EE. One candidate for this degradation is plasminogen activator which is known to degrade extracellular matrix components (Laug, De Clerck & Jones, 1983; Sheela & Barrett, 1982; Fairbairn *et al.* 1985), and which is produced by head crest (Valinsky & Le Douarin, 1985) and trunk crest (Erickson & Isseroff, unpublished). Alternatively, receptors for the BL components on the basal surface of the cells or the binding sites on the BL components themselves may be partially masked. Of course, the possibility also exists that all of these mechanisms (missing component[s] and masking) could operate together. We presently are investigating these possibilities.

As mentioned above, previous studies have shown that the BL over the dorsal portion of the neural tube is not continuous during NC cell emigration (Tosney, 1978, 1982; Newgreen & Gibbins, 1982; Erickson & Weston, 1983; Erickson, 1986). Therefore it has been suggested that breakdown of the BL is the 'trigger' to initiate crest separation from the NE. Our present study demonstrates that breakdown of the BL cannot 'trigger' NC cell emigration since BL does not become complete over the dorsal portion of the neural tube until the end of emigration. Moreover, the lack of BL by itself is not sufficient to initiate emigration because during the early stages of neurulation, the BL is absent over the dorsal portion of the neural tube and discontinuous around the lateral and ventral portions, yet no blebbing or other signs of early migration are seen.

Fig. 9. Morphological details of BL development during neurulation (see text for definition of BL). (A) Advanced neural fold stage. At this early stage of development the 'lamina densa' is ~7 nm thick. Very little material extends into the extracellular space. The 'lamina rara' is ~32 nm thick and shows very little organization of its components. (B) Terminal migratory stage. The 'lamina densa' is ~16 nm thick. Numerous projections into the extracellular space can be seen, but no organization is evident. The 'lamina rara' is ~40 nm thick and has sparsely distributed filamentous structures perpendicular to the plasma membrane. (C) Postmigratory stage. The 'lamina densa' is ~24 nm thick. The projections into the extracellular space are long and frequently contact cells or cell processes. The 'lamina rara' is ~56 nm thick; the filamentous structures are perpendicular to the plasma membrane and are regularly spaced at ~38 nm. Scale bar, 0.5 μ m.

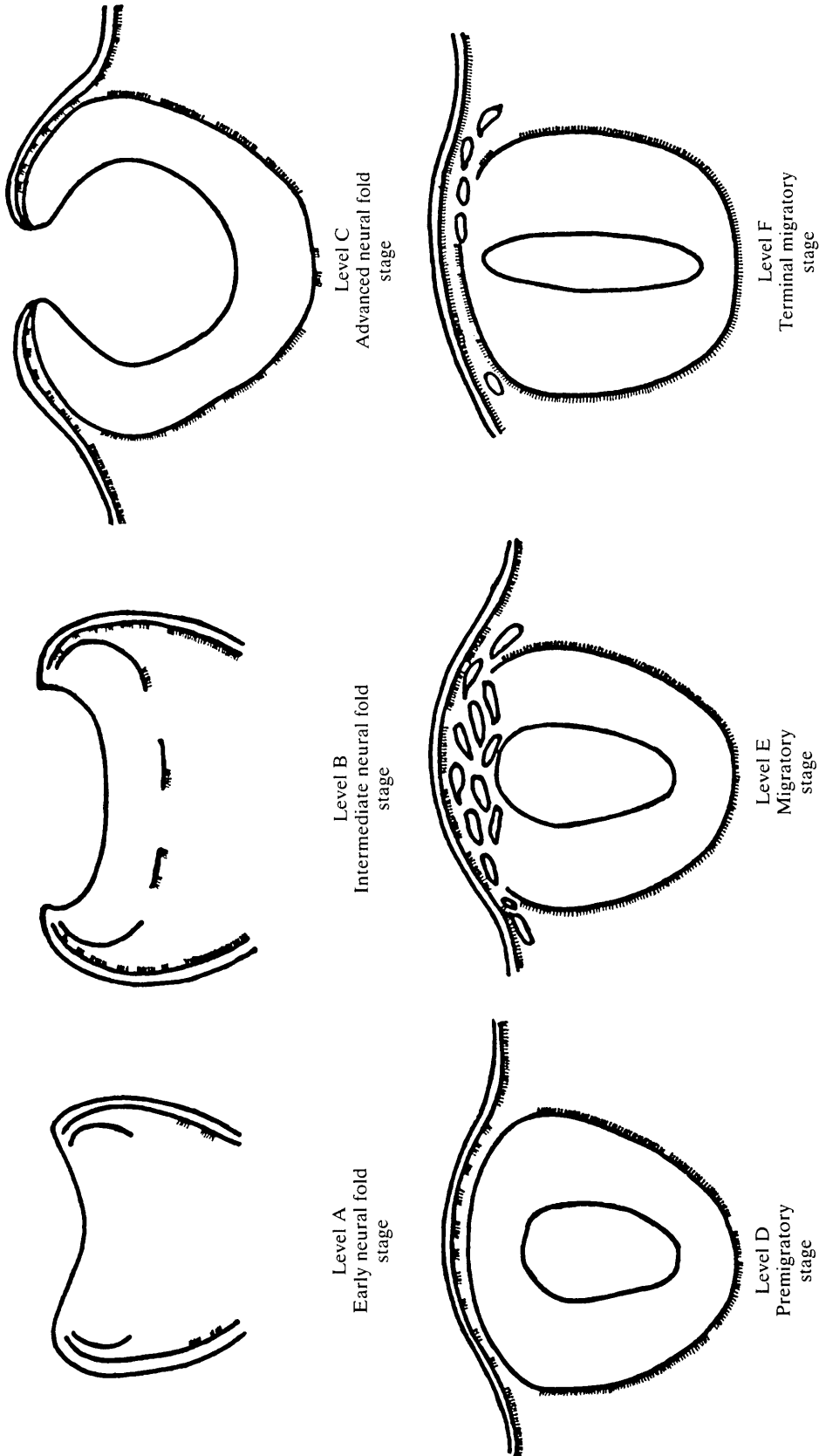


Fig. 10. Schematic representation of cross sections of a 9.5-day-old mouse embryo. Levels at which the cross sections were observed are indicated in Fig. 1. Level A, early neural fold stage; level B, intermediate neural fold stage; level C, advanced neural fold stage; level D, premigratory stage; level E, migratory stage; level F, terminal migratory stage. BL is represented by hachures beneath the epidermal ectoderm (EE) and around the neural tube (NT). The BL associated with the lateral and ventral portions of the NT becomes progressively more extensive; deposition begins on the lateral portions and then extends ventrally into the region adjacent to the notochord, becoming continuous only at migratory stages. Over the dorsal portion of the NT, however, it is completely absent until after the NC cells have emigrated. Beneath the EE, deposition of BL begins early but remains discontinuous over the NC cell region until terminal migratory stages.

We have not found the onset of migration to correlate with any obvious changes in BL development. Once begun, however, emigration continues up to the time of BL completion. It would appear, therefore, that the only direct role that BL might be playing is to terminate emigration by becoming complete and preventing further NC cell emigration.

The progressive maturation of neural tube BL, in extension as well as in complexity, appears to be another aspect of the anterior-to-posterior wave of development. Furthermore, BL deposition around the notochord and around the gut follow a similar progression (Martins-Green, unpublished results). These observations suggest that this may be a general phenomenon during embryonic development and may be found in other mammals and, indeed, in other groups of organisms.

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